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(54) **BIALLELIC MARKERS RELATED TO GENES INVOLVED IN DRUG METABOLISM**

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(57) **ABSTRACT**

The invention provides polynucleotides including biallelic markers derived from genes involved in the biotransformation of xenobiotics such as drugs and from genomic regions flanking those genes. Primers hybridizing to regions flanking these biallelic markers are also provided. This invention also provides polynucleotides and methods suitable for genotyping a nucleic acid containing sample for one or more biallelic markers of the invention. Further, the invention provides methods to detect a statistical correlation between a biallelic marker allele and a phenotype and/or between a biallelic marker haplotype and a phenotype.

BIALLELIC MARKERS RELATED TO GENES INVOLVED IN DRUG METABOLISM

RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/671,317, filed Sep. 27, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/536,178 filed Mar. 23, 2000 and International Patent Application No. PCT/IB00/00403, filed Mar. 24, 2000 both of which claim priority to U.S. Provisional Patent Application Serial No. 60/126,269 filed Mar. 25, 1999 and U.S. Provisional Patent Application Serial No. 60/131,961 filed Apr. 30, 1999. All of the above applications are hereby incorporated herein in their entirety including any figures, tables, or drawings.

FIELD OF THE INVENTION

[0002] The present invention is in the field of pharmaco-genomics, and is primarily directed to biallelic markers that are located in or in the vicinity of genes, which have an impact on the metabolism of xenobiotics such as drugs and the uses of these markers. The present invention encompasses methods of establishing associations between these markers and a phenotype such as drug response, toxicity and susceptibility to disease. The present invention also provides means to determine the genetic predisposition of individuals to such drug responses, toxicity and diseases.

BACKGROUND OF THE INVENTION

[0003] To assess the origins of individual variations in drug response, pharmacogenomics uses the genomics technologies to identify polymorphisms within genes associated with drug response. In this respect, there are three main categories of genes that may theoretically be expected to be associated with drug response, namely genes linked with the targeted disease, genes related to the drug's mode of action and genes involved in the drug's metabolism. Among these genes of pharmacogenomic importance, genes coding for drug-metabolizing enzymes have a central role.

[0004] Drug Metabolism

[0005] Drug-metabolizing enzymes are important determinants of drug disposition, safety and efficacy. The enzyme systems involved in the metabolism and the subsequent elimination from the body of environmental chemicals, food toxins and drugs are mainly localized in the liver, although every tissue examined has some metabolic activity.

[0006] In order to produce its characteristic effects, a given drug must be present in appropriate concentrations at its sites of action. The absorption, distribution, biotransformation and excretion of a drug all involve its passage across cell membranes. The lipophilic characteristics of drugs that promote their passage through biological membranes and subsequent access to their site of action reduce their elimination from the body. Renal excretion of unchanged drug plays only a modest role in the overall elimination of most therapeutic agents, since lipophilic compounds filtered through the glomerulus are largely reabsorbed through the tubular membranes. Biotransformation of drugs into more hydrophilic metabolites plays a major role in the termination of their biological activity and their elimination from the body. In general, biotransformation reactions generate more polar, inactive metabolites that are readily excreted from the body. However in some cases, metabolites with potent biological activity or toxic properties are generated and may result in adverse side effects. Metabolic biotransformation of drugs can be classified as either Phase I functionalization reactions or Phase II biosynthetic reactions. Phase I reactions introduce or expose a functional group on the parent compound, and generally result in the loss of pharmacological activity although there are some examples of retention or enhancement of activity. Phase II conjugation reactions lead to the formation of a covalent linkage between a functional group on the parent compound with glucuronic acid, sulfate, glutathione, amino acids or acetate. These highly polar conjugates are generally inactive and are excreted rapidly in the urine and feces. Within a given cell, most drug metabolizing Phase I enzymes are located primarily in the endoplasmic reticulum, while the Phase II conjugation enzyme systems are mainly cytosolic. In some cases, drugs biotransformed through a Phase I reaction in the endoplasmic reticulum are further metabolized by conjugation in the cytosolic fraction of the same cell (Hardman J. G., Goodman, Gilman A., Limbird L. E.; *Goodman & Gilman's The Pharmacological Basis of Therapeutics,* 9th edition, McGraw-Hill, N.Y., 1996).

[0007] Enzymes Involved in the Biotransformation of Xenobiotics

[0008] Besides being involved in the biotransformation of drugs, drug-metabolizing enzymes are also involved in the metabolism of xenobiotics (foreign compounds) as well as in the metabolism of endogenous compounds including steroids, vitamins and fatty acids. Foreign compounds include therapeutic agents, carcinogens, plant metabolites, environmental pollutants, foodstuffs and other dietary components as well as industrial chemicals. The biotransformation of foreign compounds (xenobiotics) is often regarded as detoxification because it usually converts compounds into more water-soluble, readily excreted substances. This tends to decrease the exposure of the organism to the compound and therefore tends to decrease toxicity. However, in some cases the reverse occurs and a metabolite is produced which is more toxic than the parent compound. For example, drug-metabolizing enzymes may activate some carcinogens, and interindividual differences in cancer susceptibilities, have been linked to polymorphisms in drug-metabolizing enzymes. There are many factors, which affect biotransformation and toxicity, such as the dose, availability of cofactors and the relative activity of the various drug-metabolizing enzymes. There may also be several competing pathways of metabolism—some leading to detoxification others to toxicity. Factors, such as genetic factors or environmental factors, which influence the balance between these competing pathways, will also determine the eventual toxicity.

[0009] As mentioned above, the metabolic conversion of drugs and other xenobiotics is enzymatic in nature. The enzyme systems involved in the biotransformation of drugs are localized in the liver, although every tissue examined has some metabolic activity. Other organs with significant metabolic capacity include the kidneys, gastrointestinal tract, skin and lungs. Following non-parenteral administration of a drug, a significant portion of the dose may be metabolically inactivated in either the liver or intestines before it

reaches the systemic circulation. This first-pass metabolism significantly limits the oral availability of highly metabolized drugs.

[0010] Cytochrome P450

[0011] The cytochrome P450 enzyme family is the major catalyst of biotransformation reactions. Since its origin, the cytochrome P450 gene family has diversified to accommodate the metabolism of a growing number of environmental chemicals, food toxins and drugs. The resulting superfamily of enzymes catalyzes a wide variety of oxidative and reductive reactions and has activity towards a chemically diverse group of substrates. Cytochrome P450 enzymes are heme-containing membrane proteins localized in the smooth endoplasmic reticulum of numerous tissues. Oxidative reactions catalyzed by the microsomal monooxygenase system require the cytochrome P450 hemoprotein, NADPH-cytochrome P450 reductase, NADPH, and molecular oxygen. Oxidative biotransformations catalyzed by cytochrome P450 monooxygenases include aromatic and side chain hydroxylation, N-, O-, S-dealkylation, N-oxidation, sulfoxidation, N-hydroxylation, deamination, dehalogenation, and desulftiration. Cytochrome P450 enzymes also catalyze a number of reductive reactions, generally under conditions of low oxygen tension. The only common structural feature of the diverse group of xenobiotics oxidized by cytochrome P450 enzymes is their high lipid solubility.

[0012] Twelve cytochrome P450 gene families have been identified in human beings, and a number of distinct cytochrome P450 enzymes often exist within a single cell. The cytochrome P450 1, 2 and 3 families (CYP1, CYP2, CYP3) encode the enzymes involved in the majority of all drug biotransfonmations, while the gene products of the remaining cytochrome P450 families are important in the metabolism of endogenous compounds such as steroids and fatty acids. CYP1A2 gene expression may play an important role in individual risk of environmental toxicity or cancer. CYP1A2 substrates include clinically important drugs such as imipramine, propranolol, paracetamol, clozapine, theophyline, caffeine and acetaimnophen. CYP1A2 is also involved in the conversion of heterocyclic amines and arylamines to their proximal carcinogenic and mutagenic forms, as well as in the metabolism of endogenous substances including estradiol and uroporphyrinogen III. Interindividual differences in susceptibility to arylamine- and heterocyclicamine-induced cancers have been linked to CYP1A2 polymorphism. CYP2C8 appears to be responsible for retinol and retinoic acid metabolism and actively catalyzes benzphetamine N-demethylation. CYP2C9 catalyzes the hydroxylation of tolbutamide, a hypoglycemic agent used in the treatment of type II diabetes mellitus, and one allelic variant of CYP2C9 accounts for the occurrence of poor metabolizers of tolbutamide. CYP2C9 may also have an important role in terminating the anti-coagulant activity of warfarin. Widespread interindividual differences in the response to warfarin have been recognized. Such variability is particularly important for drugs such as warfarin which have narrow therapeutic indices (Steward D. J. et al., *Pharmacogenetics,* 7:361-367, 1997). CYP2C9 is further involved in the oxidation of tielinic acid and several non-steroidal anti-inflammatory agents. The oxidative metabolism through CYP2C9 of tilenic acid can result in the emergence of a drug induced autoimmune hepatitis. CYP3A4 is involved in the biotransformation of a majority

of drugs and is expressed at significant levels extrahepatically. It is now recognized that extensive metabolism by CYP3A4 in the gastrointestinal tract is a significant factor contributing to the poor oral availability of many drugs (first-pass metabolism). Barbiturates, certain steroids and macrolide antibiotics can induce this enzyme. It appears to play a central role in the metabolism of the immunosuppressive cyclic peptide cyclosporin A as well as macrolide antibiotics, such as erythromycin.

[0013] Flavin-Containing Monooxygenases (FMOs)

[0014] The mammalian flavin-containing monooxygenases (FMOs) are microsomal enzymes that catalyze the NADPH-dependent oxygenation of a wide variety of drugs and other xenobiotics that possess a soft nucleophilic heteroatom, typically a nitrogen, sulfur, phosphorus or selenium atom. Of special clinical interest is the oxidation of trimethylamine in the liver by the FMO, because its deficiency causes the "Fish Odor Syndrome." Drugs oxidized by FMOs include, among others, antidepressant, antipsychotic-neuroleptic, antihypertensive drugs. FMOs have been implicated in the detoxification but also in the metabolic activation of several different environmental toxins and carcinogens.

[0015] Unlike all other known oxidases and monooxygenases, among which the well-studied cytochrome P450 monooxygenases, FMOs have the unique property of forming a stable enzyme intermediate in the absence of an oxygenatable substrate. Because the energy for catalysis is already present in the FMO enzyme before contact with the potential substrate, the fit of the substrate does not need to be as stringent as with the other enzymes. This feature, unique to FMOs among monooxygenases, is responsible for the wide range of substrates accepted by FMOs (including tertiary and secondary alkyl- and arylamines, many hydrazines, thiocarbamides, thioamides, sulfides, disulfides, thiols, among others), and determines that any soft nucleophilic xenobiotic accessible to the active enzyme will probably be oxidized by FMO in vivo. Although some FMO substrates are oxidized to less active derivatives, several soft nucleophiles are metabolized to highly reactive and potentially toxic intermediates.

[0016] The FMOs represent a multigene family. Five distinct mammalian FMO isoenzymes have been identified and cloned from various animal and human tissues: FMO1, FMO2, FMO3, FMO4 and FMO5. Human FMO2 and human FMOX were cloned and sequenced by the inventors as described in PCT Publication WO 9824914. FMOX represents a new member of the FMO gene family not previously identified in mammals. Tissue specificity and activities of the different FMOs have been thoroughly characterized. FMO1 is known to be expressed in the human kidney but is absent from the liver. In man the enzyme, is subject to developmental regulation. FMO2 is predominantly expressed in lung of all mammalian species tested. FMO3 was isolated from human liver, and accounts for the majority of FMO expressed in adult human liver.

[0017] Many of the FMO substrates may also be oxidized by the cytochrome P450 monooxygenases. However, the final oxidation products are usually different, and the nitrogen of a specific compound is rarely N-oxygenated by both types of monooxygenases. Today, a large number of drugs in human clinical trials contain a nitrogen, sulfur, phosphorous

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or some other nucleophilic functionality. Of the two major monooxygenase systems considered to be responsible for heteroatom-containing chemical and drug oxidative metabolism (CYP 450 and FMO), relatively little is known concerning the role of the FMO in human drug metabolism. Yet, given the wide range of substrates potentially oxidized by FMOs, this class of monooxygenases seems to represent a major determinant of drug safety and efficacy.

[0018] Uridine Diphosphate Glucoronosyl Transferase (UGTs)

[0019] Glucoronidation is a major detoxification pathway of Phase II metabolism that is catalyzed by the UDP-glucuronosyl transferase family of enzymes. Glucuronidation is quantitatively the most important conjugation reaction. Members of this enzyme family catalyze the conjugation of numerous endogenous substances of widely differing structures such as bilirubin, steroid hormones and fat-soluble vitamins. In general, xenobiotics become substrates for glucoronidation by first passing through Phase I metabolism, but many compounds do not require this step because they already possess reactive functionalities (e.g. hydroxyl, carboxyl, amino, sulfhydryl etc.) that are direct targets for glucuronosyl transferase. The human UGT genes appear to have evolved by a series of gene-duplication and gene-conversion events resulting in the emergence of a diversity of isoforms. They are divided into two families, UGT1 which is known to have bilirubin and phenol as substrates, and UGT2 which is known to have steroid, bile, and odorant as substrates, with these two families located on different chromosomes. The UGT2 family is divided into subfamilies UGT2A and UGT2B. The UGTs have different but sometimes overlapping substrate specificities. They catalyze the transfer of an activated glucuronic acid molecule to aromatic and aliphatic alcohols, carboxylic acids, amines and free sulthydryl groups of both exogenous and endogenous compounds, to form O- N- and S-glueuronide conjugates. The increased water solubility of the glucuronide conjugates promotes their elimination in the urine or bile. In addition to high levels of expression in the liver, UGTs are also found in the kidney, intestine, brain and skin. Glucoronidation constitutes, from a general point of view, a reaction of detoxification and elimination. It generally leads to the formation of inactive metabolites and therefore, glucoronidation can dramatically modify the pharmacological activity of a drug. Moreover, UGTs play a major role in the elimination of nucleophilic metabolites of carcinogens, such as phenols and quinols of polycyclic aromatic hydrocarbons. In this way they prevent their further oxidation to electrophiles, which may react with DNA, RNA or protein. On the other hand, glucoronidation of certain compounds facilitates metabolic activation. Aromatic amines are some of the most studied examples of the role glucoronidation plays in metabolic activation of carcinogens. Glucoronidation has also been implicated in adverse drug reactions of certain carboxylic drugs, which resulted in a toxic immunological response. Glucoronidation although generally a detoxification reaction, may occasionally be involved in increasing toxicity.

[0020] Glutathione Conjugation and Further Metabolism

[0021] Glutathione is a tripepetide (γ-glutamylcysteinylglycine, GSH) found in high concentrations in most mammalian tissues, but especially in the liver. Glutathione has

several functions including roles in metabolism, transport and catalysis. Glutathione is also important for the maintenance of the thiol moieties of proteins and for the maintenance of the reduced form of other molecules such as cysteine, coenzyme A, and antioxidants such as ascorbic acid; it is also used in the formation of deoxyribonucleic acids (Anderson M. E., *Advances in Pharmacology*, 38: 65-74, 1997). Glutathione has a major protective role in the body, as it is the major cellular antioxidant. GSH can react non-enzymatically with reactive oxygen species (ROS) and thereby protect the cell from oxidative damage. ROS have been widely implicated in the pathology of numerous diseases such as arteriosclerosis, rheumatoid arthritis, cancer, AIDS, adult respiratory distress syndrome and Parkinson's disease.

[0022] Moreover, conjugation with the tripeptide glutathione represents a major detoxification pathway for xenobiotics including drugs and carcinogens. Glutathione may react either chemically or in enzyme catalyzed reactions with a variety of compounds, which are reactive electrophilic metabolites produced in Phase I reactions. The glutathione S-transferase enzymes (GSTs) that catalyze these reactions are members of a multigene family and are expressed in virtually all tissues. Glutathione conjugates are cleaved to cysteine derivatives and subsequently are acetylated by a series of enzymes located primarily in the kidney to give N-acetylcysteine conjugates collectively referred to as mercapturic acids. Mercapturic acid derivatives are the ultimate metabolites excreted in the urine. This is a particularly important route of Phase II metabolism from the toxicological point of view, as it is often involved in the removal of reactive intermediates. Xenobiotics that act as substrates for the glutathione S-transferases (GSTs) fall into four broad categories: electropilic carbon, nitrogen, sulfur and oxygen. Examples of substrates for glutathione S-transferases include aromatic, heterocyclic, alicyclic and aliphatic epoxides; aromatic halogen and nitro compounds; alkyl halides; and unsaturated aliphatic compounds (Ballantyne, B., Marrs T. and Turner P., *General & Applied Toxicology*, Stockton Press; New York, 1993). The GSTs are also involved in the metabolism of endogenous molecules such as the leukotrienes. As mentioned above, many of the enzymes involved in xenobiotic metabolism are also involved in specific aspects of the metabolism of normal cellular biochemical constituents. Leukotrienes are important mediators and modulators of the inflammatory reaction and contribute to a number of physiological and pathological processes. Moreover, the GSTs are also capable of directly binding hydrophobic compounds such as heme, bilirubin, and steroids, which may enable them to serve as intracellular storage and transport proteins for biological substances with limited water solubility. By their catalytic activity and their capacity for binding, the GSTs provide the cell with mechanism to protect itself from the noxious effects of various xenobiotics and endogenous substances. Further, GSTs may undergo amplification in tumors and may thereby be implicated in drug resistance in cancer chemotherapy. GSTs are mostly cytosolic although, more recently, microsomal GSTs have been identified. Human microsomal GST II (MGST II) is a member of the microsomal glutathione S-transferase family. This enzyme catalyzes the production of LTC4 (leukotriene C4) from LTA4 (leukotriene A4) and reduced glutathione. Leukotrienes are derived from arachidonic acid and related fatty acids. Metabolites of arachidonic acid have

been collectively termed eicosanoids, the principal eicosanoids are prostaglandins, thromboxanes and leukotrienes (LT). Eicosanoids are among the most important chemical mediators and modulators of the inflammatory reaction and contribute to a number of physiological and pathological processes (see Hardman J. G., Goodman, Gilman A., Limbird L. E.; *Goodman & Gilman's The Pharmacological Basis of Therapeutics,* 9th edition, McGraw-Hill, N.Y., 1996). The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury, although in some situations and diseases the inflammatory response may be exaggerated and sustained for no apparent beneficial reason. This is the case in numerous chronic inflammatory diseases and allergic inflammation. Acute allergic inflammation is characterized by increased blood flow, extravasation of plasma and recruitment of leukocytes. These events are triggered by locally released inflammatory mediators including eicosanoids and more particularly leukotrienes. The participation of arachidonic acid metabolism in inflammatory diseases such as rheumatoid arthritis, asthma and acute allergy is well established. Pathological actions of leukotrienes are best understood in terms of their roles in immediate hypersensitivity and asthma. LTC4 and LTD4 are potent bronchoconstrictors, they act principally on smooth muscle in peripheral airways and are a 1000 times more potent than histamine both in vitro and in vivo. They also stimulate bronchial mucus secretion and cause mucosal edema. A complex mixture of chemical messengers is released when sensitized lung tissue is challenged by the appropriate antigen. Various prostaglandins and leukotrienes are prominent components of this mixture. Response to the leukotrienes probably dominates during allergic constriction of the airway. A particularly important role for the cysteinyl-leukotrienes (LTC4, LTD4, and LTE4) has been suggested in pathogenesis of asthma, which is now recognized as a chronic inflammatory condition. They are potent spasmogens causing a contraction of bronchiolar muscle and an increase in mucus secretion. An increased LTC4 formation has also been reported in leukocytes from patients with chronic myelogenous leukemia (Stenke et al., *Acta Oncologica,* 27:803-805, 1987) and in experimental glomerulonephritis (Petric et al., *Biochim. Biophys. Acta,* 1254:207-215, 1995).

[0023] Moreover, MGST II has the capacity to conjugate other compounds such as 1-chloro-2, 4 dinitrobenzene with glutathione and may be involved in a general metabolic system for detoxifying fatty acid epoxides (Jakobsson et al., *Journal of Biological Chemistry,* 271:22203-22210, 1996).

[0024] The resulting glutathione conjugate usually undergoes further metabolism, which involves first a removal of the glutamyl residue, catalyzed by γ-glutamyltransferase (GGT). In addition to catalyzing the initial step in the conversion of glutathione-conjugated compounds to mercapturic acids, GGT also converts LTC4 to LTD4. Interestingly, expression of GGT is often increased in cancerous tissues.

[0025] Renal dipeptidase is also implicated in the renal metabolism of glutathione and its conjugates including conjugated xenobiotics and endogenous molecules such as Leukotriene D4. Pharmacologically it is an important enzyme, for it is responsible for hydrolysis of some β-lactam antibiotics such as penem and carbapenem.

[0026] The effectiveness of the GSTs and therefore of detoxification by glutathione conjugation in general as well as the ability of the cell to resist to oxidative stress, are strongly influenced by the availability of reduced glutathione. Reduction of oxidized glutathione and de novo synthesis of glutathione, are both completely dependent on NADPH. Glutathione reductase (GSHR) maintains high levels of reduced glutathione in the cytosol in an NADPH dependent reaction. Reduced glutathione is synthesized de novo in the cytosol of most cells via the γ-glutamyl cycle; a series of tightly controlled, enzyme catalyzed reactions. The first and second step in the de novo glutathione biosynthesis are catalyzed by γ-glutamylcysteine synthetase (GLCL) and glutathione synthase (GSHS) respectively. Deficiencies in γ-glutamylcysteine synthetase and GSH synthetase are associated with hemolytic anemia and impaired central nervous system function.

[0027] Glucose 6-Phosphate Dehydrogenase (G6PDH). Phosphogluconate Dehydrogenase (PGDH) and Malate Dehydrogenase: Generation of NADPH

[0028] NADPH (nicotinamide adenine dinucleotide phosphate) serves as an electron donor in reductive biosyntheses. In the pentose phosphate pathway, NADPH is generated when glucose 6-phosphate is oxidized to ribose 5-phosphate. G6PDH and PGDH are key enzymes of the pentose phosphate pathway and directly lead to the generation of NADPH. Another major source of NADPH is the oxidative decarboxylation of malate by malic enzyme.

[0029] NADPH may be used in anabolic processes such as fatty acid biosynthesis. One of the major functions of malic enzyme may be supplying NADPH to the cytosol for the synthesis of fatty acids from acetyl CoA (coenzyme A). Further, the cytochrome P450 system is dependent on NADPH. As mentioned above, availability of NADPH is also critical for the reduction of glutathione. The connection between generation of NADPH and reduction of glutathione is clearer in tissues that have limited glycolytic metabolism, e.g. the lens and the erythrocyte. Thus the viability of the erythrocyte depends on glutathione, kept reduced by this pathway. Moreover, factors that influence the availability of reduced glutathione drastically alter the effectiveness of glutathione S-transferases therefore also affecting drug metabolism. Under most conditions saturating levels of NADPH are provided to the cell. However, certain conditions can stress the ability of the cell to provide NADPH and it may become rate limiting.

[0030] G6PDH and PGDH are present in most cells and tissues. They serve as the key enzymes of the pentose phosphate pathway that control the flow of carbon through the pathway and produce reducing equivalents as NADPH to meet cellular needs for reductive biosynthesis and to maintain the redox state of the cell at physiological levels. Deficiency of G6PDH and PGDH leads to decreased levels of NADPH and is associated with hemolytic anemia in response to oxidative stress. The red cells of G6PDH deficient persons are susceptible to hemolysis by dietary substances, and by drugs such as primaquine, sulfones, sulfonamides, nitrofurans, vitamin K analogs, acetophenetidin, chloramphenicol, and many others.

[0031] Genetic Polymorphisms in Drug Metabolizing Enzymes and Pharmacogenomics

[0032] Genetic, environmental, and physiological factors are involved in the regulation of drug biotransformation

reactions. Results obtained from epidemiological studies and experimental animal model systems have shown a wide range of phenotypic variation in the ability of individuals to metabolize drugs and environmental chemicals. While some of this variation can be attributed to different environmental exposures, it has become clear that genetic factors also play an important role in determining the response of the individual to exogenous agents. Certain allelic forms of drug-metabolizing enzymes can render the individual either more sensitive or resistant to the toxic or therapeutic effects of exogenous drugs and chemicals. Genetic factors seem to be the major determinants of the variability of drug effects and are responsible for a number of striking quantitative and qualitative differences in pharmacological activity. Genetic differences in the ability of individuals to metabolize a drug through a given pathway are an important contributor to the large interindividual differences of drug efficacy and adverse effects within a population. There are many diverse examples of xenobiotics whose toxicity is directly dependent on the activity of drug-metabolizing enzymes. Often impaired metabolism of a drug through a genetically polymorphic pathway has been associated with an increased incidence of adverse effects in the slow metabolizer population (Weber W. W., *Pharmacogenetics*, Oxford University Press, N.Y., 1997). Moreover, genetic differences in the regulation, expression and activity of genes coding for Phase I and Phase II drug-metabolizing enzymes can be crucial factors in defining cancer susceptibility and the toxic or carcinogenic power of environmental chemicals and xenobiotics. In addition, the majority of serious cases of drug-drug interactions are a result of the interference of the metabolic clearance of one drug by a coadministered drug. The interference usually occurs via inhibition or induction of drug-metabolizing enzymes. Interindividual differences in susceptibility to severe drug-drug interactions also involve drug-metabolizing enzyme polymorphism. In some cases the design of the drug takes into account the activity of drug-metabolizing enzymes. For example, prodrugs require activation by drug-metabolizing enzymes to exhibit their therapeutic activity. The activation and efficiency of such prodrugs depends on interindividual polymorphism in drug-metabolizing enzymes.

[0033] Individual differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure. Therapeutic management and drug development can be markedly improved by the identification of specific genetic polymorphisms that determine and predict patient susceptibility to diseases or patient responses to drugs. Assessing individual risk rather than population risk will lead to better targeted therapeutic strategies defining individual drug usage based on a benefit/risk prognosis. To assess the origins of individual variations in disease susceptibility or drug response, pharmacogenomics uses the genomic technologies to identify polymorphisms within genes, which are part of biological pathways involved in disease susceptibility, etiology, and development, or more specifically in drug response pathways responsible for a drug's efficacy, tolerance or toxicity. It can provide tools to refine the design of drug development by decreasing the incidence of adverse events in drug tolerance studies, by better defining patient subpopulations of responders and non-responders in efficacy studies and, by combining the results obtained therefrom, to further allow better enlightened individualized drug usage based on efficacy/tolerance prognosis. Pharmacogenomics can also pro-

vide tools to identify new targets for designing drugs and to optimize the use of already existing drugs, in order to either increase their response rate and/or exclude non-responders from corresponding treatment, or decrease their undesirable side effects and/or exclude from corresponding treatment patients with marked susceptibility to undesirable side effects.

[0034] Drug-metabolizing enzymes are highly relevant to pharmacogenomics because they are at the core of drug response, drug efficacy and toxicity. Drug-metabolizing enzymes also determine an individual's susceptibility to exogenous chemicals and to a number of diseases associated with exposure to toxic or carcinogenic chemicals.

[0035] The complexity of the pathways and enzymes that are involved in detoxification and metabolism of drugs has limited the precise identification of the drug-metabolizing enzymes, which play the causal role in pathologies or in drug response. Therapeutic management and drug development can be markedly improved by the identification of genetic markers derived from drug-metabolizing enzymes that predict patient susceptibility to diseases or patient responses to drugs.

[0036] Genetic Analysis of Complex Traits

[0037] Until recently, the identification of genes linked with detectable traits has relied mainly on a statistical approach called linkage analysis. Linkage analysis is based upon establishing a correlation between the transmission of genetic markers and that of a specific trait throughout generations within a family. Linkage analysis involves the study of families with multiple affected individuals and is useful in the detection of inherited traits, which are caused by a single gene, or possibly a very small number of genes. Linkage analysis has been successfully applied to map simple genetic traits that show clear Mendelian inheritance patterns and which have a high penetrance (the probability that a person with a given genotype will exhibit a trait). About 100 pathological trait-causing genes have been discovered using linkage analysis over the last 10 years.

[0038] But, most traits of medical relevance do not follow simple Mendelian monogenic inheritance and linkage studies have proven difficult when applied to complex genetic traits. Many complex traits such as height, blood pressure or cancer susceptibility have been known to run in families and are at least partially determined by genetic factors. However, the genes or combination of genes that underlie these observable characteristics or traits remain unknown in most cases. Such complex traits are often due to the combined action of multiple genes as well as environmental factors. Because of their low penetrance, such complex traits do not segregate in a clear-cut Mendelian manner as they are passed from one generation to the next. Drug efficacy, response and tolerance/toxicity can also be considered as multifactoral traits involving a genetic component in the same way as complex diseases. Linkage analysis is impractical when the trait under study is drug response due to the lack of availability of familial cases. In fact, the likelihood of having more than one individual in a family being exposed to the same drug at the same time is very low. Linkage analysis cannot be applied to the study of such traits for which no large informative families are available. Attempts to map complex traits have been plagued by inconclusive results, demonstrating the need for more sophisticated genetic tools.

[0039] Knowledge of genetic variation in drug-metabolizing enzymes is important for understanding why some people are more susceptible to toxicity, pathology or respond differently to drugs. Ways to identify genetic polymorphism and to analyze how they impact and predict disease susceptibility and response to treatment are needed.

[0040] Whereas a number of polymorphisms and rare mutations have been identified in drug-metabolizing enzymes (see Weber W. W., *Pharmacogenetics*, Oxford University Press, New York, 1997), genetic markers for use in determining which genes contribute to multigenic or quantitative traits and suitable methods for exploiting those markers have not been found and brought to bare on the genes coding for drug-metabolizing enzymes.

SUMMARY OF THE INVENTION

[0041] The present invention is based on the discovery of a set of novel DME-related biallelic markers. See Table 11(A-B). These markers are located in the coding regions as well as non-coding regions adjacent to genes which are involved in the metabolic conversion of drugs and other xenobiotics. The position of these markers and knowledge of the surrounding sequence has been used to design polynucleotide compositions which are useful in determining the identity of nucleotides at the marker position, as well as more complex association and haplotyping studies which are useful in determining the genetic basis for variability in drug response and adverse reactions to drugs as well as the genetic basis for disease states involving the metabolic conversion of xenobiotics such as drugs. In addition, the compositions and methods of the invention find use in the identification of the targets for the development of pharmaceutical agents and diagnostic methods, as well as the characterization of the differential efficacious responses to and side effects from pharmaceutical agents.

[0042] The present invention further stems from the isolation and characterization of the genomic sequence of the MGST-II gene including its regulatory regions and of the complete cDNA sequence encoding the MGST-II enzyme. Oligonucleotide probes and primers hybridizing specifically with a genomic sequence of MGST-II are also part of the invention. A further object of the invention consists of recombinant vectors comprising any of the nucleic acid sequences described in the present invention, and in particular of recombinant vectors comprising the promoter region of MGST-II or a sequence encoding the MGST-II enzyme, as well as cell hosts comprising said nucleic acid sequences or recombinant vectors. The invention also encompasses methods of screening of molecules which, modulate or inhibit the expression of the MGST-II gene. The invention is also directed to biallelic markers that are located within the MGST-II genomic sequence, these biallelic markers representing useful tools in order to identify a statistically significant association between specific alleles of MGST-II gene and one or several disorders related to asthma and/or hepatotoxicity.

[0043] A first embodiment of the invention encompasses polynucleotides consisting of, consisting essentially of, or comprising a contiguous span of nucleotides of a sequence selected as an individual or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; pref-

erably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; or the sequences described in any one or more of Tables 12, 13, 14, 15, 16, 17, and 18, and the complements thereof, wherein said contiguous span is at least 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 75, 100, 200, 500, or 1000 nucleotides in length, to the extent that such a length is consistent with the lengths of the particular Sequence ID. The present invention also relates to polynucleotides hybridizing under stringent or intermediate conditions to a sequence selected from the group consisting of SEQ ID Nos. SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof. In addition, the polynucleotides of the invention encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: Said contiguous span may optionally include the DME-related biallelic marker in said sequence; Optionally either the original or the alternative allele of Table 13 may be specified as being present at said DME-related biallelic marker; Optionally either the first or the second allele of Table 12 or 14 may be specified as being present at said DME-related biallelic marker; Optionally, said polynucleotide may comprise, consists of, or consist essentially of a contiguous span which ranges in length from 8, 10, 12, 15, 18 or 20 to 25, 35, 40, 50, 60, 70, or 80 nucleotides, or be specified as being 12, 15, 18, 20, 25, 35, 40, or 50 nucleotides in length and including a DME-related biallelic marker of said sequence, and optionally the original allele of Table 13 is present at said biallelic marker; Optionally, said biallelic marker may be within 6, 5, 4, 3, 2, or 1 nucleotides of the center of said polynucleotide or at the center of said polynucleotide; Optionally, the 3' end of said contiguous span may be present at the 3' end of said polynucleotide; Optionally, biallelic marker may be present at the 3' end of said polynucleotide; Optionally, the 3' end of said polynucleotide may be located within or at least 2, 4, 6, 8, 10, 12, 15, 18, 20, 25, 50, 100, 250, 500, or 1000 nucleotides upstream of a DME-related biallelic marker in said sequence, to the extent that such a distance is consistent with the lengths of the particular Sequence ID; Optionally, the 3' end of said polynucleotide may be located 1 nucleotide upstream of a DME-related biallelic marker m said sequence; and Optionally, said polynucleotide may further comprise a label.

[0044] A second embodiment of the invention encompasses any polynucleotide of the invention attached to a solid support. In addition, the polynucleotides of the invention which are attached to a solid support encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said polynucleotides may be specified as attached individually or in groups of at least 2, 5, 8, 10, 12, 15, 20, or 25 distinct polynucleotides of the invention to a single solid support; Optionally, polynucleotides other than those of the invention may attached to the same solid support as polynucleotides of the invention; Optionally, when multiple polynucleotides are attached to a solid support they may be attached at random locations, or in an ordered array; Optionally, said ordered array may be addressable.

[0045] A third embodiment of the invention encompasses the use of any polynucleotide for, or any polynucleotide for

use in, determining the identity of one or more nucleotides at a DME-related biallelic marker. In addition, the polynucleotides of the invention for use in determining the identity of one or more nucleotides at a DME-related biallelic marker encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination. Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; Optionally, said polynucleotide may comprise a sequence disclosed in the present specification; Optionally, said polynucleotide may consist of, or consist essentially of any polynucleotide described in the present specification; Optionally, said determining may be performed in a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay; Optionally, said polynucleotide may be attached to a solid support, array, or addressable array; Optionally, said polynucleotide may be labeled.

[0046] A fourth embodiment of the invention encompasses the use of any polynucleotide for, or any polynucleotide for use in, amplifying a segment of nucleotides comprising a DME-related biallelic marker. In addition, the polynucleotides of the invention for use in amplifying a segment of nucleotides comprising a DME-related biallelic marker encompass polynucleotides with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof, preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; Optionally, said DME-related biallelic marker may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected from the biallelic markers found in Tables 19, 20, 21 and 22; Optionally, said DME-related biallelic marker may be selected from the following biallelic markers: 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440, and 10-420-284; Optionally, said polynucleotide may comprise a sequence disclosed in the present specification; Optionally, said polynucleotide may consist of, or consist essentially of any polynucleotide described in the present specification; Optionally, said amplifying may be performed by a PCR or LCR. Optionally, said polynucleotide may be attached to a solid support, array, or addressable array. Optionally, said polynucleotide may be labeled.

[0047] A fifth embodiment of the invention encompasses methods of genotyping a biological sample comprising determining the identity of a nucleotide at a DME-related biallelic marker. In addition, the genotyping methods of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID

Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; Optionally, said DME-related biallelic marker may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected from the biallelic markers found in Tables 19, 20, 21 and 22; Optionally, said DME-related biallelic marker may be selected from the following biallelic markers: 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440, and 10-420-284; Optionally, said method further comprises determining the identity of a second nucleotide at said biallelic marker, wherein said first nucleotide and second nucleotide are not base paired (by Watson & Crick base pairing) to one another; Optionally, said biological sample is derived from a single individual or subject; Optionally, said method is performed in vitro; Optionally, said biallelic marker is determined for both copies of said biallelic marker present in said individual's genome; Optionally, said biological sample is derived from multiple subjects or individuals; Optionally, said method further comprises amplifying a portion of said sequence comprising the biallelic marker prior to said determining step; Optionally, wherein said amplifying is performed by PCR, LCR, or replication of a recombinant vector comprising an origin of replication and said portion in a host cell; Optionally, wherein said determining is performed by a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay.

[0048] A sixth embodiment of the invention comprises methods of estimating the frequency of an allele in a population comprising genotyping individuals from said population for a DME-related biallelic marker and determining the proportional representation of said biallelic marker in said population. In addition, the methods of estimating the frequency of an allele in a population of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; Optionally, said DME-related biallelic marker may be selected from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected from the biallelic markers found in Tables 19, 20, 21 and 22; Optionally, said DME-related biallelic marker may be selected from the following biallelic markers: 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-

440, and 10-420-284; Optionally, determining the frequency of a biallelic marker allele in a population may be accomplished by determining the identity of the nucleotides for both copies of said biallelic marker present in the genome of each individual in said population and calculating the proportional representation of said nucleotide at said DME-related biallelic marker for the population; Optionally, determining the frequency of a biallelic marker allele in a population may be accomplished by performing a genotyping method on a pooled biological sample derived from a representative number of individuals, or each individual, in said population, and calculating the proportional amount of said nucleotide compared with the total.

[0049] A seventh embodiment of the invention comprises methods of detecting an association between an allele and a phenotype, comprising the steps of a) determining the frequency of at least one DME-related biallelic marker allele in a case (trait positive) population, b) determining the frequency of said DME-related biallelic marker allele in a control population and; c) determining whether a statistically significant association exists between said genotype and said phenotype. In addition, the methods of detecting an association between an allele and a phenotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof, Optionally, said DME-related biallelic marker may be selected from the biallelic markers described in Table 11(A-B); Optionally, said control population may be a trait negative population, or a random population; Optionally, said phenotype is a response to a drug, or a side effects to a drug, or a disease involving the metabolic conversion of xenobiotics; Optionally, the identity of the nucleotides at the biallelic markers in everyone of the following sequences: SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977 is determined in steps a) and b).

[0050] An eighth embodiment of the present invention encompasses methods of estimating the frequency of a haplotype for a set of biallelic markers in a population, comprising the steps of: a) genotyping each individual in said population for at least one DME-related biallelic marker, b) genotyping each individual in said population for a second biallelic marker by determining the identity of the nucleotides at said second biallelic marker for both copies of said second biallelic marker present in the genome; and c) applying a haplotype determination method to the identities of the nucleotides determined in steps a) and b) to obtain an estimate of said frequency. In addition, the methods of estimating the frequency of a haplotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally said haplotype determination method is selected from the group consisting of asymmetric PCR amplification, double PCR amplification of specific alleles, the Clark method, and an expectation maximization algorithm; Optionally, said second biallelic marker is a DME-related biallelic marker in a sequence selected from the group consisting of the biallelic markers of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof; Optionally, said DME-related biallelic markers may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected from the biallelic markers found in Tables 19, 20, 21 and 22; Optionally, said DME-related biallelic marker may be selected from the following biallelic markers: 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440, and 10-420-284; Optionally, the identity of the nucleotides at the biallelic markers in everyone of the sequences of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977 is determined in steps a) and b).

[0051] A ninth embodiment of the present invention encompasses methods of detecting an association between a haplotype and a phenotype, comprising the steps of: a) estimating the frequency of at least one haplotype in a trait positive population according to a method of estimating the frequency of a haplotype of the invention; b) estimating the frequency of said haplotype in a control population according to the method of estimating the frequency of a haplotype of the invention; and c) determining whether a statistically significant association exists between said haplotype and said phenotype. In addition, the methods of detecting an association between a haplotype and a phenotype of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the complements thereof, Optionally, said DME-related biallelic markers may be selected individually or in any combination from the biallelic markers described in Table 11(A-B); Optionally, said DME-related biallelic marker may be selected from the biallelic markers found in Tables 19, 20, 21 and 22; Optionally, said DME-related biallelic marker may be selected from the following biallelic markers: 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440, and 10-420-284; Optionally, said control population may be a trait negative population, or a random population; Optionally, said phenotype is a response to a drug, or a side effects to a drug, or a disease involving the metabolic conversion of xenobiotics; Optionally, the identity of the nucleotides at the biallelic markers in everyone of the following sequences: SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977 is determined in steps a) and b).

[0052] A tenth embodiment of the present invention is a method of administering a drug or a treatment comprising the steps of: a) obtaining a nucleic acid sample from an individual; b) determining the identity of the polymorphic base of at least one DME-related biallelic marker which is associated with a positive response to the treatment or the drug; or at least one biallelic DME-related biallelic marker which is associated with a negative response to the treatment or the drug; and c) administering the treatment or the drug to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug. In addition, the methods of the present invention for administering a drug or a treatment encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof, or preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, 958-977, and the complements thereof; or more preferably SEQ ID Nos. 496, 498, 502, 506-508, 518, 524, 526, 530, 531, 534, 816, 838, 844-846, 850, 870, 873, and the complements thereof; Optionally, the administering step comprises administering the drug or the treatment to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

[0053] An eleventh embodiment of the present invention is a method of selecting an individual for inclusion in a clinical trial of a treatment or drug comprising the steps of: a) obtaining a nucleic acid sample from an individual; b) determining the identity of the polymorphic base of at least one DME-related biallelic marker which is associated with a positive response to the treatment or the drug, or at least one DME-related biallelic marker which is associated with a negative response to the treatment or the drug in the nucleic acid sample, and c) including the individual in the clinical trial if the nucleic acid sample contains said DME-related biallelic marker associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug. In addition, the methods of the present invention for selecting an individual for inclusion in a clinical trial of a treatment or drug encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination: Optionally, said DME-related biallelic marker may be in a sequence selected individually or in any combination from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the complements thereof, or preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, 958-977, and the complements thereof, or more preferably SEQ ID Nos. 496, 498, 502, 506-508, 518, 524, 526, 530, 531, 534, 816, 838, 844-846, 850, 870, 873, and the complements thereof; Optionally, the including step comprises administering the drug or the treatment to the individual if the nucleic acid sample contains said biallelic marker associated with a positive response to the treatment or the drug and the nucleic acid

sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

[0054] Additional embodiments are set forth in the Detailed Description of the Invention and in the Examples.

BRIEF DESCRIPTION OF THE TABLES

[0055] Table 11A is a chart containing a list of all of the DME-related biallelic markers for each gene with an indication of the gene for which the marker is in closest physical proximity, an indication of whether the markers have been validated by microsequencing (with a Y indicating that the markers have been validated by microsequencing and an N indicating that it has not), and an indication of the identity and frequency of the least common allele determined by genotyping (with a blank left to indicate that the frequency has not yet been reported for some markers). The frequencies were determined from DNA samples collected from a random US Caucasian population. When the marker was determined to be homozygous at the particular location for the random US Caucasian population, the homozygous bases were recorded in the "Genotyping Least Common Allele Frequency" column of Table 11A.

[0056] Table 11B contains all of the DME-related biallelic markers provided in Table 11A; however, they are provided in shorter, easier to search sequences of 47 nucleotides. Accordingly, Table 11A begins with SEQ ID No. 1 and ends with SEQ ID No. 484, while Table 11B begins with SEQ ID No. 494 and ends with SEQ ID No. 977 (SEQ ID Nos. 485-493 correspond to the genomic and protein sequences of the invention and are not repeated in Table 11B). Table 1 below contains the first five markers listed in the sequence listing and their corresponding SEQ ID numbers in Tables 11A and 11B to illustrate the relationship between Tables 11A and 11B:

[0057] Table 11B is the same as Table 11A in that it is a list of all of the DME-related biallelic markers for each gene with an indication of the gene for which the marker is in closest physical proximity, an indication of whether the markers have been validated by microsequencing (with a Y indicating that the markers have been validated by microsequencing and an N indicating that it has not), and an indication of the identity and frequency of the least common allele determined by genotyping (with a blank left to indicate that the frequency has not yet been reported for some markers). However, the "Biallelic Marker Position in SEQ ID No." for all of the DME-related biallelic markers provided in Table 11B is position 24 (representing the midpoint of the 47mers that make up Table 11B). The frequencies were determined from DNA samples collected from a random US Caucasian population.

[0058] Tables 12, 13, and 14 are charts containing lists of the DME-related biallelic markers. Each marker is described by indicating its SEQ ID, the biallelic marker ID, and the two most common alleles. Table 12 is a chart containing a list of biallelic markers surrounded by preferred sequences. In the column labeled, "POSITION RANGE OF PREFERRED SEQUENCE" of Table 12, regions of particularly preferred sequences are listed for each SEQ ID, which contain a DME-related biallelic marker, as well as particularly preferred regions of sequences that may not contain a DME-related biallelic marker but, which are in sufficiently

close proximity to a DME-related biallelic marker to be useful as amplification or sequencing primers.

[0059] Table 15 is a chart listing particular preferred sequences that are useful for designing some of the primers and probes of the invention. Each sequence is described by indicating its Sequence ID and the positions of the first and last nucleotides (position range) of the particular sequence in the Sequence ID.

[0060] Table 16 is a chart listing microsequencing primers which have been used to genotype DME-related biallelic markers (indicated by an *) and other preferred microsequencing primers for use in genotyping DME-related biallelic markers. Each of the primers which falls within the strand of nucleotides included in the Sequence Listing are described by indicating their Sequence ID number and the positions of the first and last nucleotides (position range) of the primers in the Sequence ID. Since the sequences in the Sequence Listing are single stranded and half the possible microsequencing primers are composed of nucleotide sequences from the complementary strand, the primers that are composed of nucleotides in the complementary strand are described by indicating their SEQ ID numbers and the positions of the first and last nucleotides to which they are complementary (complementary position range) in the Sequence ID.

[0061] Table 17 is a chart listing amplification primers which have been used to amplify polynucleotides containing one or more DME-related biallelic markers. Each of the primers which falls within the strand of nucleotides included in the Sequence Listing are described by indicating their Sequence ID number and the positions of the first and last nucleotides (position range) of the primers in the Sequence ID. Since the sequences in the Sequence Listing are single stranded and half the possible amplification primers are composed of nucleotide sequences from the complementary strand, the primers that are composed of nucleotides in the complementary strand are defined by the SEQ ID numbers and the positions of the first and last nucleotides to which they are complementary (complementary position range) in the Sequence ID.

[0062] Table 18 is a chart listing preferred probes useful in genotyping DME-related biallelic markers by hybridization assays. The probes are generally 25-mers with a DME-related biallelic marker in the center position, and described by indicating their Sequence ID number and the positions of the first and last nucleotides (position range) of the probes in the Sequence ID. The probes complementary to the sequences in each position range in each Sequence ID are also understood to be a part of this preferred list even though they are not specified separately.

[0063] Table 19 is a chart containing a list of preferred MGST-II-related biallelic markers with an indication of the frequency of both alleles determined by genotyping. Frequencies were determined in a random US Caucasian population.

[0064] Table 20 is a chart containing a list of preferred ME1-related biallelic markers with an indication of the frequency of both alleles determined by genotyping. Frequencies were determined in a random US Caucasian population.

[0065] Table 21 is a chart containing a list of preferred UGT1A7-related biallelic markers with an indication of the frequency of both alleles determined by genotyping. Frequencies were determined in a random US Caucasian population and a random French Caucasian population.

[0066] Table 22 is a chart containing a list of preferred UGT2B4-related biallelic markers with an indication of the frequency of both alleles determined by genotyping. Frequencies were determined in a random US Caucasian population and a random French Caucasian population.

[0067] Table 23 is a chart showing the results of a haplotype analysis study demonstrating an association between asthma and MGST-II-related biallelic marker haplotypes.

[0068] Table 24 is a chart showing the results of a permutation test which evaluates the statistical significance of the results obtained for the MGST2 haplotype analysis.

[0069] Table 25 is a chart showing the results of a single point allelic association analysis conducted on individuals with side effects to treatment with the anti-asthmatic drug Zyflo™ (zileuton) and MGST-II related biallelic markers.

[0070] Tables 26A and B are a chart showing the results of a haplotype analysis study demonstrating an association between side effects upon treatment with the anti-asthmatic drug Zyflo™ (zileuton) and MGST-II related biallelic marker haplotypes.

[0071] Table 27 is a table showing the results of a phenotypic permutation test which evaluates the statistical significance of the MGST-II haplotype analysis results.

[0072] Table 28 is a chart showing the results of a single point allelic association analysis conducted on individuals with side effects to treatment with the anti-asthmatic drug Zyflo™ (zileuton) and UGT1A7-related biallelic markers.

[0073] Tables 29A & B are a chart showing the results of a haplotype analysis study demonstrating an association between side effects upon treatment with the anti-asthmatic drug Zyflo™ (zileuton) and UGT1A7-related biallelic marker haplotypes.

[0074] Table 30 is a chart showing the results of a permutation test which evaluates the statistical significance of the results obtained from the UGT1A7 haplotype analysis.

[0075] Table 31 is a chart showing the results of a single point allelic association analysis conducted on individuals with side effects to treatment with the anti-asthmatic drug Zyflo™ (zileuton) and UGT2B4-related biallelic markers.

[0076] Tables 32A & B are a chart showing the results of a haplotype analysis study demonstrating an association between side effects upon, treatment with the anti-asthmatic drug Zyflo™ (zileuton) and UGT2B4-related biallelic marker haplotypes.

[0077] Table 33 is a chart showing the results of a permutation test which evaluates the statistical significance of the results obtained from the UGT2B4 haplotype analysis.

BRIEF DESCRIPTION OF THE SEQUENCES PROVIDED IN THE SEQUENCE LISTING

[0078] SEQ ID Nos. 1-484 contain the nucleotide sequence of genomic DNA fragments located in the vicinity of the candidate genes.

[0079] SEQ ID No. 485 contains the genomic sequence of the MGST-II gene comprising the 5' regulatory region (upstream untranscribed region), the exons and introns, and the 3' regulatory region (downstream untranscribed region).

[0080] SEQ ID No. 486 contains a cDNA sequence of MGST-II.

[0081] SEQ ID No. 487 contains a cDNA sequence of MGST-II corresponding to an alternative messenger RNA which is due to alternative splicing joining exon 1 to exon 3 and resulting in the absence of exon 2.

[0082] SEQ ID No. 488 contains the amino acid sequence encoded by the cDNA of SEQ ID No. 486.

[0083] SEQ ID No. 489 contains the amino acid sequence encoded by the cDNA of SEQ ID No. 487.

[0084] SEQ ID No. 490 contains a partial cDNA sequence of MGST-II sequence corresponding to a cloned partial messenger RNA.

[0085] SEQ ID No. 491 contains a partial cDNA sequence of MGST-II sequence corresponding to a cloned partial messenger RNA.

[0086] SEQ ID No. 492 contains a primer containing the additional PU 5' sequence described further in Example 1.

[0087] SEQ ID No. 493 contains a primer containing the additional RP 5' sequence described further in Example 1.

[0088] SEQ ID Nos. 494-977 contain the nucleotide sequence of genomic DNA fragments located in the vicinity of the candidate genes. These sequences are in easier to search sequences of 47 nucleotides.

DETAILED DESCRIPTION OF THE INVENTION

[0089] Advantages of the Biallelic Markers of the Present Invention

[0090] The DME-related biallelic markers of the present invention offer a number of important advantages over other genetic markers such as RFLP (Restriction fragment length polymorphism) and VNTR (Variable Number of Tandem Repeats) markers.

[0091] The first generation of markers, were RFLPs, which are variations that modify the length of a restriction fragment. But methods used to identify and to type RFLPs are relatively wasteful of materials, effort, and time. The second generation of genetic markers were VNTRs, which can be categorized as either minisatellites or microsatellites. Minisatellites are tandemly repeated DNA sequences present in units of 5-50 repeats which are distributed along regions of the human chromosomes ranging from 0.1 to 20 kilobases in length. Since they present many possible alleles, their informative content is very high. Minisatellites are scored by performing Southern blots to identify the number of tandem repeats present in a nucleic acid sample from the individual being tested. However, there are only 10^4 potential VNTRs that can be typed by Southern blotting. Moreover, both RFLP and VNTR markers are costly and time-consuming to develop and assay in large numbers.

[0092] Single nucleotide polymorphism or biallelic markers can be used in the same manner as RFLPs and VNTRs but offer several advantages. Single nucleotide polymor-

phisms are densely spaced in the human genome and represent the most frequent type of variation. An estimated number of more than 10^7 sites are scattered along the 3×10^9 base pairs of the human genome. Therefore, single nucleotide polymorphism occur at a greater frequency and with greater uniformity than RFLP or VNTR markers which means that there is a greater probability that such a marker will be found in close proximity to a genetic locus of interest. Single nucleotide polymorphisms are less variable than VNTR markers but are mutationally more stable.

[0093] Also, the different forms of a characterized single nucleotide polymorphism, such as the biallelic markers of the present invention, are often easier to distinguish and can therefore be typed easily on a routine basis. Biallelic markers have single nucleotide based alleles and they have only two common alleles, which allows highly parallel detection and automated scoring. The biallelic markers of the present invention offer the possibility of rapid, high-throughput genotyping of a large number of individuals.

[0094] Biallelic markers are densely spaced in the genome, sufficiently informative and can be assayed in large numbers. The combined effects of these advantages make biallelic markers extremely valuable in genetic studies. Biallelic markers can be used in linkage studies in families, in allele sharing methods, in linkage disequilibrium studies in populations, in association studies of case-control populations. An important aspect of the present invention is that biallelic markers allow association studies to be performed to identify genes involved in complex traits. Association studies examine the frequency of marker alleles in unrelated case- and control-populations and are generally employed in the detection of polygenic or sporadic traits. Association studies may be conducted within the general population and are not limited to studies performed on related individuals in affected families (linkage studies). Biallelic markers in different genes can be screened in parallel for direct association with disease or response to a treatment. This multiple gene approach is a powerful tool for a variety of human genetic studies as it provides the necessary statistical power to examine the synergistic effect of multiple genetic factors on a particular phenotype, drug response, sporadic trait, or disease state with a complex genetic etiology.

[0095] Candidate Genes of the Present Invention

[0096] Different approaches can be employed to perform association studies: genome-wide association studies, candidate region association studies and candidate gene association studies. Genome-wide association studies rely on the screening of genetic markers evenly spaced and covering the entire genome. Candidate region association studies rely on the screening of genetic markers evenly spaced covering a region identified as linked to the trait of interest. The candidate gene approach is based on the study of genetic markers specifically derived from genes potentially involved in a biological pathway related to the trait of interest. In the present invention, genes involved in drug metabolism have been chosen as candidate genes. As mentioned above, these genes are highly relevant to pharmacogenetics because they are at the core of drug response, drug efficacy and toxicity, moreover, drug-metabolizing enzymes also determine an individuals susceptibility to exogenous chemicals and to a number of diseases associated with exposure to toxic or carcinogenic chemicals. The candidate gene analysis clearly

provides a short-cut approach to the identification of genes and gene polymorphisms related to a particular trait when some information concerning the biology of the trait is available. However, it should be noted that all of the biallelic markers disclosed in the instant application can be employed as part of genome-wide association studies or as part of candidate region association studies and such uses are specifically contemplated in the present invention and claims. All of the markers are known to be in close proximity to the genes with which they are listed in Table 11(A-B). For a portion of the markers, the precise position of the marker with respect to the various coding and non-coding elements of the genes has also been determined.

[0097] The following is a table of abbreviations for the candidate genes as they appear throughout the specification:

[0098] Definitions

[0099] As used interchangeably herein, the terms "nucleic acids," "oligonucleotides" and "polynucleotides" include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The term "nucleotide" as used herein as an adjective to describe molecules comprising RNA, DNA, or RNA/DNA hybrid sequences of any length in single-stranded or duplex form. The term "nucleotide" is also used herein as a noun to refer to individual nucleotides or varieties of nucleotides, meaning a molecule, or individual unit in a larger nucleic acid molecule, comprising a purine or pyrimidine, a ribose or deoxyribose sugar moiety, and a phosphate group, or phosphodiester linkage in the case of nucleotides within an oligonucleotide or polynucleotide. Although the term "nucleotide" is also used herein to encompass "modified nucleotides" which comprise at least one modifications (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar, for examples of analogous linking groups, purine, pyrimidines, and sugars see for example PCT publication No. WO 95/04064, the disclosure of which is incorporated herein by reference in its entirety. However, the polynucleotides of the invention are preferably comprised of greater than 50% conventional deoxyribose nucleotides, and most preferably greater than 90% conventional deoxyribose nucleotides. The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, ex vivo generation, or a combination thereof, as well as utilizing any purification methods known in the art.

[0100] Throughout the present specification, the expression "nucleotide sequence" may be employed to designate indifferently a polynucleotide or a nucleic acid. More precisely, the expression "nucleotide sequence" encompasses the nucleic material itself and is thus not restricted to the sequence information (i.e. the succession of letters chosen among the four base letters) that biochemically characterizes a specific DNA or RNA molecule.

[0101] The term "polypeptide" refers to a polymer of amino without regard to the length of the polymer; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude prost-expression modifications of polypeptides, for example, polypeptides which include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term polypeptide. Also included within the definition are polypeptides which contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

[0102] The term "recombinant polypeptide" is used herein to refer to polypeptides that have been artificially designed and which comprise at least two polypeptide sequences that are not found as contiguous polypeptide sequences in their initial natural environment, or to refer to polypeptides which have been expressed from a recombinant polynucleotide.

[0103] As used herein, the term "isolated" requires that the material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated. Specifically excluded from the definition of "isolated" are: naturally occurring chromosomes (e.g., chromosome spreads) artificial chromosome libraries, genomic libraries, and cDNA libraries that exist either as an in vitro nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an in vitro heterogeneous preparation or plated as a heterogeneous population of single colonies. Also specifically excluded are the above libraries wherein the 5' EST makes up less than 5% of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including said whole cell preparations which are mechanically sheared or enzymaticly digested). Further specifically excluded are the above whole cell preparations as either an in vitro preparation or as a heterogeneous mixture separated by electrophoresis (including blot transfers of the same) wherein the polynucleotide of the invention have not been further separated from the heterologous polynucleotides in the electrophoresis medium (e.g., further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

[0104] As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Individual 5' EST clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. The sequences obtained from these clones could not be obtained directly either from the library or from total human DNA. The cDNA clones are not naturally occurring as such, but rather are obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The conversion of mRNA into a cDNA library involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection. Thus, creating a cDNA library from messenger RNA and subsequently isolating individual clones from that library results in an approximately 10^4-10^6 fold purification of the native message. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Alternatively, purification may be expressed as "at least" a percent purity relative to heterologous poly-

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nucleotides (DNA, RNA or both). As a preferred embodiment, the polynucleotides of the present invention are at least; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 96%, 98%, 99%, or 100% pure relative to heterologous polynucleotides. As a further preferred embodiment the polynucleotides have an "at least" purity ranging from any number, to the thousandth position, between 90% and 100% (e.g., 5' EST at least 99.995% pure) relative to heterologous polynucleotides. Additionally, purity of the polynucleotides may be expressed as a percentage (as described above) relative to all materials and compounds other than the carrier solution. Each number, to the thousandth position, may be claimed as individual species of purity.

[0105] As used herein, the term "non-human animal" refers to any non-human vertebrate, birds and more usually mammals, preferably primates, farm animals such as swine, goats, sheep, donkeys, and horses, rabbits or rodents, more preferably rats or mice. As used herein, the term "animal" is used to refer to any vertebrate, preferable a mammal. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

[0106] The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

[0107] The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., polynucleotide as defined herein) which can be used to identify a specific polynucleotide sequence present in samples, said nucleic acid segment comprising a nucleotide sequence complementary of the specific polynucleotide sequence to be identified.

[0108] The terms "trait" and "phenotype" are used interchangeably herein and refer to any visible, detectable or otherwise measurable property of an organism such as symptoms of, or susceptibility to a disease for example. Typically the terms "trait" or "phenotype" are used herein to refer to symptoms of, or susceptibility to a disease; or to refer to an individual's response to a drug; or to refer to symptoms of, or susceptibility to side effects to a drug. In addition, the terms "trait" or "phenotype" may be used herein to refer to symptoms of, or susceptibility to a disease involving arachidonic acid metabolism; or to refer to an individual's response to an agent acting on arachidonic acid metabolism; or to refer to symptoms of, or susceptibility to side effects to an agent acting on arachidonic acid metabolism.

[0109] The term "allele" is used herein to refer to variants of a nucleotide sequence. A biallelic polymorphism has two forms. Typically the first identified allele is designated as the original allele whereas other alleles are designated as alternative alleles. Diploid organisms may be homozygous or heterozygous for an allelic form.

[0110] The term "heterozygosity rate" is used herein to refer to the incidence of individuals in a population, which are heterozygous at a particular allele. In a biallelic system the heterozygosity rate is on average equal to $2P_a(1-P_a)$, where P_a is the frequency of the least common allele. In order to be useful in genetic studies a genetic marker should

have an adequate level of heterozygosity to allow a reasonable probability that a randomly selected person will be heterozygous.

[0111] The term "genotype" as used herein refers the identity of the alleles present in an individual or a sample. In the context of the present invention a genotype preferably refers to the description of the biallelic marker alleles present in an individual or a sample. The term "genotyping" a sample or an individual for a biallelic marker consists of determining the specific allele or the specific nucleotide carried by an individual at a biallelic marker.

[0112] The term "mutation" as used herein refers to a difference in DNA sequence between or among different genomes or individuals which has a frequency below 1%.

[0113] The term "haplotype" refers to a combination of alleles present in an individual or a sample. In the context of the present invention a haplotype preferably refers to a combination of biallelic marker alleles found in a given individual and which may be associated with a phenotype.

[0114] The term "polymorphism" as used herein refers to the occurrence of two or more alternative genomic sequences or alleles between or among different genomes or individuals. "Polymorphic" refers to the condition in which two or more variants of a specific genomic sequence can be found in a population. A "polymorphic site" is the locus at which the variation occurs. A single nucleotide polymorphism is a single base pair change. Typically a single nucleotide polymorphism is the replacement of one nucleotide by another nucleotide at the polymorphic site. Deletion of a single nucleotide or insertion of a single nucleotide, also give rise to single nucleotide polymorphisms. In the context of the present invention "single nucleotide polymorphism" preferably refers to a single nucleotide substitution. Typically, between different genomes or between different individuals, the polymorphic site may be occupied by two different nucleotides.

[0115] The terms "biallelic polymorphism" and "bialielic marker" are used interchangeably herein to refer to a polymorphism having two alleles at a fairly high frequency in the population, preferably a single nucleotide polymorphism. A "biallelic marker allele" refers to the nucleotide variants present at a biallelic marker site. Typically the frequency of the less common allele of the biallelic markers of the present invention has been validated to be greater than 1%, preferably the frequency is greater than 10%, more preferably the frequency is at least 20% (i.e. heterozygosity rate of at least 0.32), even more preferably the frequency is at least 30% (i.e. heterozygosity rate of at least 0.42). A biallelic marker wherein the frequency of the less common allele is 30% or more is termed a "high quality biallelic marker."

[0116] The location of nucleotides in a polynucleotide with respect to the center of the polynucleotide are described herein in the following manner. When a polynucleotide has an odd number of nucleotides, the nucleotide at an equal distance from the 3' and 5' ends of the polynucleotide is considered to be "at the center" of the polynucleotide, and any nucleotide immediately adjacent to the nucleotide at the center, or the nucleotide at the center itself is considered to be "within 1 nucleotide of the center." With an odd number of nucleotides in a polynucleotide any of the five nucleotides positions in the middle of the polynucleotide would be

considered to be within 2 nucleotides of the center, and so on. When a polynucleotide has an even number of nucleotides, there would be a bond and not a nucleotide at the center of the polynucleotide. Thus, either of the two central nucleotides would be considered to be "within 1 nucleotide of the center" and any of the four nucleotides in the middle of the polynucleotide would be considered to be "within 2 nucleotides of the center", and so on. For polymorphisms which involve the substitution, insertion or deletion of 1 or more nucleotides, the polymorphism, allele or biallelic marker is "at the center" of a polynucleotide if the difference between the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 3' end of the polynucleotide, and the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 5' end of the polynucleotide is zero or one nucleotide. If this difference is 0 to 3, then the polymorphism is considered to be "within 1 nucleotide of the center." If the difference is 0 to 5, the polymorphism is considered to be "within 2 nucleotides of the center." If the difference is 0 to 7, the polymorphism is considered to be "within 3 nucleotides of the center," and so on. For polymorphisms which involve the substitution, insertion or deletion of 1 or more nucleotides, the polymorphism, allele or biallelic marker is "at the center" of a polynucleotide if the difference between the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 3' end of the polynucleotide, and the distance from the substituted, inserted, or deleted polynucleotides of the polymorphism and the 5' end of the polynucleotide is zero or one nucleotide. If this difference is 0 to 3, then the polymorphism is considered to be "within 1 nucleotide of the center." If the difference is 0 to 5, the polymorphism is considered to be "within 2 nucleotides of the center." If the difference is 0 to 7, the polymorphism is considered to be "within 3 nucleotides of the center," and so on.

[0117] The term "upstream" is used herein to refer to a location which, is toward the 5' end of the polynucleotide from a specific reference point.

[0118] The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another by virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds (See Stryer, L., *Biochemistry,* 4th edition, 1995).

[0119] The terms "complementary" or "complement thereof" are used herein to refer to the sequences of polynucleotides which is capable of forming Watson & Crick base pairing with another specified polynucleotide throughout the entirety of the complementary region. This term is applied to pairs of polynucleotides based solely upon their sequences and not any particular set of conditions under which the two polynucleotides would actually bind.

[0120] A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell required to initiate the specific transcription of a gene.

[0121] A sequence which is "operably linked" to a regulatory sequence such as a promoter means that said regulatory element is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the nucleic acid of interest.

[0122] As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. More precisely, two DNA molecules (such as a polynucleotide containing a promoter region and a polynucleotide encoding a desired polypeptide or polynucleotide) are said to be "operably linked" if the nature of the linkage between the two polynucleotides does not (1) result in the introduction of a frame-shift mutation or (2) interfere with the ability of the polynucleotide containing the promoter to direct the transcription of the coding polynucleotide.

[0123] The terms "disease involving the metabolic conversion of xenobiotics" refers to susceptibility to a condition or to a condition linked to any of the genes listed in Table 11(A-B). "Disease involving the metabolic conversion of xenobiotics" further refers to a condition involving the biotransformation of drugs and other xenobiotics such as environmental chemicals, food toxins, plant metabolites, carcinogens and industrial chemicals. Such conditions include susceptibility to the toxic or carcinogenic effect of exogenous compounds. "Disease involving the metabolic conversion of xenobiotics" also refers to disorders in the metabolism of some endogenous compounds such as the metabolism of steroids, vitamins, fatty acids and eicosanoids such as leukotrienes involving any of the drug-metabolizing enzymes shown in Table 11(A-B). "Disease involving the metabolic conversion of xenobiotics" includes, but is not limited to, disorders involving the cytochrome P450 enzyme family, the flavin containing monooxygenases, glucoronidation, the metabolism of glutathione, the pentose pathway and the generation of NADPH.

[0124] The term "disease involving arachidonic acid metabolism" refers to a condition linked to disturbances in expression, production or cellular response to eicosanoids such as prostaglandins, thromboxanes, prostacyclins, leukotrienes or hydroperoxyeicosaetrenoic acids. A disease involving arachidonic acid metabolism further refers to a condition involving one or several enzymes of the distinct enzyme systems contributing to arachidonate metabolism including particularly the 5-lipoxygenase pathway. "Diseases involving arachidonic acid metabolism" also include chronic inflammatory diseases, acute allergic inflammation and inflammatory conditions such as pain, fever, hypersensitivity, asthma, psoriasis and arthritis. "Diseases involving arachidonic acid metabolism" also include disorders in platelet function, blood pressure, thrombosis, renal function, host defense mechanism, hemostasis, smooth muscle tone, male infertility, primary dysmenorrhea, disorders in parturition, and disorders in tissue injury repair, as well as disorders in cellular function and development. "Diseases involving arachidonic acid metabolism" also include diseases such as gastrointestinal ulceration, coronary and cerebrovascular syndromes, glomerular immune injury and cancer. Preferably the terms "disease involving arachidonic acid metabolism" refer to a disease including diseases such as cancer, prostate cancer, breast cancer, psoriasis and inflammatory diseases. Preferably the terms "disease involving arachidonic acid metabolism" refer to a disease involving the 5-lipoxygenase pathway and the biosynthesis of the leukotrienes. More preferably the terms "disease involving arachidonic acid metabolism" refer to a disease involving

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the synthesis of leukotriene C4 (LTC4) and refers to disturbances in expression, activity or function of the human MGST-II enzyme.

[0125] As used herein the term "DME-related biallelic marker" relates to a set of biallelic markers located in or in the vicinity of the genes disclosed in Table 11(A-B) and further relates to biallelic markers in linkage disequilibrium with the biallelic markers disclosed in Table 11(A-B). The term DME-related biallelic marker encompasses all of the biallelic markers disclosed in Table 11(A-B).

[0126] The invention also concerns MGST-II-related biallelic markers. The term "MGST-II-related biallelic marker" is used interchangeably herein to relate to all biallelic markers in linkage disequilibrium with the biallelic markers of the MGST-II gene. The term MGST-II-related biallelic marker includes both the genic and non-genic biallelic markers described in Table 3.

[0127] The term "non-genic" is used herein to describe MGST-II-related biallelic markers, as well as polynucleotides and primers which occur outside the nucleotide positions shown in the human MGST-II genomic sequence of SEQ ID No. 485. The term "genic" is used herein to describe MGST-II-related biallelic markers as well as polynucleotides and primers which do occur in the nucleotide positions shown in the human MGST-II genomic sequence of SEQ ID No. 485.

[0128] The terms "agent acting on arachidonic acid metabolism" refers to a drug or a compound modulating the activity or concentration of one or several enzymes of the distinct enzyme systems contributing to arachidonate metabolism including particularly the 5-lipoxygenase pathway. "Agent acting on arachidonic acid metabolism" also refers to compounds modulating the formation and action of the eicosanoids including particularly the leukotrienes.

[0129] The terms "response to a drug" refer to drug efficacy, including but not limited to ability to metabolize a therapeutic compound, to the ability to convert a pro-drug to an active drug, and to the pharmacokinetics (absorption, distribution, elimination) and the pharmacodynamics (receptor-related) of a drug in an individual.

[0130] The terms "response to an agent acting on arachidonic acid metabolism" refer to drug efficacy, including but not limited to ability to metabolize a compound, to the ability to convert a pro-drug to an active drug, and to the pharmacokinetics (absorption, distribution, elimination) and the pharmacodynamics (receptor-related) of a drug in an individual.

[0131] The terms "side effects to a drug" refer to adverse effects of therapy resulting from extensions of the principal pharmacological action of the drug or to idiosyncratic adverse reactions resulting from an interaction of the drug with unique host factors. "Side effects to a drug" include, but are not limited to, adverse reactions such as dermatologic, hematologic or hepatologic toxicities and further includes gastric and intestinal ulceration, disturbance in platelet function, renal injury, generalized urticaria, bronchoconstriction, hypotension, and shock.

[0132] The terms "side effects to an agent acting on arachidonic acid metabolism" refer to adverse effects of therapy resulting from extensions of the principal pharma-

cological action of the drug or to idiosyncratic adverse reactions resulting from an interaction of the drug with unique host factors. The terms "side effects to an agent acting on arachidonic acid metabolism" include, but are not limited to, adverse reactions such as dermatologic, hematologic or hepatologic toxicities.

[0133] The term "sequence described in Table 11(A-B)" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 11(A-B). The SEQ ID that contains each "sequence described in Table 11(A-B)" is provided in the column labeled, "SEQ ID NO." The column labeled "Gene" indicates the gene for which the marker is in closest physical proximity, an indication of whether the markers have been validated by microsequencing (with a Y indicating that the markers have been validated by microsequencing and an N indicating that it has not), and an indication of the identity and frequency of the least common allele determined by genotyping (with a blank left to indicate that the frequency has not yet been reported for some markers). The frequencies were determined from DNA samples collected from a random US Caucasian population.

[0134] The term "sequence described in Table 11B" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 11B. The SEQ ID that contains each "sequence described in Table 11B" is provided in the column labeled, "SEQ ID NO." The column labeled "Gene" indicates the gene for which the marker is in closest physical proximity, an indication of whether the markers have been validated by microsequencing (with a Y indicating that the markers have been validated by microsequencing and an N indicating that it has not), and an indication of the identity and frequency of the least common allele determined by genotyping (with a blank left to indicate that the frequency has not yet been reported for some markers). The frequencies were determined from DNA samples collected from a random US Caucasian population. The "Biallelic Marker location in SEQ ID No." indicates the biallelic marker location within the 47 nucleotide sequence. In Table 11B, this location is 24 for all of the markers.

[0135] The term "sequence described in Table 12" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 12. The SEQ ID that contains each "sequence described in Table 12" is provided in the column labeled, "SEQ ID NO." The range of nucleotide positions within the Sequence ID of which each sequence consists is provided in the same row as the Sequence ID in a column labeled, "POSITION RANGE OF PREFERRED SEQUENCE". It should be noted that some of the Sequence ID numbers have multiple sequence ranges listed, because they contain multiple "sequences described in Table 12." Unless otherwise noted the term "sequence described in Table 12" is to be construed as encompassing sequences that contain either of the two alleles listed in the columns labeled, "1ST ALLELE" and "2ND ALLELE" at the position identified in field<222>of the allele feature in the appended Sequence Listing for each Sequence ID number referenced in Table 12.

[0136] The term "sequence described in Table 13" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 13.

Unless otherwise noted, the "sequences described in Table 13" consist of the entire sequence of each Sequence ID provided in the column labeled, "SEQ ID NO." Also unless otherwise noted the term "sequence described in Table 13" is to be construed as encompassing sequences that contain either of the two alleles listed in the columns labeled, "ORIGINAL ALLELE" and "ALTERNATIVE ALLELE" at the position identified in field<222>of the allele feature in the appended Sequence Listing for each Sequence ID number referenced in Table 13.

[0137] The term "sequence described in Table 14" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 14. Unless otherwise noted, the "sequences described in Table 14" consist of the entire sequence of each Sequence ID provided in the column labeled, "SEQ ID NO." Also unless otherwise noted the term "sequence described in Table 14" is to be construed as encompassing sequences that contain either of the two alleles listed in the columns labeled, "1ST ALLELE" and "2ND ALLELE" at the position identified in field <222>of the allele feature in the appended Sequence Listing for each Sequence ID number referenced in Table 14.

[0138] The term "sequence described in Table 15" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 15. The SEQ ID that contains each "sequence described in Table 15" is provided in the column labeled, "SEQ ID NO." The range of nucleotide positions within the Sequence ID of which each sequence consists is provided in the same row as the Sequence ID in a column labeled, "POSITION RANGE OF PREFERRED SEQUENCE". It should be noted that some of the Sequence ID numbers have multiple sequence ranges listed, because they contain multiple "sequences described in Table 15."

[0139] The term "sequence described in Table 16" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 16. The SEQ ID that contains each "sequence described in Table 16" is provided in the column labeled, "SEQ ID" The range of nucleotide positions within the Sequence ID of which half of the sequences consists is provided in the same row as the Sequence ID in a column labeled, "POSITION RANGE OF MICROSEQUENCING PRIMERS". The remaining half of the sequences described in Table 16 are complementary to the range of nucleotide positions within the Sequence ID provided in the same row as the Sequence ID in a column labeled, "COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS".

[0140] The term "sequence described in Table 17" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 17. The SEQ ID that contains each "sequence described in Table 17" is provided in the column labeled, "SEQ ID" The range of nucleotide positions within the Sequence ID of which half of the sequences consists is provided in the same row as the Sequence ID in a column labeled, "POSITION RANGE OF AMPLIFICATION PRIMERS". The remaining half of the sequences described in Table 17 are complementary to the range of nucleotide positions within the Sequence ID provided in the same row as the Sequence ID in a column labeled, "COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS".

[0141] The term "sequence described in Table 18" is used herein to refer to the entire collection of nucleotide sequences or any individual sequence defined in Table 18. The SEQ ID that contains each "sequence described in Table 18" is provided in the column labeled, "SEQ ID". The range of nucleotide positions within the Sequence ID of which each sequence consists is provided in the same row as the Sequence ID in a column labeled, "POSITION RANGE OF PROBES". The sequences which are complementary to the ranges listed in the column labeled, "POSITION RANGE OF PROBES" are also encompassed by the term, "sequence described in Table 18." Unless otherwise noted the term "sequence described in Table 18" is to be construed as encompassing sequences that contain either of the two alleles listed in the allele feature in the sequence listing.

[0142] The terms "biallelic marker described in Table" and "allele described in Table" are used herein to refer to any or all alleles which are listed in the allele feature in the appended Sequence Listing for each Sequence ID number referenced in the particular Table being mentioned.

[0143] Variants and Fragments

[0144] The invention also relates to variants and fragments of the polynucleotides described herein, particularly of a MGST-II gene containing one or more biallelic markers according to the invention.

[0145] Variants of polynucleotides, as the term is used herein, are polynucleotides that differ from a reference polynucleotide. A variant of a polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical. Variants of polynucleotides according to the invention include, without being limited to, nucleotide sequences which are at least 95% identical, preferably at least 99% identical, more particularly at least 99.5% identical, and most preferably at least 99.8% identical to a polynucleotide selected from the group consisting of the polynucleotides of a sequence from any sequence in the Sequence Listing as well as sequences which are complementary thereto or to any polynucleotide fragment of at least 8 consecutive nucleotides of a sequence from any sequence in the Sequence Listing. Nucleotide changes present in a variant polynucleotide may be silent, which means that they do not alter the amino acids encoded by the polynucleotide. However, nucleotide changes may also result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. In the context of the present invention, particularly preferred embodiments are those in which the polynucleotides encode polypeptides which retain substantially the same biological function or activity as the mature MGST-II protein, or those in which the polynucleotides encode-

polypeptides which maintain or increase a particular bio-logical activity, while reducing a second biological activity. A polynucleotide fragment is a polynucleotide having a sequence that is entirely the same as part but not all of a given nucleotide sequence, preferably the nucleotide sequence of a MGST-II gene, and variants thereof. The fragment can be a portion of an exon or of an intron of a MGST-II gene. It can also be a portion of the regulatory regions of MGST-II, preferably of the promoter sequence of the MGST-II gene. Such fragments may be "free-standing", i.e. not part of or fused to other polynucleotides, or they may be comprised within a single larger polynucleotide of which they form a part or region. Indeed, several of these fragments may be present within a single larger polynucleotide.

[0146] Identity Between Nucleic Acids or Polypeptides

[0147] The terms "percentage of sequence identity" and "percentage homology" are used interchangeably herein to refer to comparisons among polynucleotides and polypep-tides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal align-ment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, divid-ing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Homology is evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, *Proc. Natl. Acad. Sci.* 85(8):2444-2448, 1988; Altschul et al., *J. Mol. Biol.* 215(3):403-410, 1990; Thompson et al., *Nucleic Acids Res.* 22(2):4673-4680, 1994; Higgins et al., *Methods Enzymol.* 266:383402, 1996; Altschul et al., *Nature Genetics* 3:266-272, 1993, the disclosures of which are incorporated herein by reference in their entireties). In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") which is well known in the art (See, e.g., Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 87:2267-2268, 1990; Altschul et al., *J. Mol. Biol.* 215(3):403-410, 1990; Altschul et al., *Nature Genetics* 3:266-272, 1993; Altschul et al., *Nuc. Acids Res.* 25:3389-3402, 1997, the disclosures of which are incorporated herein by reference in their entire-ties). In particular, five specific BLAST programs are used to perform the following task:

[0148] (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence data-base;

[0149] (2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

[0150] (3) BLASTX compares the six-frame concep-tual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

[0151] (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

[0152] (5) TBLASTX compares the six-frame trans-lations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence data-base.

[0153] The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are prefer-ably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet et al., *Science* 256:1443-1445, 1992; Henikoff and Henikoff, *Pro-teins* 17:49-61, 1993, the disclosures of which are incorpo-rated herein by reference in their entireties). Less preferably, the PAM or PAM250 matrices may also be used (See, e.g., Schwartz and Dayhoff, eds., *Matrices for Detecting Dis-tance Relationships: Atlas of Protein Sequence and Struc-ture, Washington:National Biomedical Research Founda-tion,* 1978, the disclosure of which is incorporated herein by reference in its entirety). The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (see, e.g., Karlin and Altschul, 1990).

[0154] Stringent Hybridization Conditions

[0155] By way of example and not limitation, procedures using conditions of high stringency are as follows: Prehy-bridization of filters containing DNA is carried out for 8 h to overnight at 65° C. in buffer composed of 6×SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65° C., the preferred hybridization temperature, in prehybridization mixture con-taining 100 μg/ml denatured salmon sperm DNA and 5-20× 10⁶ cpm of ³²P-labeled probe. Alternatively, the hybridiza-tion step can be performed at 65° C. in the presence of SSC buffer, 1×SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37° C. for 1 h in a solution containing 2×SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1×SSC at 50° C. for 45 min. Alternatively, filter washes can be performed in a solution containing 2×SSC and 0.1% SDS, or 0.5×SSC and 0.1% SDS, or 0.1×SSC and 0.1% SDS at 68° C. for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. Other conditions of high stringency which may be used are well known in the art and as cited in Sambrook et al., 1989; and Ausubel et al., 1989. These hybridization conditions are suitable for a nucleic acid molecule of about 20 nucleotides in length. There is no need to say that the hybridization conditions described above are to be adapted according to the length of the desired nucleic acid, following techniques well known to the one skilled in the art. The suitable

hybridization conditions may for example be adapted according to the teachings disclosed in the book of Hames and Higgins (*NucleicAcid Hybridization: A Practical Approach*, IRL Press, Oxford, 1985) or in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989), the disclosures of which are incorporated herein by reference in their entireties.

[0156] I. Biallelic Markers and Polynucleotides Comprising Biallelic Markers

[0157] A. Polynucleotides of the Present Invention

[0158] The present invention encompasses polynucleotides for use as primers and probes in the methods of the invention. These polynucleotides may consist of, consist essentially of, or comprise a contiguous span of nucleotides of a sequence from any sequence in the Sequence Listing as well as sequences which are complementary thereto ("complements thereof"). The "contiguous span" may be at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. It should be noted that the polynucleotides of the present invention are not limited to having the exact flanking sequences surrounding the polymorphic bases which, are enumerated in the Sequence Listing. Rather, it will be appreciated that the flanking sequences surrounding the biallelic markers, or any of the primers of probes of the invention which, are more distant from the markers, may be lengthened or shortened to any extent compatible with their intended use and the present invention specifically contemplates such sequences. It will be appreciated that the polynucleotides referred to in the Sequence Listing may be of any length compatible with their intended use. Also the flanking regions outside of the contiguous span need not be homologous to native flanking sequences which actually occur in human subjects. The addition of any nucleotide sequence, which is compatible with the nucleotides intended use is specifically contemplated. The contiguous span may optionally include the DME-related biallelic marker in said sequence. Biallelic markers generally consist of a polymorphism at one single base position. Each biallelic marker therefore corresponds to two forms of a polynucleotide sequence which, when compared with one another, present a nucleotide modification at one position. Usually, the nucleotide modification involves the substitution of one nucleotide for another. Optionally either the original or the alternative allele of the biallelic markers disclosed in Table 13, or the first or second allele disclosed in Table 12 and 13 may be specified as being present at the DME-related biallelic marker. Optionally, the biallelic markers may be specified as 12-421-135, 12-442-133, 12-449-63, 12-454-242, 12-463-230, 12-462-199, 10-430-287, 12-718-432, 12-269-301, 2-13-398, 2-28-132, 2-39-27, 2-45-155, 2-4-391, 12-345-410, 10-358-353, 10-360-190, 10-365-374, 10-367-58, 12-468-424, 12-481-293, 12-499-86, 12-500-217, 12-511-101, 12-586-443, 12-593-287, 12-795-383, 10-494-332, 12-659-251, 12-912-419, 12-914-28, 12-624-307 which consist of more complex polymorphisms including insertions/deletions of at least one nucleotide. Optionally either the original or the alternative allele of these biallelic markers may be specified as being present at the DME-related biallelic marker. Preferred polynucleotides may consist of, consist essentially of, or com-

prise a contiguous span of nucleotides of a sequence from SEQ ID No. 929-961 well as sequences which are complementary thereto. The "contiguous span" may be at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID. The contiguous span may optionally comprise a biallelic marker selected from the group consisting of biallelic markers 12-421-135, 12-442-133, 12-449-63, 12-454-242, 12-463-230, 12-462-199, 10-430-287, 12-718-432, 12-269-301, 2-13-398, 2-28-132, 2-39-27, 245-155, 24-391, 12-345-410, 10-358-353, 10-360-190, 10-365-374, 10-367-58, 12-468-424, 12-481-293, 12-499-86, 12-500-217, 12-511-101, 12-586-443, 12-593-287, 12-795-383, 10-494-332, 12-659-251, 12-912-419, 12-914-28, 12-624-307.

[0159] The preferred polynucleotides of the invention include the sequence ranges included in any one the sequence ranges of Tables 12, and 15 to 18 individually or in groups consisting of all the possible combinations of the ranges of included in Tables 12, and 15 to 18. The preferred polynucleotides of the invention also include fragments of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 consecutive nucleotides of the sequence ranges included in any one of the sequence ranges of Tables 12, and 15 to 18 to the extent that fragments of these lengths are consistent with the lengths of the particular sequence range. The preferred polynucleotides of the invention also include fragments of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 consecutive nucleotides of the sequence complementary to the sequence ranges included in any one of the sequence ranges of Tables 12, and 15 to 18 to the extent that fragments of these lengths are consistent with the lengths of the particular sequence range.

[0160] Preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a sequence selected from the group consisting of the sequences of SEQ ID Nos. 485-487, 490-493, 495, 496, 498-523, 930-934, and 965, and the complements thereof.

[0161] Particularly preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 485, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of the following nucleotide positions of SEQ ID No. 485: 1 to 7667, 7726 to 20264, 20365 to 36918, 36991 to 45180, 45263 to 45741, and 45980 to 49327, and the complements thereof. Other particularly preferred polynucleotides of the present invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 486, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of nucleotide positions 1 to 198 of SEQ ID No. 486 and the complements thereof. Other particularly preferred polynucleotides of the present invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 487, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of

nucleotide positions 1 to 198 of SEQ ID No. 487 and the complements thereof. Other particularly preferred polynucleotides of the present invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 490, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of nucleotide positions 1 to 198 of SEQ ID No. 490 and the complements thereof. Other preferred polynucleotides of the present invention include polynucleotides comprising, consisting of, or consisting essentially of a nucleotide sequence of SEQ ID No. 491.

[0162] Particularly preferred polynucleotides of the present invention include purified, isolated or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a sequence selected from the group consisting of SEQ ID SEQ ID Nos. 3, 5, 9, 13-15, 25, 31, 33, 37, 38, 41, 323, 345, 351-353, 357, 377, and 380, or the complements thereof; or more preferably SEQ ID Nos. 496, 498, 502, 506-508, 518, 524, 526, 530, 531, 534, 816, 838, 844-846, 850, 870, and 873, or the complements thereof, wherein said span includes a MGST-II-related biallelic marker. Optionally either allele of the biallelic markers described above in the definition of MGST-II-related biallelic marker is specified as being present at the MGST-II-related biallelic marker.

[0163] Additional preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotide comprising a contiguous span of at least 12 nucleotides of SEQ ID No. 485 or the complementary sequence thereof, wherein said contiguous span comprises a nucleotide selected from the group consisting of a T at position 36971, a C at position 45214 or a T at position 45741 of SEQ ID No. 485. Additional preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides from a sequence of SEQ ID No. 486, wherein said contiguous span comprises a T at position 426, a C at position 478 or a T at position 526 of SEQ ID No. 486; or the complement thereof. Additional preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides from a sequence of SEQ ID No. 487, wherein said contiguous span comprises a T at position 325, a C at position 378 or a T at position 426 of SEQ ID No. 487; or the complements thereof.

[0164] Table 3(A-D) contains a list of preferred MGST-II-related biallelic markers. Each marker is described by indicating its Marker ID, the position of the marker in the SEQ ID and the two most common alleles.

[0165] The invention also relates to polynucleotides that hybridize, under conditions of high or intermediate stringency, to a polynucleotide of a sequence from any sequence in the Sequence Listing as well as sequences, which are complementary thereto. Preferably such polynucleotides are at least 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides in length, to the extent that a polynucleotide of these lengths is consistent with the lengths of the particular Sequence ID. Preferred polynucleotides comprise a DME-

related biallelic marker. Optionally either the original or the alternative allele of the biallelic markers disclosed in Table 13 may be specified as being present at the DME-related biallelic marker. Conditions of high and intermediate stringency are further described in "Methods of Genotyping DNA Samples for Biallelic Markers-Hybridization Assay Methods."

[0166] The present invention further embodies isolated, purified, and recombinant polynucleotides which encode MGST-II polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of SEQ ID No. 488. The present invention further embodies isolated, purified, and recombinant polynucleotides which encode the variant MGST-II polypeptides of SEQ ID Nos. 488 and 489. The present invention further embodies isolated, purified, and recombinant polynucleotides which encode a variant MGST-II polypeptide comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of SEQ ID No. 489. The present invention further embodies isolated, purified, and recombinant polynucleotides which encode polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of SEQ ID No. 488 wherein said contiguous span comprises a His residue at amino acid position 93. The present invention further embodies isolated, purified, and recombinant polynucleotides which encode polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of amino acid positions 1-108 of SEQ ID No. 488.

[0167] The primers of the present invention may be designed from the disclosed sequences for any method known in the art. A preferred set of primers is fashioned such that the 3' end of the contiguous span of identity with the sequences of the Sequence Listing is present at the 3' end of the primer. Such a configuration allows the 3' end of the primer to hybridize to a selected nucleic acid sequence and dramatically increases the efficiency of the primer for amplification or sequencing reactions. In a preferred set of primers the contiguous span is found in one of the sequences described in Table 15. Allele specific primers may be designed such that a biallelic marker is at the 3' end of the contiguous span and the contiguous span is present at the 3' end of the primer. Such allele specific primers tend to selectively prime an amplification or sequencing reaction so long as they are used with a nucleic acid sample that contains one of the two alleles present at a biallelic marker. The 3' end of primer of the invention may be located within or at least 2, 4, 6, 8, 10, 12, 15, 18, 20, 25, 50, 100, 250, 500, or 1000, to the extent that this distance is consistent with the particular Sequence ID, nucleotides upstream of a DME-related biallelic marker in said sequence or at any other location which is appropriate for their intended use in sequencing, amplification or the location of novel sequences or markers. A list of preferred amplification primers is disclosed in Table 17. Primers with their 3' ends located 1 nucleotide upstream of a DME-related biallelic marker have a special utility as microsequencing assays. Preferred microsequencing primers are described in Table 16.

[0168] The probes of the present invention may be designed from the disclosed sequences for any method known in the art, particularly methods which allow for testing if a particular sequence or marker disclosed herein is present. A preferred set of probes may be designed for use in the hybridization assays of the invention in any manner known in the art such that they selectively bind to one allele of a biallelic marker, but not the other under any particular set of assay conditions. Preferred hybridization probes may consists of, consist essentially of, or comprise a contiguous span which ranges in length from 8, 10, 12, 15, 18 or 20 to 25, 35, 40, 50, 60, 70, or 80 nucleotides, or be specified as being 12, 15, 18, 20, 25, 35, 40, or 50 nucleotides in length and including a DME-related biallelic marker of said sequence. Optionally the original allele or alternative allele disclosed in Table 13 and 14 may be specified as being present at the biallelic marker site. Optionally, said biallelic marker may be within 6, 5, 4, 3, 2, or 1 nucleotides of the center of the hybridization probe or at the center of said probe. A particularly preferred set of hybridization probes is disclosed in Table 18 or a sequence complementary thereto.

[0169] Any of the polynucleotides of the present invention can be labeled, if desired, by incorporating a label detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive substances, fluorescent dyes or biotin. Preferably, polynucleotides are labeled at their 3' and 5' ends. A label can also be used to capture the primer, so as to facilitate the immobilization of either the primer or a primer extension product, such as amplified DNA, on a solid support. A capture label is attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid's phase reagent's specific binding member (e.g. biotin and streptavidin). Therefore depending upon the type of label carried by a polynucleotide or a probe, it may be employed to capture or to detect the target DNA. Further, it will be understood that the polynucleotides, primers or probes provided herein, may, themselves, serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of a primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where a polynucleotide probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the target. In the case where a polynucleotide primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase. DNA Labeling techniques are well known to the skilled technician.

[0170] Any of the polynucleotides, primers and probes of the present invention can be conveniently immobilized on a solid support. Solid supports are known to those skilled in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, duracytes® and others. The solid support is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and duracytes are all suitable examples. Suitable methods for immobilizing

nucleic acids on solid phases include ionic, hydrophobic, covalent interactions and the like. A solid support, as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid support can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid support and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid support material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, duracytes® and other configurations known to those of ordinary skill in the art. The polynucleotides of the invention can be attached to or immobilized on a solid support individually or in groups of at least 2, 5, 8, 10, 12, 15, 20, or 25 distinct polynucleotides of the inventions to a single solid support. In addition, polynucleotides other than those of the invention may attached to the same solid support as one or more polynucleotides of the invention.

[0171] Any polynucleotide provided herein may be attached in overlapping areas or at random locations on the solid support. Alternatively the polynucleotides of the invention may be attached in an ordered array wherein each polynucleotide is attached to a distinct region of the solid support which does not overlap with the attachment site of any other polynucleotide. Preferably, such an ordered array of polynucleotides is designed to be "addressable" where the distinct locations are recorded and can be accessed as part of an assay procedure. Addressable polynucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. The knowledge of the precise location of each polynucleotides location makes these "addressable" arrays particularly useful in hybridization assays. Any addressable array technology known in the art can be employed with the polynucleotides of the invention. One particular embodiment of these polynucleotide arrays is known as the Genechips™, and has been generally described in U.S. Pat. No. 5,143,854; PCT publications WO 90/15070 and 92/10092, the disclosures of which are incorporated herein by reference in their entirety. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis (Fodor et al., Science, 251:767-777, 1991). The immobilization of arrays of oligonucleotides on solid supports has been rendered possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSIPS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. Examples of VLSIPS™ technologies are provided in U.S. Pat. Nos. 5,143,854 and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO

95/11995, the disclosures of which are incorporated herein by reference in their entirety, which describe methods for forming oligonucleotide arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and sequence information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212 and WO 97/31256, the disclosures of which are incorporated herein by reference in their entirety.

[0172] Oligonucleotide arrays may comprise at least one of the sequences selected from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the sequences complementary thereto; or more preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the sequences complementary thereto; or a fragment thereof of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for determining whether a sample contains one or more alleles of the biallelic markers of the present invention. Oligonucleotide arrays may also comprise at least one of the sequences selected from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the sequences complementary thereto; or more preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the sequences complementary thereto; or a fragment thereof of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for amplifying one or more alleles of the biallelic markers of Table 11. In other embodiments, arrays may also comprise at least one of the sequences selected from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the sequences complementary thereto; or more preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the sequences complementary thereto; or a fragment thereof of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 consecutive nucleotides, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for conducting microsequencing analyses to determine whether a sample contains one or more alleles of the biallelic markers of the invention. In still further embodiments, the oligonucleotide array may comprise at least one of the sequences selecting from the group consisting of SEQ ID Nos. 1-38, 40-54, 56-353, 355-463, and 465-487, and the sequences complementary thereto; or more preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, and 958-977, and the sequences complementary thereto; or a fragment thereof of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides in length, to the extent that fragments of these lengths is consistent with the lengths of the particular Sequence ID, for determining whether a sample contains one or more alleles of the biallelic markers of the present invention.

[0173] The present invention also encompasses diagnostic kits comprising one or more polynucleotides of the invention, optionally with a portion or all of the necessary reagents and instructions for genotyping a test subject by determining the identity of a nucleotide at a DME-related biallelic marker. The polynucleotides of a kit may optionally be attached to a solid support, or be part of an array or addressable array of polynucleotides. The kit may provide for the determination of the identity of the nucleotide at a marker position by any method known in the art including, but not limited to, a sequencing assay method, a microsequencing assay method, a hybridization assay method, or an allele specific amplification method. Optionally such a kit may include instructions for scoring the results of the determination with respect to the test subjects' risk of contracting a diseases involving the metabolic conversion of xenobiotics, or likely response to a drug, or chances of suffering from side effects to a drug, including hepatotoxicity.

[0174] B. Genomic Sequences of the MGST-II Gene and Biallelic Markers

[0175] The present invention encompasses the genomic sequence of the MGST-II gene of SEQ ID No. 485. The MGST-II genomic sequences comprise exons and introns. Particularly preferred genomic sequences of the MGST-II gene of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 485, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of the following nucleotide positions of SEQ ID No. 485: 1 to 7667, 7726 to 20264, 20365 to 36918, 36991 to 45180, 45263 to 45741, and 45980 to 49327, and the complements thereof. The nucleic acids defining the MGST-II intronic polynucleotides may be used as oligonucleotide primers or probes in order to detect the presence of a copy of the MGST-II gene in a test sample, or alternatively in order to amplify a target nucleotide sequence within the MGST-II sequences.

[0176] The present invention further provides MGST-II intron and exon polynucleotide sequences including biallelic markers. Particularly preferred polynucleotides of the present invention include purified, isolated or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a sequence of SEQ ID No. 485; or the complements thereof; wherein said span includes a MGST-II-related biallelic marker. Preferred polynucleotides comprise at least one biallelic marker selected from the group consisting of biallelic markers 10-286-289, 10-287-116, 10-286-375, 12-425-57, 12-421-135, 12-421-140, 10-523-232, 10-289-201, 10-290-37, 10-290-326 and 10-290-328. The present invention also provides polynucleotides which, may be used as primers and probes in order to amplify fragments carrying biallelic markers or in order to detect biallelic marker alleles.

[0177] Regulatory Sequences

[0178] The genomic sequence of the MGST-II gene contains regulatory sequences both in the non-coding 5'-flanking region and in the non-coding 3'-flanking region that border the MGST-II transcribed region containing the 5 exons of this gene. 5'-regulatory sequences of the MGST-II gene comprise the polynucleotide sequences located between the nucleotide in position 1 and the nucleotide in position 7466 of the nucleotide sequence of SEQ ID No.

485. 3'-regulatory sequences of the MGST-II gene comprise the polynucleotide sequences located between the nucleotide in position 45980 and the nucleotide in position 49327 of the nucleotide sequence of SEQ ID No. 485. Particularly preferred regulatory polynucleotides of the present invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 485, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of the following nucleotide positions of SEQ ID No. 485: 1 to 7466 and 45966 to 49312; and the complements thereof.

[0179] The promoter activity of the regulatory regions contained in the MGST-II genomic sequence of polynucleotide sequence of SEQ ID No. 485 can be assessed by any method known in the art. Methods for identifying the polynucleotide fragments of SEQ ID No. 485 involved in the regulation of the expression of the MGST-II gene are well-known to those skilled in the art (see Sambrook et al., Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989). An example of a typical method, that can be used, involves a recombinant vector carrying a reporter gene and genomic sequences from the MGST-II genomic sequence of SEQ ID No. 485. Briefly, the expression of the reporter gene (for example beta galactosidase or chloramphenicol acetyl transferase) is detected when placed under the control of a biologically active polynucleotide fragment. Genomic sequences located upstream of the first exon of the MGST-II gene may be cloned into any suitable promoter reporter vector, such as the pSEAP-Basic, pSEAP-Enhancer, pβgal-Basic, pβgal-Enhancer, or pEGFP-1 Promoter Reporter vectors available from Clontech, or pGL2-basic or pGL3-basic promoterless luciferase reporter gene vector from Promega. Each of these promoter reporter vectors include multiple cloning sites positioned upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline phosphatase, luciferase, beta galactosidase, or green fluorescent protein. The sequences upstream the first MGST-II exon are inserted into the cloning sites upstream of the reporter gene in both orientations and introduced into an appropriate host cell. The level of reporter protein is assayed and compared to the level obtained with a vector lacking an insert in the cloning site. The presence of an elevated expression level in the vector containing the insert with respect to the control vector indicates the presence of a promoter in the insert.

[0180] Promoter sequences within the 5' non-coding regions of the MGST-II gene may be further defined by constructing nested 5' and/or 3' deletions using conventional techniques such as Exonuclease III or appropriate restriction endonuclease digestion. The resulting deletion fragments can be inserted into the promoter reporter vector to determine whether the deletion has reduced or obliterated promoter activity, such as described, for example, by Coles et al. (*Hum. Mol. Genet.,* 7:791-800, 1998). In this way, the boundaries of the promoters may be defined. If desired, potential individual regulatory sites within the promoter may be identified using site directed mutagenesis or linker scanning to obliterate potential transcription factor binding sites within the promoter individually or in combination. The effects of these mutations on transcription levels may be determined by inserting the mutations into cloning sites in promoter reporter vectors. This type of assays are well

known to those skilled in the art and are further described in WO 97/17359, U.S. Pat. No. 5,374,544, EP 582 796, U.S. Pat. No. 5,698,389, U.S. Pat. No. 5,643,746, U.S. Pat. No. 5,502,176, and U.S. Pat. No. 5,266,488.

[0181] The activity and the specificity of the promoter of the MGST-II gene can further be assessed by monitoring the expression level of a detectable polynucleotide operably linked to the MGST-II promoter in different types of cells and tissues. The detectable polynucleotide may be either a polynucleotide that specifically hybridizes with a predefined oligonucleotide probe, or a polynucleotide encoding a detectable protein, including a MGST-II polypeptide or a fragment or a variant thereof. This type of assay is well known to those skilled in the art and is described in U.S. Pat. No. 5,502,176 and U.S. Pat. No. 5,266,488 for example.

[0182] Polynucleotides carrying the regulatory elements located both at the 5' end and at the 3' end of the MGST-II coding region may be advantageously used to control the transcriptional and translational activity of an heterologous polynucleotide of interest, said polynucleotide being heterologous as regards to the MGST-II regulatory region.

[0183] Thus, the present invention also concerns a purified, isolated, and recombinant nucleic acid comprising a polynucleotide which, is selected from the group consisting of, the polynucleotide sequences located between the nucleotide in position 1 and the nucleotide in position 7466 of the nucleotide sequence of SEQ ID No. 485; or a sequence complementary thereto or a biologically active fragment thereof.

[0184] By a "biologically active" fragment of SEQ ID No. 485 according to the present invention is intended a polynucleotide comprising or alternatively consisting of a fragment of said polynucleotide which is functional as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide in a recombinant cell host.

[0185] For the purpose of the invention, a nucleic acid or polynucleotide is "functional" as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide if said regulatory polynucleotide contains nucleotide sequences which contain transcriptional and translational regulatory information, and such sequences are "operably linked" to nucleotide sequences which encode the desired polypeptide or the desired polynucleotide.

[0186] The regulatory polynucleotides according to the invention may be advantageously part of a recombinant expression vector that may be used to express a coding sequence in a desired host cell or host organism.

[0187] C. MGST-II cDNA Comprising Biallelic Markers and Variant MGST-II cDNA

[0188] The present invention provides a MGST-II cDNA of SEQ ID No. 486. The Open Reading Frame encoding the MGST-II protein spans from the nucleotide in position 202 to the nucleotide in position 642 of the polynucleotide sequence of SEQ ID No. 486. The cDNA of SEQ ID No. 486 also includes a 5'-UTR region and a 3'-UTR region.

[0189] Particularly preferred cDNA polynucleotides of the present invention include purified, isolated or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a sequence of SEQ ID No. 486,

or the complements thereof, wherein said span includes a MGST-II-related biallelic marker. Preferred cDNA fragments comprise a biallelic marker selected from the group consisting of 10-286-289 (position 98), 10-286-345 (position 153), 10-286-375 (position 183), 10-523-232 (position 426), 10-289-201 (position 478) and 10-290-37 (position 526). Additional preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides from a sequence of SEQ ID No. 486, wherein said contiguous span comprises a T at position 426, a C at position 478 or a T at position 526 of SEQ ID No. 486; or the complement thereof. Most biallelic polymorphisms represent silent nucleotide substitutions but biallelic marker 10-289-201 is associated with an amino acid change in the corresponding MGST-II polypeptide (TYR replaced by ARG in position 93 of the polypeptide). Moreover, one biallelic marker allele of marker 10-290-37 is associated with a stop codon and the corresponding variant cDNA encodes a truncated MGST-II polypeptide including amino acids 1 to 108.

[0190] The present invention further provides a variant MGST-II cDNA of SEQ ID No. 487. corresponding to an alternative splicing form which results in the deletion of exon 2. This alternative splicing of MGST-II yields the variant MGST-II polypeptide of SEQ ID No. 489. MGST-II polypeptides of the present invention are further described below. Preferred cDNAs of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides from a sequence of SEQ ID No. 487; or the complements thereof. Additional preferred polynucleotides of the invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides from a sequence of SEQ ID No. 487, wherein said contiguous span comprises a T at position 325, a C at position 378 or a T at position 426 of SEQ ID No. 487; or the complements thereof. The new exon 1/exon 3 junction sequence of the splice variant MGST-II cDNA, more particularly the nucleotide sequence comprised between the nucleotide in position 106 and the nucleotide in position 374 of the nucleic acid of SEQ ID No. 486 corresponds to the nucleotide sequence of an EST that has been obtained from a human cDNA library. Polynucleotides comprising this EST of a sequence from SEQ ID No. 490 are also part of the invention.

[0191] The above disclosed polynucleotides that contain the coding sequence of the MGST-II gene and of MGST-II variants may be expressed in a desired host cell or a desired host organism, when this polynucleotide is placed under the control of suitable expression signals. The expression signals may be either the expression signals contained in the regulatory regions in the MGST-II gene of the invention or in contrast the signals may be exogenous regulatory nucleic sequences. Such a polynucleotide, when placed under the suitable expression signals, may also be inserted in a vector for its expression and/or amplification.

[0192] Another preferred cDNA fragment comprises the 5'-UTR region (regulatory) beginning at position 1 and ending at position 201 of SEQ ID Nos. 486 and 487.

Preferably said 5'-UTR region comprises a biallelic marker selected from the group consisting of biallelic markers 10-286-289, 10-286-345 and 10-286-375. Particularly preferred 5'-UTR polynucleotides of the present invention include isolated, purified or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No. 486, wherein said contiguous span comprises at least 1, 2, 3, 4, 5 or 10 of nucleotide positions 1 to 198 of SEQ ID No. 486; and the complements thereof. The 5'-end sequence of the MGST-II cDNA, more particularly the nucleotide sequence comprised between the nucleotide in position 1 and the nucleotide in position 357 of the nucleic acid of SEQ ID No. 486 corresponds to the nucleotide sequence of a 5'-EST that has been obtained from a human cDNA library. Polynucleotides comprising this 5'-EST of a sequence from SEQ ID No. 490 are also part of the invention.

[0193] The polynucleotide disclosed above that contains the coding sequence of the MGST-II gene of the invention may be expressed in a desired host cell or a desired host organism, when this polynucleotide is placed under the control of suitable expression signals. The expression signals may be either the expression signals contained in the regulatory regions in the MGST-II gene of the invention or may be exogenous regulatory nucleic sequences. Such a polynucleotide, when placed under the suitable expression signals, may also be inserted in a vector for its expression.

[0194] A further object of the invention consists of an isolated polynucleotide comprising:

[0195] a) a nucleic acid comprising a regulatory nucleotide sequence from a sequence of SEQ ID No. 485;

[0196] b) a polynucleotide encoding a desired polypeptide or a nucleic acid of interest, operably linked to the nucleic acid defined in (a) above; and

[0197] c) Optionally, a nucleic acid comprising a 5'-UTR regulatory polynucleotide, preferably a 5'-UTR regulatory polynucleotide sequence of a sequence of SEQ ID No. 486.

[0198] The polypeptide encoded by the nucleic acid described above may be of various nature or origin, encompassing proteins of prokaryotic or eukaryotic origin. Among the polypeptides expressed under the control of a MGST-II regulatory region, there may be cited bacterial, fungal or viral antigens. Also encompassed are eukaryotic proteins such as intracellular proteins, for example "house keeping" proteins, membrane-bound proteins, for example receptors, and secreted proteins, for example cytokines. In a specific embodiment, the desired polypeptide may be the MGST-II protein, especially the proteins of the amino acid sequence of SEQ ID Nos. 488 and 489.

[0199] The desired nucleic acids encoded by the above described polynucleotide, usually a RNA molecule, may be complementary to a desired coding polynucleotide, for example to the MGST-II coding sequence, and thus useful as an antisense polynucleotide.

[0200] Such a polynucleotide may be included in a recombinant expression vector in order to express the desired polypeptide or the desired nucleic acid in host cell or in a host organism.

[0201] D. Polynucleotide Constructs, Recombinant Vectors, Host Cells and Transgenic Animals

[0202] Polynucleotide Constructs

[0203] The terms "polynucleotide construct" and "recombinant polynucleotide" are used interchangeably herein to refer to linear or circular, purified or isolated polynucleotides that have been artificially designed and which comprise at least two nucleotide sequences that are not found as contiguous nucleotide sequences in their initial natural environment.

[0204] 1. DNA constructs for Expressing the MGST-II Gene in Recombinant Host Cells and in Transgenic Animals

[0205] In order to study the physiological and phenotype consequences of a lack of synthesis of the MGST-II protein, both at the cellular level and at the multicellular organism level, in particular as regards to disorders related to abnormal cell proliferation, notably cancers, the invention also encompasses DNA constructs and recombinant vectors enabling a conditional expression of a specific allele of the MGST-II genomic sequence or cDNA A first preferred DNA construct is based on the tetracycline resistance operon tet from *E. coli* transposon Tn110 for controlling the MGST-II gene expression, such as described by Gossen et al. (*Science,* 268:1766-1769, 1995). Such a DNA construct contains seven tet operator sequences from Tn10 (tetop) that are fused to either a minimal promoter or a 5'-regulatory sequence of the MGST-II gene, said minimal promoter or said MGST-II regulatory sequence being operably linked to a polynucleotide of interest that codes either for a sense or an antisense oligonucleotide or for a polypeptide, including a MGST-II polypeptide or a peptide fragment thereof This DNA construct is functional as a conditional expression system for the nucleotide sequence of interest when the same cell also comprises a nucleotide sequence coding for either the wild type (tTA) or the mutant (rTA) repressor fused to the activating domain of viral protein VP16 of herpes simplex virus, placed under the control of a promoter, such as the HCMVIE1 enhancer/promoter or the MMTV-LTR. Indeed, a preferred DNA construct of the invention will comprise both the polynucleotide containing the tet operator sequences and the polynucleotide containing a sequence coding for the tTA or the rTA repressor. In the specific embodiment wherein the conditional expression DNA construct contains the sequence encoding the mutant tetracycline repressor rTA, the expression of the polynucleotide of interest is silent in the absence of tetracycline and induced in its presence.

[0206] 2. DNA Constructs Allowing Homologous Recombination: Replacement Vectors

[0207] A second preferred DNA construct will comprise, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the MGST-II genomic sequence; (b) a nucleotide sequence comprising a positive selection marker, such as the marker for neomycine resistance (neo); and (c) a second nucleotide sequence that is comprised in the MGST-II genomic sequence, and is located on the genome downstream the first MGST-II nucleotide sequence (a).

[0208] In a preferred embodiment, this DNA construct also comprises a negative selection marker located upstream the nucleotide sequence (a) or downstream the nucleotide sequence (c). Preferably, the negative selection marker consists of the thymidine kinase (tk) gene (Thomas et al., *Cell,* 44:419-428, 1986), the hygromycine beta gene (Te Riele et al., *Nature,* 348:649-651, 1990), the hprt gene (Van der Lugt et al., *Gene,* 105:263-267, 1991; Reid et al., *Proc. Natl. Acad. Sci. USA,* 87:4299-4303, 1990) or the Diphteria toxin A fragment (Dt-A) gene (Nada et al., *Cell,* 73:1125-1135, 1993; Yagi et al., *Proc. Natl; Acad. Sci. USA,* 87:9918-9922, 1990). Preferably, the positive selection marker is located within a MGST-II exon sequence so as to interrupt the sequence encoding a MGST-II protein. These replacement vectors are further described by Mansour et al. (*Nature,* 336:348-352, 1988) and Koller et al. (*Ann. Rev. Immunol.,* 10:705-730, 1992). The first and second nucleotide sequences (a) and (c) may be indifferently located within a MGST-II regulatory sequence, an intronic sequence, an exon sequence or a sequence containing both regulatory and/or intronic and/or exon sequences. The size of the nucleotide sequences (a) and (c) is ranging from 1 to 50 kb, preferably from 1 to 10 kb, more preferably from 2 to 6 kb and most preferably from 2 to 4 kb.

[0209] 3. DNA Constructs Allowing Homologous Recombination: Cre-loxP System

[0210] These new DNA constructs make use of the site specific recombination system of the P1 phage. The P1 phage possesses a recombinase called Cre which, interacts specifically with a 34 base pairs loxP site. The loxP site is composed of two palindromic sequences of 13 bp separated by a 8 bp conserved sequence (Hoess et al., *Nucleic Acids Res.,* 14:2287-2300, 1986). The recombination by the Cre enzyme between two loxP sites having an identical orientation leads to the deletion of the DNA fragment.

[0211] The Cre-loxP system used in combination with a homologous recombination technique has been first described by Gu et al. (*Cell,* 73:1155-1164, 1993). Briefly, a nucleotide sequence of interest to be inserted in a targeted location of the genome harbors at least two loxP sites in the same orientation and located at the respective ends of a nucleotide sequence to be excised from the recombinant genome. The excision event requires the presence of the recombinase (Cre) enzyme within the nucleus of the recombinant cell host. The recombinase enzyme may be brought at the desired time either by (a) incubating the recombinant cell hosts in a culture medium containing this enzyme, by injecting the Cre enzyme directly into the desired cell, such as described by Araki et al. (*Proc. Natl; Acad. Sci. USA,* 92: 160-164, 1995), or by lipofection of the enzyme into the cells, such as described by Baubonis et al. (*Nucleic Acids Res.,* 21:2025-2029, 1993); (b) transfecting the cell host with a vector comprising the Cre coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter being optionally inducible, said vector being introduced in the recombinant cell host, such as described by Gu et al. (*Cell,* 73:1155-1164, 1993) and Sauer et al. (*Proc. Natl; Acad. Sci. USA,* 85:5166-5170, 1988); (c) introducing in the genome of the cell host a polynucleotide comprising the Cre coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter is optionally inducible, and said polynucleotide being inserted in the genome of the cell host either by a random insertion event or an homologous recombination event, such as described by Gu et al. (*Science,* 265:103-106, 1994).

[0212] In the specific embodiment wherein the vector containing the sequence to be inserted in the MGST-II gene by homologous recombination is constructed in such a way that selectable markers are flanked by loxP sites of the same orientation, it is possible, by treatment by the Cre enzyme, to eliminate the selectable markers while leaving the MGST-II sequences of interest that have been inserted by an homologous recombination event. Again, two selectable markers are needed: a positive selection marker to select for the recombination event and a negative selection marker to select for the homologous recombination event. Vectors and methods using the Cre-loxP system are further described by Zou et al. (*Curr. Biol.,* 4:1099-1103, 1994).

[0213] Thus, a third preferred DNA construct of the invention comprises, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the MGST-II genomic sequence; (b) a nucleotide sequence comprising a polynucleotide encoding a positive selection marker, said nucleotide sequence comprising additionally two sequences defining a site recognized by a recombinase, such as a loxP site, the two sites being placed in the same orientation; and (c) a second nucleotide sequence that is comprised in the MGST-II genomic sequence, and is located on the genome downstream of the first MGST-II nucleotide sequence (a).

[0214] The sequences defining a site recognized by a recombinase, such as a loxP site, are preferably located within the nucleotide sequence (b) at suitable locations bordering the nucleotide sequence for which the conditional excision is sought. In one specific embodiment, two loxP sites are located at each side of the positive selection marker sequence, in order to allow its excision at a desired time after the occurrence of the homologous recombination event.

[0215] In a preferred embodiment of a method using the third DNA construct described above, the excision of the polynucleotide fragment bordered by the two sites recognized by a recombinase, preferably two loxP sites, is performed at a desired time, due to the presence within the genome of the recombinant cell host of a sequence encoding the Cre enzyme operably linked to a promoter sequence, preferably an inducible promoter, more preferably a tissue-specific promoter sequence and most preferably a promoter sequence which is both inducible and tissue-specific, such as described by Gu et al. (*Science,* 265:103-106, 1994).

[0216] The presence of the Cre enzyme within the genome of the recombinant cell host may result of the breeding of two transgenic animals, the first transgenic animal bearing the MGST-II-derived sequence of interest containing the loxP sites as described above and the second transgenic animal bearing the Cre coding sequence operably linked to a suitable promoter sequence, such as described by Gu et al. (*Science,* 265:103-106, 1994).

[0217] Spatio-temporal control of the Cre enzyme expression may also be achieved with an adenovirus based vector that contains the Cre gene thus allowing infection of cells, or in vivo infection of organs, for delivery of the Cre enzyme, such as described by Anton et al. (*J. Virol.,* 69:4600-4606, 1995) and Kanegae et al. (*Nucleic Acids Res.,* 23:3816-3821, 1995).

[0218] The DNA constructs described above may be used to introduce a desired nucleotide sequence of the invention, preferably a MGST-II genomic sequence or a MGST-II

cDNA sequence, and most preferably an altered copy of a MGST-II genomic or cDNA sequence, within a predetermined location of the targeted genome, leading either to the generation of an altered copy of a targeted gene (knock-out homologous recombination) or to the replacement of a copy of the targeted gene by another copy sufficiently homologous to allow an homologous recombination event to occur (knock-in homologous recombination).

[0219] Recombinant Vectors

[0220] The term "vector" is used herein to designate either a circular or a linear DNA or RNA molecule, which is either double-stranded or single-stranded, and which comprise at least one polynucleotide of interest that is sought to be transferred in a cell host or in a unicellular or multicellular host organism.

[0221] The present invention encompasses a family of recombinant vectors that comprise a regulatory polynucleotide derived from the MGST-II genomic sequence, or a coding polynucleotide from the MGST-II genomic sequence. Consequently, the present invention further deals with a recombinant vector comprising either a regulatory polynucleotide comprised in the nucleic acid of SEQ ID Nos. 485 and 486 or a polynucleotide comprising the MGST-II coding sequence or both.

[0222] In a first preferred embodiment, a recombinant vector of the invention is used to amplify the inserted polynucleotide derived from a MGST-II genomic sequence selected from the group consisting of the nucleic acids of SEQ ID No. 485 or a MGST-II cDNA, for example the cDNA of SEQ ID Nos. 486 and 487 in a suitable host cell, this polynucleotide being amplified each time the recombinant vector replicates. Generally, a recombinant vector of the invention may comprise any of the polynucleotides described herein, including regulatory sequences and coding sequences, as well as any MGST-II primer or probe as defined above.

[0223] A second preferred embodiment of the recombinant vectors according to the invention consists of expression vectors comprising either a regulatory polynucleotide or a coding nucleic acid of the invention, or both. Within certain embodiments, expression vectors are employed to express the MGST-II polypeptide which can be then purified and, for example be used in ligand screening assays or as an immunogen in order to raise specific antibodies directed against the MGST-II protein. In other embodiments, the expression vectors are used for constructing transgenic animals and also for gene therapy. Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements, such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in host cells. Dominant drug selection markers for establishing permanent, stable cell clones expressing the products are generally included in the expression vectors of the invention, as they are elements that link expression of the drug selection markers to expression of the polypeptide.

[0224] More particularly, the present invention relates to expression vectors which include nucleic acids encoding a MGST-II protein, preferably the MGST-II protein of the amino acid sequence of SEQ ID Nos. 488 and 489, under the control of a regulatory sequence selected among the MGST-

II regulatory polynucleotides of SEQ ID Nos. 485 and 486, or alternatively under the control of an exogenous regulatory sequence. Consequently, preferred expression vectors of the invention are selected from the group consisting of: (a) the MGST-II regulatory sequence comprised therein drives the expression of a coding polynucleotide operably linked thereto; (b) the MGST-II coding sequence is operably linked to regulation sequences allowing its expression in a suitable cell host and/or host organism. Additionally, the recombinant expression vector described above may also comprise a nucleic acid comprising a 5'-regulatory polynucleotide or a 3'-regulatory polynucleotide, preferably a 5'-regulatory polynucleotide or a 3'-regulatory polynucleotide of the MGST-II gene. The MGST-II 5'-regulatory polynucleotide may also comprise the 5'-UTR sequence contained in the nucleotide sequence of SEQ ID Nos. 486 and 487; or a biologically active fragment or variant thereof The invention also pertains to a recombinant expression vector useful for the expression of the MGST-II coding sequence, wherein said vector comprises any of the MGST-II cDNAs or cDNA variants described above; or fragments thereof.

[0225] Some of the elements which, can be found in the vectors of the present invention are described in further detail in the following sections.

[0226] 1. General Features of the Expression Vectors of the Invention

[0227] A recombinant vector according to the invention comprises, but is not limited to, a YAC (Yeast Artificial Chromosome), a BAC (Bacterial Artificial Chromosome), a phage, a phagemid, a cosmid, a plasmid or even a linear DNA molecule which may consist of a chromosomal, non-chromosomal, semi-synthetic and synthetic DNA. Such a recombinant vector can comprise a transcriptional unit comprising an assembly of:

[0228] (1) a genetic element or elements having a regulatory role in gene expression, for example promoters or enhancers. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp in length that act on the promoter to increase the transcription.

[0229] (2) a structural or coding sequence which is transcribed into mRNA and eventually translated into a polypeptide, said structural or coding sequence being operably linked to the regulatory elements described in (1); and

[0230] (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, when a recombinant protein is expressed without a leader or transport sequence, it may include a N-terminal residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

[0231] Generally, recombinant expression vectors will include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably a leader sequence capable of directing secretion of the translated protein into the periplasmic space or the extracellular medium. In a specific embodiment wherein the vector is adapted for transfecting and expressing desired sequences in mammalian host cells, preferred vectors will comprise an origin of replication in the desired host, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5'-flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example SV40 origin, early promoter, enhancer, splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

[0232] The in vivo expression of a MGST-II polypeptide of SEQ ID Nos. 488 and 489 may be useful in order to correct a genetic defect related to the expression of the native gene in a host organism or to the production of a biologically inactive MGST-II protein.

[0233] Consequently, the present invention also deals with recombinant expression vectors mainly designed for the in vivo production of the MGST-II polypeptide of SEQ ID Nos. 488 and 489 or fragments or variants thereof by the introduction of the appropriate genetic material in the organism of the patient to be treated. This genetic material may be introduced in vitro in a cell that has been previously extracted from the organism, the modified cell being subsequently reintroduced in the said organism, directly in vivo into the appropriate tissue.

[0234] 2. Regulatory Elements

[0235] Promoters

[0236] The suitable promoter regions used in the expression vectors according to the present invention are chosen taking into account the cell host in which the heterologous gene has to be expressed. The particular promoter employed to control the expression of a nucleic acid sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell, such as, for example, a human or a viral promoter.

[0237] A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed. Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

[0238] Promoter regions can be selected from any desired gene using, for example, CAT (chloramphenicol transferase) vectors and more preferably pKK232-8 and pCM7 vectors.

[0239] Preferred bacterial promoters are the Lacd, LacZ, the T3 or T7 bacteriophage RNA polymerase promoters, the gpt, lambda PR, PL and trp promoters (EP 0036776), the polyhedrin promoter, or the p10 protein promoter from baculovirus (Kit Novagen) (Smith et al., 1983; O'Reilly et al., 1992), the lambda PR promoter or also the trc promoter.

[0240] Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-L. Selection of a convenient vector and promoter is well within the level of ordinary skill in the art.

[0241] The choice of a promoter is well within the ability of a person skilled in the field of genetic egineering. For example, one may refer to the book of Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).

[0242] Other Regulatory Elements:

[0243] Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed such as human growth hormone and SV40 polyadenylation signals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

[0244] The vector containing the appropriate DNA sequence as described above, more preferably MGST-II gene regulatory polynucleotide, a polynucleotide encoding the MGST-II polypeptides of SEQ ID Nos. 488 and 489 or both of them, can be utilized to transform an appropriate host to allow the expression of the desired polypeptide or polynucleotide.

[0245] 3. Selectable Markers

[0246] Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. The selectable marker genes for selection of transformed host cells are preferably dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for *S. cerevisiae* or tetracycline, rifampicin or ampicillin resistance in *E. coli*, or levan saccharase for mycobacteria, this latter marker being a negative selection marker.

[0247] 4. Preferred Vectors

[0248] Bacterial Vectors

[0249] As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and a bacterial origin of replication derived from commercially available plasmids comprising genetic elements of pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia, Uppsala, Sweden), and GEM1 (Promega Biotec, Madison, Wis., USA). Large numbers of other suitable vectors are known to those of skill in the art, and commercially available, such as the following bacterial vectors: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pQE-30 (QIAexpress).

[0250] Bacteriophage Vectors

[0251] The P1 bacteriophage vector may contain large inserts ranging from about 80 to about 100 kb. The construction of P1 bacteriophage vectors such as p158 or p158/neo8 have been described by Sternberg (*Mamm. Genome*, 5:397404, 1994). Recombinant P1 clones comprising MGST-II nucleotide sequences may be designed for inserting large polynucleotides of more than 40 kb (Linton et al., *J. Clin. Invest.*, 92:3029-3037, 1993). To generate P1 DNA for transgenic experiments, a preferred protocol is the protocol described by McCormick et al. (*Genet. Anal. Tech. Appl.*, 11:158-164, 1994). Briefly, *E. coli* (preferably strain NS3529) harboring the P1 plasmid are grown overnight in a suitable broth medium containing 25 gg/ml of kanamycin. The P1 DNA is prepared from the *E. coli* by alkaline lysis using the Qiagen Plasmid Maxi kit (Qiagen, Chatsworth, Calif., USA), according to the manufacturer's instructions. The P1 DNA is purified from the bacterial lysate on two Qiagen-tip 500 columns, using the washing and elution buffers contained in the kit. A phenol/chloroform extraction is then performed before precipitating the DNA with 70% ethanol. After solubilizing the DNA in TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA), the concentration of the DNA is assessed by spectrophotometry.

[0252] When the goal is to express a P1 clone comprising MGST-II nucleotide sequences in a transgenic animal, typically in transgenic mice, it is desirable to remove vector sequences from the P1 DNA fragment, for example by cleaving the P1 DNA at rare-cutting sites within the P1 polylinker (SfiI, NotI or SalI). The P1 insert is then purified from vector sequences on a pulsed-field agarose gel, using methods similar using methods similar to those originally reported for the isolation of DNA from YACs (Schedl et al., 1993a; Peterson et al., 1993). At this stage, the resulting purified insert DNA can be concentrated, if necessary, on a Millipore Ultrafree-MC Filter Unit (Millipore, Bedford, Mass., USA—30,000 molecular weight limit) and then dialyzed against microinjection buffer (10 mM Tris-HCl, pH 7.4; 250 μM EDTA) containing 100 mM NaCl, 30 μM spermine, 70 μM spermidine on a microdyalisis membrane (type VS, 0.025 μM from Millipore). The intactness of the purified P1 DNA insert is assessed by electrophoresis on 1% agarose (Sea Kem GTG; FMC Bio-products) pulse-field gel and staining with ethidium bromide.

[0253] Baculovirus Vectors

[0254] A suitable vector for the expression of the MGST-II polypeptides of SEQ ID Nos. 488 and 489 is a baculovirus vector that can be propagated in insect cells and in insect cell lines. A specific suitable host vector system is the pVL1392/1393 baculovirus transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC N°CRL 1711) which is derived from *Spodoptera frugiperda*.

[0255] Other suitable vectors for the expression of the MGST-II polypeptides of SEQ ID Nos. 488 and 489 in a baculovirus expression system include those described by Chai et al. (*Biotech. Appl. Biochem.*, 18:259-273, 1993), Vlasak et al. (*Eur. J. Biochem.*, 135: 123-126,1983) and Lenhard et al. (*Gene*, 169: 187-190,1996).

[0256] Viral Vectors

[0257] Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery systems of choice for the transfer of exogenous

polynucleotides in vivo, particularly to mammals, including humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host.

[0258] Particularly preferred retroviruses for the preparation or construction of retroviral in vitro or in vitro gene delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus and Rous Sarcoma virus. Particularly preferred Murine Leukemia Viruses include the 4070A and the 1504A viruses, Abelson (ATCC No VR-999), Friend (ATCC No VR-245), Gross (ATCC No. VR-590), Rauscher (ATCC No VR-998) and Moloney Murine Leukemia Virus (ATCC No VR-190; PCT Application No WO 94/24298). Particularly preferred Rous Sarcoma Viruses include Bryan high titer (ATCC Nos. VR-334, VR-657, VR-726, VR-659 and VR-728). Other preferred retroviral vectors are those described in Roth et al. (*Nature Medicine*, 2:985-991, 1996), PCT Application No. WO 93/25234 and PCT Application No. WO 94/06920.

[0259] Yet another viral vector system that is contemplated by the invention consists in the adeno-associated virus (AAV). The adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al., *Current Topics in Microbiol. Immunol.*, 158:97-129, 1992). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (McLaughlin et al., *Am. J. Hum. Genet.*, 59: 561-569, 1989). One advantageous feature of AAV derives from its reduced efficacy for transducing primary cells relative to transformed cells.

[0260] BAC Vectors:

[0261] The bacterial artificial chromosome (BAC) cloning system (Shizuya et al., 1992) has been developed to stably maintain large fragments of genomic DNA (100-300 kb) in *E. coli*. A preferred BAC vector consists of pBeloBAC11 vector that has been described by Kim et al. (*Genomics*, 34:213-218,1996). BAC libraries are prepared with this vector using size-selected genomic DNA that has been partially digested using enzymes that permit ligation into either the Bam HI or HindIII sites in the vector. Flanking these cloning sites are T7 and SP6 RNA polymerase transcription initiation sites that can be used to generate end probes by either RNA transcription or PCR methods. After the construction of a BAC library in *E. coli*, BAC DNA is purified from the host cell as a supercoiled circle. Converting these circular molecules into a linear form precedes both size determination and introduction of the BACs into recipient cells. The cloning site is flanked by two Not I sites, permitting cloned segments to be excised from the vector by Not I digestion. Alternatively, the DNA insert contained in the pBeloBAC11 vector may be linearized by treatment of the BAC vector with the commercially available enzyme lambda terminase that leads to the cleavage at the unique cosN site, but this cleavage method results in a full length BAC clone containing both the insert DNA and the BAC sequences.

[0262] 5. Delivery of the Recombinant Vectors

[0263] In order to effect expression of the polynucleotides and polynucleotide constructs of the invention, these con-

structs must be delivered into a cell. This delivery may be accomplished in vitro, as in laboratory procedures for transforming cell lines, or in vivo or ex vivo, as in the treatment of certain diseases states. One mechanism is viral infection where the expression construct is encapsidated in an infectious viral particle. Several non-viral methods for the transfer of polynucleotides into cultured mammalian cells are also contemplated by the present invention, and include, without being limited to, calcium phosphate precipitation (Chen et al., *Proc. Natl. Acad. Sci. USA*, 94:10756-10761, 1987), DEAE-dextran (Gopal, *Mol. Cell. Biol.*, 5:1188-1190, 1985), electroporation (Tur-Kaspa et al., *Mol. Cell. Biol.*, 6:716-7181986), direct microinjection (Harland et al., 1985), DNA-loaded liposomes (Nicolau et al., 1982; Fraley et al., 1979), and receptor-mediate transfection (Wu and Wu, 1987; 1988). Some of these techniques may be successfully adapted for in vivo or ex vivo use.

[0264] Once the expression polynucleotide has been delivered into the cell, it may be stably integrated into the genome of the recipient cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle.

[0265] One specific embodiment for a method for delivering a protein or peptide to the interior of a cell of a vertebrate in vivo comprises the step of introducing a preparation comprising a physiologically acceptable carrier and a naked polynucleotide operatively coding for the polypeptide of interest into the interstitial space of a tissue comprising the cell, whereby the naked polynucleotide is taken up into the interior of the cell and has a physiological effect. This is particularly applicable for transfer in vitro but it may be applied to in vivo as well.

[0266] Compositions for use in vitro and in vivo comprising a "naked" polynucleotide are described in PCT application No. WO 90/11092 (Vical Inc.) and also in PCT application No. WO 95/11307.

[0267] In still another embodiment of the invention, the transfer of a naked polynucleotide of the invention, including a polynucleotide construct of the invention, into cells may be proceeded with a particle bombardment (biolistic), said particles being DNA-coated microprojectiles accelerated to a high velocity allowing them to pierce cell membranes and enter cells without killing them, such as described by Klein et al. (*Nature* 327:70-73, 1987).

[0268] In a further embodiment, the polynucleotide of the invention may be entrapped in a liposome (Ghosh and Bacchawat, *Targeting of liposomes to hepatocytes, In: Liver Diseases, Targeted diagnosis and therapy using specific rceptors and ligands*, Marcel Dekeker, New York, 87-104, 1991; Wong et al., *Gene* 10:87-94, 1980; Nicolau et al., *Biochim. Biophys. Acta.* 721:185-190, 1982).

[0269] In a specific embodiment, the invention provides a composition for the in vivo production of the MGST-II protein or polypeptide described herein. It comprises a naked polynucleotide operatively coding for this polypep-

tide, in solution in a physiologically acceptable carrier, and suitable for introduction into a tissue to cause cells of the tissue to express the said protein or polypeptide.

[0270] The amount of vector to be injected to the desired host organism varies according to the site of injection. As an indicative dose, it will be injected between 0.1 and 100 μg of the vector in an animal body, preferably a mammal body, for example a mouse body.

[0271] In another embodiment of the invention, the vector may be introduced in vitro in a host cell, preferably in a host cell previously harvested from the animal to be treated and more preferably a somatic cell such as a muscle cell. In a subsequent step, the cell that has been transformed with the vector coding for the desired MGST-II polypeptide or the desired fragment thereof is reintroduced into the animal body in order to deliver the recombinant protein within the body either locally or systemically.

[0272] Host Cells

[0273] Another embodiment of the invention consists of a host cell that has been transformed or transfected with one of the polynucleotides described therein, and more precisely a polynucleotide either comprising a MGST-II regulatory polynucleotide or the coding sequence of the MGST-II polypeptide having the amino acid sequence of SEQ ID Nos. 488 and 489. The embodiment includes host cells that are transformed (prokaryotic cells) or that are transfected (eukaryotic cells) with a recombinant vector such as one of those described above. Generally, a recombinant host cell of the invention comprises any one of the polynucleotides or the recombinant vectors described therein.

[0274] A preferred recombinant host cell according to the invention comprises a polynucleotide selected from the following group of polynucleotides:

[0275] a) a purified or isolated nucleic acid encoding a MGST-II polypeptide, or a polypeptide fragment or variant thereof.

[0276] b) a purified or isolated nucleic comprising at least 8, preferably at least 15, more preferably at least 25, consecutive nucleotides of the nucleotide sequence SEQ ID No. 485, a nucleotide sequence complementary thereto, or a variant thereof.

[0277] c) a purified or isolated nucleic acid comprising at least 8 consecutive nucleotides, preferably at least 15, more preferably at least 25 of the nucleotide sequence SEQ ID Nos. 486 and 487, a nucleotide sequence complementary thereto or a variant thereof.

[0278] d) a purified or isolated nucleic acid comprising an exon of the MGST-II gene, a sequence complementary thereto or a fragment or a variant thereof.

[0279] e) a purified or isolated nucleic acid comprising a combination of at least two exons of the MGST-II gene, or the sequences complementary thereto wherein the polynucleotides are arranged within the nucleic acid, from the 5' end to the 3'end of said nucleic acid, in the same order than in SEQ ID No. 485.

[0280] f) a purified or isolated nucleic acid comprising the nucleotide sequence SEQ ID No. 485 or the sequences complementary thereto or a biologically active fragment thereof.

[0281] g) a purified or isolated nucleic acid comprising the nucleotide sequence SEQ ID No. 486, or the sequence complementary thereto or a biologically active fragment thereof.

[0282] h) a polynucleotide consisting of:

[0283] (1) a nucleic acid comprising a regulatory polynucleotide of SEQ ID No. 485 or the sequences complementary thereto or a biologically active fragment thereof

[0284] (2) a polynucleotide encoding a desired polypeptide or nucleic acid.

[0285] (3) Optionally, a nucleic acid comprising a regulatory polynucleotide of SEQ ID No. 485, or the sequence complementary thereto or a biologically active fragment thereof.

[0286] i) a DNA construct as described previously in the present specification.

[0287] Another preferred recombinant cell host according to the present invention is characterized in that its genome or genetic background (including chromosome, plasmids) is modified by the nucleic acid coding for the MGST-II polypeptide of SEQ ID Nos. 488 and 489 or fragments or variants thereof.

[0288] Preferred host cells used as recipients for the expression vectors of the invention are the following:

[0289] a) Prokaryotic host cells: *Eschericlhia coli* strains (I.E. DH5-α strain), *Bacillus subtilis, Salmonella typhimurium*, and strains from species like Pseudomonas, Streptomyces and Staphylococcus.

[0290] b) Eukaryotic host cells: HeLa cells (ATCC N°CCL2; N°CCL2.1; N°CCL2.2), Cv 1 cells (ATCC N°CCL70), COS cells (ATCC N°CRL1650; N°CRL1651), Sf-9 cells (ATCC N°CRL1711), C127 cells (ATCC N°CRL-1804), 3T3 (ATCC N°CRL-6361), CHO (ATCC N°CCL-61), human kidney 293.(ATCC N° 45504; N°CRL-1573) and BHK (ECACC N° 84100501; N° 84111301)

[0291] c) Other mammalian host cells:

[0292] The MGST-II gene expression in mammalian, and typically human, cells may be rendered defective, or alternatively it may be proceeded with the insertion of a MGST-II genomic or cDNA sequence with the replacement of the MGST-II gene counterpart in the genome of an animal cell by a MGST-II polynucleotide according to the invention. These genetic alterations may be generated by homologous recombination events using specific DNA constructs that have been previously described.

[0293] One kind of cell host that is commonly used is mammal zygotes, such as murine zygotes. For example, murine zygotes may undergo microinjection with a purified DNA molecule of interest, for example a purified DNA molecule that has previously been adjusted to a concentration range from 1 ng/ml (for BAC inserts) 3 ng/μl (for P1 bacteriophage inserts) in 10 mM Tris-HCl, pH 7.4, 250 μM EDTA containing 100 mM NaCl, 30 μM spermine, and70 μM spermidine. When the DNA to be microinjected has a large size, polyamines and high salt concentrations can be

used in order to avoid mechanical breakage of this DNA, as described by Schedl et al (*Nucleic Acids Res.* 21:4783-4787, 1993).

[0294] Anyone of the polynucleotides of the invention, including the DNA constructs described herein, may be introduced in an embryonic stem (ES) cell line, preferably a mouse ES cell line. ES cell lines are derived from pluripotent, uncommitted cells of the inner cell mass of pre-implantation blastocysts. Preferred ES cell lines are the following: ES-E14TG2a (ATCC n° CRL-1821), ES-D3 (ATCC n° CRL1934 and n° CRL-11632), YS001 (ATCC n° CRL-11776), 36.5 (ATCC n° CRL-11116). To maintain ES cells in an uncommitted state, they are cultured in the presence of growth inhibited feeder cells which, provide the appropriate signals to preserve this embryonic phenotype and serve as a matrix for ES cell adherence. Preferred feeder cells consist of primary embryonic fibroblasts that are established from tissue of day 13-day 14 embryos of virtually any mouse strain, that are maintained in culture, such as described by Abbondanzo et al. (*Methods in Enzymology,* Academic Press, New York, 803-823, 1993) and are inhibited in growth by irradiation, such as described by Robertson ("Embryo-Derived StemCell Lines," E. J. Robertson Ed. *Teratocarcinomas and Embrionic Stem Cells: A Practical Approach.* IRL Press, Oxford, 71, 1987), or by the presence of an inhibitory concentration of LIF, such as described by Pease and Williams (*Exp. Cell. Res.* 190:09-211, 1990).

[0295] The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

[0296] Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period.

[0297] Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

[0298] Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by one skilled in the art.

[0299] Transgenic Animals

[0300] The terms "transgenic animals" or "host animals" are used herein designate animals that have their genome genetically and artificially manipulated so as to include one of the nucleic acids according to the invention. Preferred animals are non-human mammals and include those belonging to a genus selected from Mus (e.g. mice), Rattus (e.g. rats) and Oryctogalus (e.g. rabbits) which have their genome artificially and genetically altered by the insertion of a nucleic acid according to the invention.

[0301] The transgenic animals of the invention all include within a plurality of their cells a cloned recombinant or synthetic DNA sequence, more specifically one of the purified or isolated nucleic acids comprising a MGST-II coding sequence, a MGST-II regulatory polynucleotide or a DNA sequence encoding an antisense polynucleotide such as described in the present specification.

[0302] Preferred transgenic animals according to the invention contains in their somatic cells and/or in their germ line cells a polynucleotide selected from the following group of polynucleotides:

[0303] a) a purified or isolated nucleic acid encoding a MGST-II polypeptide, or a polypeptide fragment or variant thereof.

[0304] b) a purified or isolated nucleic comprising at least 8, preferably at least 15, more preferably at least 25, consecutive nucleotides of the nucleotide sequence SEQ ID No. 485, a nucleotide sequence complementary thereto.

[0305] c) a purified or isolated nucleic acid comprising at least 8 consecutive nucleotides, preferably at least 15, more preferably at least 25 of the nucleotide sequence SEQ ID Nos. 486 and 487, a nucleotide sequence complementary thereto.

[0306] d) a purified or isolated nucleic acid comprising an exon of the MGST-II gene, a sequence complementary thereto or a fragment or a variant thereof.

[0307] e) a purified or isolated nucleic acid comprising a combination of at least two exons of the MGST-II gene, or the sequences complementary thereto wherein the polynucleotides are arranged within the nucleic acid, from the 5' end to the 3'end of said nucleic acid, in the same order than in SEQ ID No. 485.

[0308] f) a purified or isolated nucleic acid comprising the nucleotide sequence SEQ ID No. 485 or the sequences complementary thereto or a biologically active fragment thereof.

[0309] g) a purified or isolated nucleic acid comprising the nucleotide sequence SEQ ID No. 486, or the sequence complementary thereto or a biologically active fragment thereof.

[0310] h) a polynucleotide consisting of:

[0311] (1) a nucleic acid comprising a regulatory polynucleotide of SEQ ID No. 485 or the sequences complementary thereto or a biologically active fragment thereof

[0312] (2) a polynucleotide encoding a desired polypeptide or nucleic acid.

[0313] (3) Optionally, a nucleic acid comprising a regulatory polynucleotide of SEQ ID No. 486, or the sequence complementary thereto or a biologically active fragment thereof.

[0314] i) a DNA construct as described previously in the present specification.

[0315] The transgenic animals of the invention thus contain specific sequences of exogenous genetic material such as the nucleotide sequences described above in detail.

[0316] In a first preferred embodiment, these transgenic animals may be good experimental models in order to study the diverse pathologies related to cell differentiation, in particular concerning the transgenic animals within the genome of which has been inserted one or several copies of

a polynucleotide encoding a native MGST-II protein, or alternatively a mutant MGST-II protein.

[0317] In a second preferred embodiment, these transgenic animals may express a desired polypeptide of interest under the control of the regulatory polynucleotides of the MGST-II gene, leading to good yields in the synthesis of this protein of interest, and eventually a tissue specific expression of this protein of interest.

[0318] The design of the transgenic animals of the invention may be made according to the conventional techniques well known from the one skilled in the art. For more details regarding the production of transgenic animals, and specifically transgenic mice, it may be referred to U.S. Pat. No. 4,873,191, issued Oct. 10, 1989, U.S. Pat. No. 5,464,764 issued Nov. 7, 1995 and U.S. Pat. No. 5,789,215, issued Aug. 4, 1998, these documents being herein incorporated by reference to disclose methods producing transgenic mice.

[0319] Transgenic animals of the present invention are produced by the application of procedures which result in an animal with a genome that has incorporated exogenous genetic material. The procedure involves obtaining the genetic material, or a portion thereof, which encodes either a MGST-II coding sequence, a MGST-II regulatory polynucleotide or a DNA sequence encoding a MGST-II antisense polynucleotide such as described in the present specification.

[0320] A recombinant polynucleotide of the invention is inserted into an embryonic or ES stem cell line. The insertion is preferably made using electroporation, such as described by Thomas et al. (*Cell* 51:503-512, 1987). The cells subjected to electroporation are screened (e.g. by selection via selectable markers, by PCR or by Southern blot analysis) to find positive cells which have integrated the exogenous recombinant polynucleotide into their genome, preferably via an homologous recombination event. An illustrative positive-negative selection procedure that may be used according to the invention is described by Mansour et al. (*Nature* 336:348-352, 1988).

[0321] Then, the positive cells are isolated, cloned and injected into 3.5 days old blastocysts from mice, such as described by Bradley ("Production and Analysis of Chimaeric Mice," E. J. Robertson (Ed.), *Teratocarcinomas and embryonic stem cells: A practical approach* IRL Press, Oxford, 113, 1987). The blastocysts are then inserted into a female host animal and allowed to grow to term.

[0322] Alternatively, the positive ES cells are brought into contact with embryos at the 2.5 days old 8-16 cell stage (morulae) such as described by Wood et al. (*Proc. Natl. Acad. Sci. U.S.A.* 90:4582-4585, 1993) or by Nagy et al. (*Proc. Natl. Acad. Sci. USA.* 90: 8424-8428, 1993), the ES cells being internalized to colonize extensively the blastocyst including the cells which will give rise to the germ line.

[0323] The offspring of the female host are tested to determine which animals are transgenic e.g. include the inserted exogenous DNA sequence and which are wild-type. Thus, the present invention also concerns a transgenic animal containing a nucleic acid, a recombinant expression vector or a recombinant host cell according to the invention.

[0324] A further object of the invention consists of recombinant host cells obtained from a transgenic animal described herein.

[0325] Recombinant cell lines may be established in vitro from cells obtained from any tissue of a transgenic animal according to the invention, for example by transfection of primary cell cultures with vectors expressing onc-genes such as SV40 large T antigen, as described by Chou (*Mol. Endocrinol.* 3:1511-1514, 1989) and Shay et al. (*Biochem. Biophys. Acta.* 1072:1-7, 1991).

[0326] E. MGST-II Polypeptides

[0327] The term "MGST-II polypeptides" is used herein to embrace all of the proteins and polypeptides of the present invention. Also forming part of the invention are polypeptides encoded by the polynucleotides of the invention, as well as fusion polypeptides comprising such polypeptides. The invention embodies MGST-II proteins from humans, including isolated or purified MGST-II proteins consisting, consisting essentially, or comprising the sequence of SEQ ID Nos. 488 and 489. It should be noted the MGST-II proteins of the invention are based on the naturally-occurring variants of the amino acid sequence of human MGST-II.

[0328] In a first embodiment, the present invention provides a variant MGST-II protein; wherein the Tyr residue of amino acid position 93 has been replaced with a His residue. Variant proteins and the fragments thereof which contain amino acid position 93 are collectively referred to herein as "93-His variants." More particularly, the present invention embodies isolated, purified, and recombinant polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of SEQ ID No. 488, wherein said contiguous span comprises a His residue at amino acid position 93. In this amino acid substitution the original residue (Tyr) is replaced by a non-equivalent amino acid (His) presenting different chemical properties.

[0329] The present invention further provides another naturally-occurring variant of the MGST-II protein that consists or consists essentially of amino acids 1-109 of SEQ ID No. 488. This variant MGST-II polypeptide corresponds to one allele of biallelic marker 10-290-37.

[0330] Another naturally-occurring variant of the MGST-II protein of the present invention is encoded by a cDNA obtained by alternative splicing. MGST-II cDNAs and cDNA variants are further described above. This variant polypeptide of a sequence from SEQ ID No. 489 is identical to the MGST-II protein of SEQ ID No. 488 from amino acid position 1 to amino acid position 19 but comprises 11 additional amino acids. The present invention embodies isolated, purified, and recombinant polypeptides comprising, consisting of or consisting essentially of an amino acid sequence from SEQ ID No. 489. Moreover, the present invention embodies isolated, purified, and recombinant polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, or 30 amino acids of SEQ ID No. 489, wherein said contiguous span comprises a least one of amino acid positions 20 to 30 of SEQ ID No. 489.

[0331] All the variant MGST-II polypeptides described above most probably show alterations in the activity, specificity and function of the MGST-II enzyme. In preferred embodiments the polypeptides of the present invention comprise the site of a mutation or functional mutation,

including a deletion, substitution or truncation in the amino acid sequence in the MGST-II protein.

[0332] MGST-II proteins are preferably isolated from human or mammalian tissue samples or expressed from human or mammalian genes. The MGST-II polypeptides of the invention can be made using routine expression methods known in the art. The polynucleotide encoding the desired polypeptide, is ligated into an expression vector suitable for any convenient host. Both eukaryotic and prokaryotic host systems are used in forming recombinant polypeptides. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification is by any technique known in the art, for example, differential extraction, salt fractionation, chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins.

[0333] In addition, shorter protein fragments are produced by chemical synthesis. Alternatively the proteins of the invention are extracted from cells or tissues of humans or non-human animals. Methods for purifying proteins are known in the art, and include the use of detergents or chaotropic agents to disrupt particles followed by differential extraction and separation of the polypeptides by ion exchange chromatography, affinity chromatography, sedimentation according to density, and gel electrophoresis.

[0334] Any MGST-II cDNA of the invention is used to express MGST-II proteins and polypeptides. The nucleic acid encoding the MGST-II protein or polypeptide to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The MGST-II insert in the expression vector may comprise the full coding sequence for the MGST-II protein or a portion thereof. For example, the MGST-II derived insert may encode a polypeptide comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of SEQ ID No. 488, wherein said contiguous span comprises a His residue at amino acid position 93. The MGST-II derived insert may further encode a polypeptide comprising, consisting of or consisting essentially of an amino acid sequence from amino acid positions 1-108 of SEQ ID No. 488. The MGST-II derived insert may further encode a polypeptide comprising, consisting of or consisting essentially of an amino acid sequence from SEQ ID No. 489. The MGST-II derived insert may also encode a polypeptide comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, or 30 amino acids of SEQ ID No. 489, wherein said contiguous span comprises a least one of amino acid positions 20 to 30 of SEQ ID No. 489.

[0335] The expression vector is any of the mammalian, yeast, insect or bacterial expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, Mass.), Stratagene (La Jolla, Calif.), Promega (Madison, Wis.), and Invitrogen (San Diego, Calif.). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence is optimized for the particular expression organism in which the expression vector is introduced, as explained by Hatfield, et al., U.S. Pat. No. 5,082,767.

[0336] In one embodiment, the entire coding sequence of the MGST-II cDNA through the poly A signal of the cDNA is operably linked to a promoter in the expression vector. Alternatively, if the nucleic acid encoding a portion of the MGST-II protein lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. Similarly, if the insert from the MGST-II cDNA lacks a poly A signal, this sequence can be added to the construct by, for example, splicing out the Poly A signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allows efficient stable transfection. The vector includes the Herpes Simplex Thymidine Kinase promoter and the selectable neomycin gene. The nucleic acid encoding the MGST-II protein or a portion thereof is obtained by PCR from a bacterial vector containing a MGST-II cDNA of the present invention using oligonucleotide primers complementary to the MGST-II cDNA or portion thereof and containing restriction endonuclease sequences for Pst I incorporated into the 5'primer and BglI at the 5' end of the corresponding cDNA 3' primer, taking care to ensure that the sequence encoding the MGST-II protein or a portion thereof is positioned properly with respect to the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII.

[0337] The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, N.Y.) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Mo.).

[0338] Alternatively, the nucleic acids encoding the MGST-II protein or a portion thereof is cloned into pED6dpc2 (Genetics Institute, Cambridge, Mass.). The resulting pED6dpc2 constructs is transfected into a suitable host cell, such as COS 1 cells. Methotrexate resistant cells are selected and expanded.

[0339] The above procedures may also be used to express a mutant MGST-II protein responsible for a detectable phenotype or a portion thereof

[0340] The expressed proteins are purified using conventional purification techniques such as ammonium sulfate precipitation or chromatographic separation based on size or charge. The protein encoded by the nucleic acid insert may also be purified using standard immunochromatography techniques. In such procedures, a solution containing the expressed MGST-II protein or portion thereof, such as a cell extract, is applied to a column having antibodies against the MGST-II protein or portion thereof is attached to the chromatography matrix. The expressed protein is allowed to bind the immunochromatography column. Thereafter, the column is washed to remove non-specifically bound proteins. The specifically bound expressed protein is then released from the column and recovered using standard techniques.

[0341] To confirm expression of the MGST-II protein or a portion thereof, the proteins expressed from host cells containing an expression vector containing an insert encoding

the MGST-II protein or a portion thereof can be compared to the proteins expressed in host cells containing the expression vector without an insert. The presence of a band in samples from cells containing the expression vector with an insert which is absent in samples from cells containing the expression vector without an insert indicates that the MGST-II protein or a portion thereof is being expressed. Generally, the band will have the mobility expected for the MGST-II protein or portion thereof. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

[0342] Antibodies capable of specifically recognizing the expressed MGST-II protein or a portion thereof, are described below.

[0343] If antibody production is not possible, the nucleic acids encoding the MGST-II protein or a portion thereof is incorporated into expression vectors designed for use in purification schemes employing chimeric polypeptides. In such strategies the nucleic acid encoding the MGST-II protein or a portion thereof is inserted in frame with the gene encoding the other half of the chimera. The other half of the chimera is β-globin or a nickel binding polypeptide encoding sequence. A chromatography matrix having antibody to β-globin or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites is engineered between the β-globin gene or the nickel binding polypeptide and the MGST-II protein or portion thereof. Thus, the two polypeptides of the chimera are separated from one another by protease digestion.

[0344] One useful expression vector for generating β-globin chimerics is pSG5 (Stratagene), which encodes rabbit β-globin. Intron II of the rabbit β-globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis et al., (Basic Methods in Molecular Biology, L. G. Davis, M. D. Dibner, and J. F. Battey, ed., Elsevier Press, NY, 1986) and many of the methods are available from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from the construct using in vitro translation systems such as the In vitro Express™ Translation Kit (Stratagene).

[0345] F. Production of Antibodies Against MGST-II Polypeptides

[0346] Any MGST-II polypeptide or whole protein may be used to generate antibodies capable of specifically binding to expressed MGST-II protein or fragments thereof or variants thereof. Preferably the antibody compositions of the invention are capable of specifically binding to the 93-His variant of the MGST-II protein. Alternatively the antibody compositions of the present invention are capable of specifically binding the variant MGST-II polypeptide of SEQ ID No. 489. A preferred embodiment of the invention encompasses isolated or purified antibody compositions capable of selectively binding, or which are capable of binding to an epitope-containing fragment of a polypeptide of the invention, wherein said epitope comprises at least one amino acid position selected from the group consisting of His residue at amino acid position 93 of SEQ ID No. 488 and of amino acid

positions 20-30 of SEQ ID No. 489. For an antibody composition to specifically bind to these MGST-II variants it must demonstrate at least a 5%, 10%, 15%, 20%, 25%, 50%, or 100% greater binding affinity for fill length MGST-II variants in an ELISA, RIA, or other antibody-based binding assay than to full length MGST-II protein described in SEQ ID No. 488. Affinity of the antibody composition for the epitope can further be determined by preparing competitive binding curves, as described, for example, by Fisher, D. (Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) *Amer. Soc. For Microbiol.*, Washington, D.C., 1980).

[0347] The present invention also contemplates the use of variant MGST-II polypeptides in the manufacture of antibodies. In a preferred embodiment such polypeptides are useful in the manufacture of antibodies to detect the presence and absence of the 93-His variant and of the MGST-II variant of SEQ ID No. 489.

[0348] Non-human animals or mammals, whether wild-type or transgenic, which express a different species of MGST-II than the one to which antibody binding is desired, and animals which do not express MGST-II (i.e. an MGST-II knock out animal as described in herein) are particularly useful for preparing antibodies. MGST-II knock out animals will recognize all or most of the exposed regions of MGST-II as foreign antigens, and therefore produce antibodies with a wider array of MGST-II epitopes. Moreover, smaller polypeptides with only 10 to 30 amino acids may be useful in obtaining specific binding to the 93-His variant and to the MGST-II variant of SEQ ID No. 489. In addition, the humoral immune system of animals which produce a species of MGST-II that resembles the antigenic sequence will preferentially recognize the differences between the animal's native MGST-II species and the antigen sequence, and produce antibodies to these unique sites in the antigen sequence. Such a technique will be particularly useful in obtaining antibodies that specifically bind to the 93-His variant and to the MGST-II variant of SEQ ID No. 489. The preparation of antibody compositions is further described in Example 6.

[0349] Antibody preparations prepared according to the present invention are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body. The antibodies of the invention may be labeled, either by a radioactive, a fluorescent or an enzymatic label. Consequently, the invention is also directed to a method for detecting specifically the presence of a variant MGST-II polypeptide according to the invention in a biological sample, said method comprising the following steps: a) bringing into contact the biological sample with a polyclonal or monoclonal antibody that specifically binds a variant MGST-II polypeptide or to a peptide fragment or variant thereof, and b) detecting the antigen-antibody complex formed. The invention also concerns a diagnostic kit for detecting in vitro the presence of a variant MGST-II polypeptide according to the present invention in a biological sample, wherein said kit comprises: a) a polyclonal or monoclonal antibody that specifically binds a variant

MGST-II polypeptide or to a peptide fragment or variant thereof, optionally labeled; b) a reagent allowing the detection of the antigen-antibody complexes formed, said reagent carrying optionally a label, or being able to be recognized itself by a labeled reagent, more particularly in the case when the above-mentioned monoclonal or polyclonal antibody is not labeled by itself.

[0350] II. Methods for De Novo Identification of Biallelic Markers

[0351] Large fragments of human DNA, carrying genes of interest involved in the biotransformation of xenobiotics such as therapeutic drugs; were cloned, sequenced and screened for biallelic markers. Biallelic markers within the candidate genes themselves as well as markers located on the same genomic fragment were identified. It will be clear to one of skill in the art that large fragments of human genomic DNA may be obtained from any appropriate source and may be cloned into a number of suitable vectors.

[0352] In a preferred embodiment of the invention, BAC (Bacterial Artificial Chromosomes) vectors were used to construct DNA libraries covering the entire human genome. Specific amplification primers were designed for each candidate gene and the BAC library was screened by PCR until there was at least one positive BAC clone per candidate gene. Genomic sequence, screened for biallelic markers, was generated by sequencing ends of BAC subclones. Details of a preferred embodiment are provided in Example 1. As a preferred alternative to sequencing the ends of an adequate number of BAC subclones, high throughput deletion-based sequencing vectors, which allow the generation of a high quality sequence information covering fragments of about 6 kb, may be used. Having sequence fragments longer than 2.5 or 3 kb enhances the chances of identifying biallelic markers therein. Methods of constructing and sequencing a nested set of deletions are disclosed in the related U.S. patent application entitled "High Throughput DNA Sequencing Vector" (Ser. No. 09/058,746).

[0353] In another embodiment of the invention, genomic sequences of candidate genes were available in public databases allowing direct screening for biallelic markers.

[0354] Any of a variety of methods can be used to screen a genomic fragment for single nucleotide polymorphisms such as differential hybridization with oligonucleotide probes, detection of changes in the mobility measured by gel electrophoresis or direct sequencing of the amplified nucleic acid. A preferred method for identifying biallelic markers involves comparative sequencing of genomic DNA fragments from an appropriate number of unrelated individuals.

[0355] In a first embodiment, DNA samples from unrelated individuals are pooled together, following which the genomic DNA of interest is amplified and sequenced. The nucleotide sequences thus obtained are then analyzed to identify significant polymorphisms. One of the major advantages of this method resides in the fact that the pooling of the DNA samples substantially reduces the number of DNA amplification reactions and sequencing reactions, which must be carried out. Moreover, this method is sufficiently sensitive so that a biallelic marker obtained thereby usually demonstrates a sufficient frequency of its less common allele to be useful in conducting association studies. Usually, the frequency of the least common allele of a biallelic marker identified by this method is at least 10%.

[0356] In a second embodiment, the DNA samples are not pooled and are therefore amplified and sequenced individually. This method is usually preferred when biallelic markers need to be identified in order to perform association studies within candidate genes. Preferably, highly relevant gene regions such as promoter regions or exon regions may be screened for biallelic markers. A biallelic marker obtained using this method may show a lower degree of informativeness for conducting association studies, e.g. if the frequency of its less frequent allele may be less than about 10%. Such a biallelic marker will however be sufficiently infomative to conduct association studies and it will further be appreciated that including less informative biallelic markers in the genetic analysis studies of the present invention, may allow in some cases the direct identification of causal mutations, which may, depending on their penetrance, be rare mutations.

[0357] The following is a description of the various parameters of a preferred method used by the inventors for the identification of the biallelic markers of the present invention.

[0358] A. Genomic DNA Samples

[0359] The genomic DNA samples from which the biallelic markers of the present invention are generated are preferably obtained from unrelated individuals corresponding to a heterogeneous population of known ethnic background. The number of individuals from whom DNA samples are obtained can vary substantially, preferably from about 10 to about 1000, more preferably from about 50 to about 200 individuals. Usually, DNA samples are collected from at least about 100 individuals in order to have sufficient polymorphic diversity in a given population to identify as many markers as possible and to generate statistically significant results.

[0360] As for the source of the genomic DNA to be subjected to analysis, any test sample can be foreseen without any particular limitation. These test samples include biological samples, which can be tested by the methods of the present invention described herein, and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; fixed tissue specimens including tumor and non-tumor tissue and lymph node tissues; bone marrow aspirates and fixed cell specimens. The preferred source of genomic DNA used in the present invention is from peripheral venous blood of each donor. Techniques to prepare genomic DNA from biological samples are well known to the skilled technician. Details of a preferred embodiment are provided in Example 1. The person skilled in the art can choose to amplify pooled or unpooled DNA samples.

[0361] B. DNA Amplification

[0362] The identification of biallelic markers in a sample of genomic DNA may be facilitated through the use of DNA amplification methods. DNA samples can be pooled or unpooled for the amplification step. DNA amplification techniques are well known to those skilled in the art. Various methods to amplify DNA fragments carrying biallelic markers are further described hereinafter in III.B. The PCR

technology is the preferred amplification technique used to identify new biallelic markers.

[0363] In a first embodiment, biallelic markers are identified using genomic sequence information generated by the inventors. Genomic DNA fragments, such as the inserts of the BAC clones described above, are sequenced and used to design primers for the amplification of 500 bp fragments. These 500 bp fragments are amplified from genomic DNA and are scanned for biallelic markers. Primers may be designed using the OSP software (Hillier L. and Green P., 1991), the disclosures of which are incorporated herein by reference in their entirety. All primers may contain, upstream of the specific target bases, a common oligonucleotide tail that serves as a sequencing primer. Those skilled in the art are familiar with primer extensions, which can be used for these purposes.

[0364] In another embodiment of the invention, genomic sequences of candidate genes are available in public databases allowing direct screening for biallelic markers. Preferred primers, useful for the amplification of genomic sequences encoding the candidate genes, focus on promoters, exons and splice sites of the genes. A biallelic marker present in these functional regions of the gene have a higher probability to be a causal mutation.

[0365] Preferred primers include those disclosed in Table 17.

[0366] C. Sequencing of Amplified Genomic DNA and Identification of Single Nucleotide Polymorphisms

[0367] The amplification products generated as described above, are then sequenced using any method known and available to the skilled technician. Methods for sequencing DNA using either the dideoxy-mediated method (Sanger method) or the Maxam-Gilbert method are widely known to those of ordinary skill in the art. Such methods are for example disclosed in Maniatis et al. (Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Second Edition, 1989), the disclosure of which is incorporated herein by reference in its entirety. Alternative approaches include hybridization to high-density DNA probe arrays as described in Chee et al. (*Science* 274, 610, 1996), the disclosure of which is incorporated herein by reference in its entirety.

[0368] Preferably, the amplified DNA is subjected to automated dideoxy terminator sequencing reactions using a dye-primer cycle sequencing protocol. The products of the sequencing reactions are run on sequencing gels and the sequences are determined using gel image analysis. The polymorphism search is based on the presence of superimposed peaks in the electrophoresis pattern resulting from different bases occurring at the same position. Because each dideoxy terminator is labeled with a different fluorescent molecule, the two peaks corresponding to a biallelic site present distinct colors corresponding to two different nucleotides at the same position on the sequence. However, the presence of two peaks can be an artifact due to background noise. To exclude such an artifact, the two DNA strands are sequenced and a comparison between the peaks is carried out. In order to be registered as a polymorphic sequence, the polymorphism has to be detected on both strands.

[0369] The above procedure permits those amplification products, which contain biallelic markers to be identified.

The detection limit for the frequency of biallelic polymorphisms detected by sequencing pools of 100 individuals is approximately 0.1 for the minor allele, as verified by sequencing pools of known allelic frequencies. However, more than 90% of the biallelic polymorphisms detected by the pooling method have a frequency for the minor allele higher than 0.25. Therefore, the biallelic markers selected by this method have a frequency of at least 0.1 for the minor allele and less than 0.9 for the major allele. Preferably at least 0.2 for the minor allele and less than 0.8 for the major allele, more preferably at least 0.3 for the minor allele and less than 0.7 for the major allele, thus a heterozygosity rate higher than 0.18, preferably higher than 0.32, more preferably higher than 0.42.

[0370] In another embodiment, biallelic markers are detected by sequencing individual DNA samples, the frequency of the minor allele of such a biallelic marker may be less than 0.1.

[0371] The markers carried by the same fragment of genomic DNA, such as the insert in a BAC clone, need not necessarily be ordered with respect to one another within the genomic fragment to conduct association studies. However, in some embodiments of the present invention, the order of biallelic markers carried by the same fragment of genomic DNA are determined.

[0372] D. Validation of the Biallelic Markers of the Present Invention

[0373] The polymorphisms are evaluated for their usefulness as genetic markers by validating that both alleles are present in a population. Validation of the biallelic markers is accomplished by genotyping a group of individuals by a method of the invention and demonstrating that both alleles are present. Microsequencing is a preferred method of genotyping alleles. The validation by genotyping step may be performed on individual samples derived from each individual in the group or by genotyping a pooled sample derived from more than one individual. The group can be as small as one individual if that individual is heterozygous for the allele in question. Preferably the group contains at least three individuals, more preferably the group contains five or six individuals, so that a single validation test will be more likely to result in the validation of more of the biallelic markers that are being tested. It should be noted, however, that when the validation test is performed on a small group it may result in a false negative result if as a result of sampling error none of the individuals tested carries one of the two alleles. Thus, the validation process is less useful in demonstrating that a particular initial result is an artifact, than it is at demonstrating that there is a bona fide biallelic marker at a particular position in a sequence. For an indication of whether a particular biallelic marker has been validated see Table 11(A-B). All of the genotyping, haplotyping, association, and interaction study methods of the invention may optionally be performed solely with validated biallelic markers.

[0374] E. Evaluation of the Frequency of the Biallelic Markers of the Present Invention

[0375] The validated biallelic markers are further evaluated for their usefulness as genetic markers by determining the frequency of the least common allele at the biallelic marker site. The determination of the least common allele is

accomplished by genotyping a group of individuals by a method of the invention and demonstrating that both alleles are present. This determination of frequency by genotyping step may be performed on individual samples derived from each individual in the group or by genotyping a pooled sample derived from more than one individual. The group must be large enough to be representative of the population as a whole. Preferably the group contains at least 20 individuals, more preferably the group contains at least 50 individuals, most preferably the group contains at least 100 individuals. Of course the larger the group the greater the accuracy of the frequency determination because of reduced sampling error. For an indication of the frequency for the less common allele of a particular biallelic marker of the invention see Table 11(A-B). A biallelic marker wherein the frequency of the less common allele is 30% or more is termed a "high quality biallelic marker." All of the genotyping, haplotyping, association, and interaction study methods of the invention may optionally be performed solely with high quality biallelic markers.

[0376] III. Methods of Genotyping an Individual for Biallelic Markers

[0377] Methods are provided to genotype a biological sample for one or more biallelic markers of the present invention, all of which may be performed in vitro. Such methods of genotyping comprise determining the identity of a nucleotide at a DME-related biallelic marker by any method known in the art. These methods find use in genotyping case-control populations in association studies as well as individuals in the context of detection of alleles of biallelic markers which, are known to be associated with a given trait, in which case both copies of the biallelic marker present in individual's genome are determined so that an individual may be classified as homozygous or heterozygous for a particular allele.

[0378] These genotyping methods can be performed nucleic acid samples derived from a single individual or pooled DNA samples.

[0379] Genotyping can be performed using similar methods as those described above for the identification of the biallelic markers, or using other genotyping methods such as those further described below. In preferred embodiments, the comparison of sequences of amplified genomic fragments from different individuals is used to identify new biallelic markers whereas microsequencing is used for genotyping known biallelic markers in diagnostic and association study applications.

[0380] A. Source of DNA for Genotyping

[0381] Any source of nucleic acids, in purified or non-purified form, can be utilized as the starting nucleic acid, provided it contains or is suspected of containing the specific nucleic acid sequence desired. DNA or RNA may be extracted from cells, tissues, body fluids and the like as described above in II.A. "Genomic DNA Samples." While nucleic acids for use in the genotyping methods of the invention can be derived from any mammalian source, the test subjects and individuals from which nucleic acid samples are taken are generally understood to be human.

[0382] B. Amplification of DNA Fragments Comprising Biallelic Markers

[0383] Methods and polynucleotides are provided to amplify a segment of nucleotides comprising one or more biallelic marker of the present invention. It will be appreciated that amplification of DNA fragments comprising biallelic markers may be used in various methods and for various purposes and is not restricted to genotyping. Nevertheless, many genotyping methods, although not all, require the previous amplification of the DNA region carrying the biallelic marker of interest. Such methods specifically increase the concentration or total number of sequences that span the biallelic marker or include that site and sequences located either distal or proximal to it. Diagnostic assays may also rely on amplification of DNA segments carrying a biallelic marker of the present invention.

[0384] Amplification of DNA may be achieved by any method known in the art. The established PCR (polymerase chain reaction) method or by developments thereof or alternatives. Amplification methods which can be utilized herein include but are not limited to Ligase Chain Reaction (LCR) as described in EP A 320 308 and EP A 439 182, Gap LCR (Wolcott, M. J., Clin. Mcrobiol. Rev. 5:370-386), the so-called "NASBA" or "3SR" technique described in Guatelli J. C. et al. (*Proc. Natl. Acad. Sci. USA* 87:1874-1878, 1990) and in Compton J. (*Nature* 350:91-92, 1991), Q-beta amplification as described in European Patent Application no 4544610, strand displacement amplification as described in Walker et al. (*Clin. Chem.* 42:9-13, 1996) and EP A 684 315 and, target mediated amplification as described in PCT Publication WO 9322461, the disclosures of which are incorporated herein by reference in their entirety.

[0385] LCR and Gap LCR are exponential amplification techniques, both depend on DNA ligase to join adjacent primers annealed to a DNA molecule. In Ligase Chain Reaction (LCR), probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. A method for multiplex LCR has also been described (WO 9320227), the disclosure of which is incorporated herein by reference in its entirety. Gap LCR (GLCR) is a version of LCR where the probes are not adjacent but are separated by 2 to 3 bases.

[0386] For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or,

to use a single enzyme for both steps as described in U.S. Pat. No. 5,322,770 or, to use Asyminetric Gap LCR (RT-AGLCR) as described by Marshall R. L. et al. (*PCR Methods and Applications* 4:80-84, 1994), the disclosures of which are incorporated herein by reference in their entirety. AGLCR is a modification of GLCR that allows the amplification of RNA.

[0387] Some of these amplification methods are particularly suited for the detection of single nucleotide polymorphisms and allow the simultaneous amplification of a target sequence and the identification of the polymorphic nucleotide as it is further described in "IIIC. Methods of Genotyping DNA samples for Biallelic Markers."

[0388] The PCR technology is the preferred amplification technique used in the present invention. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering White, B. A. Ed. in *Methods in Molecular Biology* 67: Humana Press, Totowa (1997) and the publication entitled "PCR Methods and Applications" (1991, Cold Spring Harbor Laboratory Press), the disclosure of which is incorporated herein by reference in its entirety. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites. PCR has further been described in several patents including U.S. Pat. Nos. 4,683, 195, 4,683,202 and 4,965,188, the disclosures of which are incorporated herein by reference in their entirety.

[0389] The identification of biallelic markers as described above allows the design of appropriate oligonucleotides, which can be used as primers to amplify DNA fragments comprising the biallelic markers of the present invention. Amplification can be performed using the primers initially used to discover new biallelic markers which are described herein or any set of primers allowing the amplification of a DNA fragment comprising a biallelic marker of the present invention. Primers can be prepared by any suitable method. As for example, direct chemical synthesis by a method such as the phosphodiester method of Narang S. A. et al. (*Methods Enzymol.* 68:90-98, 1979), the phosphodiester method of Brown E. L. et al. (*Methods Enzymol.* 68:109-151, 1979), the diethylphosphoramidite method of Beaucage et al. (*Tetrahedron Lett.* 22:1859-1862, 1981) and the solid support method described in EP 0 707 592, the disclosures of which are incorporated herein by reference in their entirety.

[0390] In some embodiments the present invention provides primers for amplifying a DNA fragment containing one or more biallelic markers of the present invention. Preferred amplification primers are listed in Table 17. It will be appreciated that the primers listed are merely exemplary and that any other set of primers which produce amplification products containing one or more biallelic markers of the present invention.

[0391] The primers are selected to be substantially complementary to the different strands of each specific sequence to be amplified. The length of the primers of the present invention can range from 8 to 100 nucleotides, preferably from 8 to 50, 8 to 30 or more preferably 8 to 25 nucleotides. Shorter primers tend to lack specificity for a target nucleic acid sequence and generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. Longer primers are expensive to produce and can sometimes self-hybridize to form hairpin structures. The formation of stable hybrids depends on the melting temperature (Tm) of the DNA. The Tm depends on the length of the primer, the ionic strength of the solution and the G+C content. The higher the G+C content of the primer, the higher is the melting temperature because G:C pairs are held by three H bonds whereas A:T pairs have only two. The G+C content of the amplification primers of the present invention preferably ranges between 10 and 75%, more preferably between 35 and 60%, and most preferably between 40 and 55%. The appropriate length for primers under a particular set of assay conditions may be empirically determined by one of skill in the art.

[0392] The spacing of the primers determines the length of the segment to be amplified. In the context of the present invention amplified segments carrying biallelic markers can range in size from at least about 25 bp to 35 kbp. Amplification fragments from 25-3000 bp are typical, fragments from 50-1000 bp are preferred and fragments from 100-600 bp are highly preferred. It will be appreciated that amplification primers for the biallelic markers may be any sequence which allow the specific amplification of any DNA fragment carrying the markers. Amplification primers may be labeled or immobilized on a solid support as described in I.

[0393] C. Methods of Genotyping DNA Samples for Biallelic Markers

[0394] Any method known in the art can be used to identify the nucleotide present at a biallelic marker site. Since the biallelic marker allele to be detected has been identified and specified in the present invention, detection will prove simple for one of ordinary skill in the art by employing any of a number of techniques. Many genotyping methods require the previous amplification of the DNA region carrying the biallelic marker of interest. While the amplification of target or signal is often preferred at present, ultrasensitive detection methods which do not require amplification are also encompassed by the present genotyping methods. Methods well-known to those skilled in the art that can be used to detect biallelic polymorphisms include methods such as, conventional dot blot analyzes, single strand conformational polymorphism analysis (SSCP) described by Orita et al. (*Proc. Natl. Acad. Sci. U.S.A* 86:27776-2770, 1989), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, mismatch cleavage detection, and other conventional techniques as described in Sheffield, V. C. et al. (*Proc. Natl. Acad. Sci. USA* 49:699-706, 1991), White et al. (*Genomics* 12:301-306, 1992), Grompe, M. et al. (*Proc. Natl. Acad. Sci. USA* 86:5855-5892, 1989) and Grompe, M. (*Nature Genetics* 5:111-117, 1993), the disclosures of which are incorporated herein by reference in their entirety. Another method for determining the identity of the nucleotide present at a particular polymorphic site employs a specialized exonuclease-resistant nucleotide derivative as

described in U.S. Pat. No. 4,656,127, the disclosure of which is incorporated herein by reference in its entirety.

[0395] Preferred methods involve directly determining the identity of the nucleotide present at a biallelic marker site by sequencing assay, enzyme-based mismatch detection assay, or hybridization assay. The following is a description of some preferred methods. A highly preferred method is the microsequencing technique. The term "sequencing assay" is used herein to refer to polymerase extension of duplex primer/template complexes and includes both traditional sequencing and microsequencing.

[0396] Sequencing Assays

[0397] The nucleotide present at a polymorphic site can be determined by sequencing methods. In a preferred embodiment, DNA samples are subjected to PCR amplification before sequencing as described above. DNA sequencing methods are described in IIC.

[0398] Preferably, the amplified DNA is subjected to automated dideoxy terminator sequencing reactions using a dye-primer cycle sequencing protocol. Sequence analysis allows the identification of the base present at the biallelic marker site.

[0399] Microsequencing Assays

[0400] In microsequencing methods, a nucleotide at the polymorphic site that is unique to one of the alleles in a target DNA is detected by a single nucleotide primer extension reaction. This method involves appropriate microsequencing primers which, hybridize just upstream of a polymorphic base of interest in the target nucleic acid. A polymerase is used to specifically extend the 3' end of the primer with one single ddNTP (chain terminator) complementary to the selected nucleotide at the polymorphic site. Next the identity of the incorporated nucleotide is determined in any suitable way.

[0401] Typically, microsequencing reactions are carried out using fluorescent ddNTPs and the extended microsequencing primers are analyzed by electrophoresis on ABI 377 sequencing machines to determine the identity of the incorporated nucleotide as described in EP 412 883. Alternatively capillary electrophoresis can be used in order to process a higher number of assays simultaneously. An example of a typical microsequencing procedure that can be used in the context of the present invention is provided in Example 2.

[0402] Different approaches can be used to detect the nucleotide added to the microsequencing primer. A homogeneous phase detection method based on fluorescence resonance energy transfer has been described by Chen and Kwok (*Nucleic Acids Research* 25:347-353 1997) and Chen et al. (*Proc. Natl Acad. Sci. USA* 94/20 10756-10761,1997), the disclosures of which are incorporated herein by reference in their entirety. In this method amplified genomic DNA fragments containing polymorphic sites are incubated with a 5'-fluorescein-labeled primer in the presence of allelic dye-labeled dideoxyribonucleoside triphosphates and a modified Taq polymerase. The dye-labeled primer is extended one base by the dye-terminator specific for the allele present on the template. At the end of the genotyping reaction, the fluorescence intensities of the two dyes in the reaction mixture are analyzed directly without separation or purification. All these steps can be performed in the same tube and the fluorescence changes can be monitored in real time. Alternatively, the extended primer may be analyzed by MALDI-TOF Mass Spectrometry. The base at the polymorphic site is identified by the mass added onto the microsequencing primer (see Haff L. A. and Smirnov I. P., *Genome Research,* 7:378-388, 1997), the disclosures of which are incorporated herein by reference in their entirety.

[0403] Microsequencing may be achieved by the established microsequencing method or by developments or derivatives thereof. Alternative methods include several solid-phase microsequencing techniques. The basic microsequencing protocol is the same as described previously, except that the method is conducted as a heterogenous phase assay, in which the primer or the target molecule is immobilized or captured onto a solid support. To simplify the primer separation and the terminal nucleotide addition analysis, oligonucleotides are attached to solid supports or are modified in such ways that permit affinity separation as well as polymerase extension. The 5' ends and internal nucleotides of synthetic oligonucleotides can be modified in a number of different ways to permit different affinity separation approaches, e.g., biotinylation. If a single affinity group is used on the oligonucleotides, the oligonucleotides can be separated from the incorporated terminator regent. This eliminates the need of physical or size separation. More than one oligonucleotide can be separated from the terminator reagent and analyzed simultaneously if more than one affinity group is used. This permits the analysis of several nucleic acid species or more nucleic acid sequence information per extension reaction. The affinity group need not be on the priming oligonucleotide but could alternatively be present on the template. For example, immobilization can be carried out via an interaction between biotinylated DNA and streptavidin-coated microtitration wells or avidin-coated polystyrene particles. In the same manner oligonucleotides or templates may be attached to a solid support in a high-density format. In such solid phase microsequencing reactions, incorporated ddNTPs can be radiolabeled (Syvänen, *Clinica Chimica Acta* 226:225-236, 1994) or linked to fluorescein (Livak and Hainer, *Human Mutation* 3:379-385, 1994), the disclosures of which are incorporated herein by reference in their entirety. The detection of radiolabeled ddNTPs can be achieved through scintillation-based techniques. The detection of fluorescein-linked ddNTPs can be based on the binding of antifluorescein antibody conjugated with alkaline phosphatase, followed by incubation with a chromogenic substrate (such as p-nitrophenyl phosphate). Other possible reporter-detection pairs include: ddNTP linked to dinitrophenyl (DNP) and anti-DNP alkaline phosphatase conjugate (Harju et al., *Clin. Chem.* 39/11 2282-2287, 1993) or biotinylated ddNTP and horseradish peroxidase-conjugated streptavidin with o-phenylenediamine as a substrate (WO 92/15712), the disclosures of which are incorporated herein by reference in their entirety. As yet another alternative solid-phase microsequencing procedure, Nyren et al. (*Analytical Biochemistry* 208:171-175, 1993) described a method relying on the detection of DNA polymerase activity by an enzymatic luminometric inorganic pyrophosphate detection assay (ELIDA).

[0404] Pastinen et al. (*Genome research* 7:606-614, 1997), the disclosure of which is incorporated herein by reference in its entirety, describe a method for multiplex detection of single nucleotide polymorphism in which the solid phase

minisequencing principle is applied to an oligonucleotide array format. High-density arrays of DNA probes attached to a solid support (DNA chips) are further described in III.C.5.

[0405] In one aspect the present invention provides polynucleotides and methods to genotype one or more biallelic markers of the present invention by performing a microsequencing assay. Preferred microsequencing primers include those being featured Table 16. It will be appreciated that the microsequencing primers listed in Table 16 are merely exemplary and that, any primer having a 3' end immediately adjacent to a polymorphic nucleotide may be used. Similarly, it will be appreciated that microsequencing analysis may be performed for any biallelic marker or any combination of biallelic markers of the present invention. One aspect of the present invention is a solid support which includes one or more microsequencing primers listed in Table 16, or fragments comprising at least 8, at least 12, at least 15, or at least 20 consecutive nucleotides thereof and having a 3' terminus immediately upstream of the corresponding biallelic marker, for determining the identity of a nucleotide at biallelic marker site.

[0406] Mismatch Detection Assays Based on Polymerases and Ligases

[0407] In one aspect the present invention provides polynucleotides and methods to determine the allele of one or more biallelic markers of the present invention in a biological sample, by mismatch detection assays based on polymerases and/or ligases. These assays are based on the specificity of polymerases and ligases. Polymerization reactions places particularly stringent requirements on correct base pairing of the 3' end of the amplification primer and the joining of two oligonucleotides hybridized to a target DNA sequence is quite sensitive to mismatches close to the ligation site, especially at the 3' end. The terms "enzyme based mismatch detection assay" are used herein to refer to any method of determining the allele of a biallelic marker based on the specificity of ligases and polymerases. Preferred methods are described below. Methods, primers and various parameters to amplify DNA fragments comprising biallelic markers of the present invention are further described above in III.B.

[0408] Allele Specific Amplification

[0409] Discrimination between the two alleles of a biallelic marker can also be achieved by allele specific amplification, a selective strategy, whereby one of the alleles is amplified without amplification of the other allele. This is accomplished by placing a polymorphic base at the 3' end of one of the amplification primers. Because the extension forms from the 3'end of the primer, a mismatch at or near this position has an inhibitory effect on amplification. Therefore, under appropriate amplification conditions, these primers only direct amplification on their complementary allele. Designing the appropriate allele-specific primer and the corresponding assay conditions are well with the ordinary skill in the art.

[0410] Ligation/Amplification Based Methods

[0411] The "Oligonucleotide Ligation Assay" (OLA) uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target molecules. One of the oligonucleotides is biotinylated, and the other is detectably labeled. If the precise comple-

mentary sequence is found in a target molecule, the oligonucleotides will hybridize such that their termini abut, and create a ligation substrate that can be captured and detected. OLA is capable of detecting biallelic markers and may be advantageously combined with PCR as described by Nickerson D. A. et al. (*Proc. Natl. Acad. Sci. U.S.A.* 87:8923-8927, 1990)., the disclosure of which is incorporated herein by reference in its entirety In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA.

[0412] Other methods which are particularly suited for the detection of biallelic markers include LCR (ligase chain reaction), Gap LCR (GLCR) which are described above in III.B. As mentioned above LCR uses two pairs of probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides, is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependant ligase. In accordance with the present invention, LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a biallelic marker site. In one embodiment, either oligonucleotide will be designed to include the biallelic marker site. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide(s) that is complementary to the biallelic marker on the oligonucleotide. In an alternative embodiment, the oligonucleotides will not include the biallelic marker, such that when they hybridize to the target molecule, a "gap" is created as described in WO 90/01069, the disclosure of which is incorporated herein by reference in its entirety. This gap is then "filled" with complementary dNTPs (as mediated by DNA polymerase), or by an additional pair of oligonucleotides. Thus at the end of each cycle, each single strand has a complement capable of serving as a target during the next cycle and exponential allele-specific amplification of the desired sequence is obtained.

[0413] Ligase/Polymerase-mediated Genetic Bit Analysis™ is another method for determining the identity of a nucleotide at a preselected site in a nucleic acid molecule (WO 95/21271), the disclosure of which is incorporated herein by reference in its entirety. This method involves the incorporation of a nucleoside triphosphate that is complementary to the nucleotide present at the preselected site onto the terminus of a primer molecule, and their subsequent ligation to a second oligonucleotide. The reaction is monitored by detecting a specific label attached to the reaction's solid phase or by detection in solution.

[0414] Hybridization Assay Methods

[0415] A preferred method of determining the identity of the nucleotide present at a biallelic marker site involves nucleic acid hybridization. The hybridization probes, which can be conveniently used in such reactions, preferably include the probes defined herein. Any hybridization assay may be used including Southern hybridization, Northern hybridization, dot blot hybridization and solid-phase hybridization (see Sambrook et al., Molecular Cloning—A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989), the disclosure of which is incorporated herein by reference in its entirety.

[0416] Hybridization refers to the formation of a duplex structure by two single stranded nucleic acids due to

complementary base pairing. Hybridization can occur between exactly complementary nucleic acid strands or between nucleic acid strands that contain minor regions of mismatch. Specific probes can be designed that hybridize to one form of a biallelic marker and not to the other and therefore are able to discriminate between different allelic forms. Allele-specific probes are often used in pairs, one member of a pair showing perfect match to a target sequence containing the original allele and the other showing a perfect match to the target sequence containing the alternative allele. Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the alleles. Stringent, sequence specific hybridization conditions, under which a probe will hybridize only to the exactly complementary target sequence are well known in the art (Sambrook et al., Molecular Cloning—A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989), the disclosures of which are incorporated herein by reference in their entirety. Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5° C. lower than the thennal melting point (Tm) for the specific sequence at a defined ionic strength and pH. By way of example and not limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65 C in buffer composed of 6×SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65° C., the preferred hybridization temperature, in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20×10^6 cpm of ^{32}P-labeled probe. Alternatively, the hybridization step can be performed at 65° C. in the presence of SSC buffer, 1×SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37° C. for 1 h in a solution containing 2×SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1×SSC at 50° C. for 45 min. Alternatively, filter washes can be performed in a solution containing 2×SSC and 0.1% SDS, or 0.5×SSC and 0.1% SDS, or 0.1×SSC and 0.1% SDS at 68° C. for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. By way of example and not limitation, procedures using conditions of intermediate stringency are as follows: Filters containing DNA are prehybridized, and then hybridized at a temperature of 60° C. in the presence of a 5×SSC buffer and labeled probe. Subsequently, filters washes are performed in a solution containing 2×SSC at 50° C. and the hybridized probes are detectable by autoradiography. Other conditions of high and intermediate stringency which may be used are well known in the art and as cited in Sambrook et al. (Molecular Cloning—A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., 1989) and Ausubel et al. (Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y., 1989).

[0417] Although such hybridizations can be performed in solution, it is preferred to employ a solid-phase hybridization assay. The target DNA comprising a biallelic marker of the present invention may be amplified prior to the hybridization reaction. The presence of a specific allele in the sample is determined by detecting the presence or the absence of stable hybrid duplexes formed between the probe and the target DNA. The detection of hybrid duplexes can be carried out by a number of methods. Various detection assay formats are well known which utilize detectable labels bound to either the target or the probe to enable detection of the hybrid duplexes. Typically, hybridization duplexes are separated from unhybridized nucleic acids and the labels bound to the duplexes are then detected. Those skilled in the art will recognize that wash steps may be employed to wash away excess target DNA or probe. Standard heterogeneous assay formats are suitable for detecting the hybrids using the labels present on the primers and probes.

[0418] Two recently developed assays allow hybridization-based allele discrimination with no need for separations or washes (see Landegren U. et al., Genome Research, 8:769-776,1998), the disclosure of which is incorporated herein by reference in its entirety. The TaqMan assay takes advantage of the 5' nuclease activity of Taq DNA polymerase to digest a DNA probe annealed specifically to the accumulating amplification product. TaqMan probes are labeled with a donor-acceptor dye pair that interacts via fluorescence energy transfer. Cleavage of the TaqMan probe by the advancing polymerase during amplification dissociates the donor dye from the quenching acceptor dye, greatly increasing the donor fluorescence. All reagents necessary to detect two allelic variants can be assembled at the beginning of the reaction and the results are monitored in real time (see Livak et al., Nature Genetics, 9:341-342, 1995), the disclosure of which is incorporated herein by reference in its entirety. In an alternative homogeneous hybridization-based procedure, molecular beacons are used for allele discriminations. Molecular beacons are hairpin-shaped oligonucleotide probes that report the presence of specific nucleic acids in homogeneous solutions. When they bind to their targets they undergo a conformational reorganization that restores the fluorescence of an internally quenched fluorophore (Tyagi et al., Nature Biotechnology, 16:49-53, 1998)., the disclosure of which is incorporated herein by reference in its entirety

[0419] The polynucleotides provided herein can be used in hybridization assays for the detection of biallelic marker alleles in biological samples. These probes are characterized in that they preferably comprise between 8 and 50 nucleotides, and in that they are sufficiently complementary to a sequence comprising a biallelic marker of the present invention to hybridize thereto and preferably sufficiently specific to be able to discriminate the targeted sequence for only one nucleotide variation. The GC content in the probes of the invention usually ranges between 10 and 75%, preferably between 35 and 60%, and more preferably between 40 and 55%. The length of these probes can range from 10, 15, 20, or 30 to at least 100 nucleotides, preferably from 10 to 50, more preferably from 18 to 35 nucleotides. A particularly preferred probe is 25 nucleotides in length. Preferably the biallelic marker is within 4 nucleotides of the center of the polynucleotide probe. In particularly preferred probes the biallelic marker is at the center of said polynucleotide. Shorter probes may lack specificity for a target nucleic acid sequence and generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. Longer probes are expensive to produce and can sometimes self-hybridize to form hairpin structures. Methods for the

synthesis of oligonucleotide probes have been described above and can be applied to the probes of the present invention.

[0420] Preferably the probes of the present invention are labeled or immobilized on a solid support. Labels and solid supports are further described in I. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example peptide nucleic acids which are disclosed in International Patent Application WO 92/20702, d, morpholino analogs which are described in U.S. Pat. Nos. 5,185,444; 5,034,506 and 5,142,047, the disclosures of which are incorporated herein by reference in their entirety. The probe may have to be rendered "non-extendable" in that additional dNTPs cannot be added to the probe. In and of themselves analogs usually are non-extendable and nucleic acid probes can be rendered non-extendable by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified, U.S. patent application Ser. No. 07/049,061 filed Apr. 19, 1993 describes modifications, which can be used to render a probe non-extendable.

[0421] The probes of the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA or Northern hybridization to mRNA. The probes can also be used to detect PCR amplification products. By assaying the hybridization to an allele specific probe, one can detect the presence or absence of a biallelic marker allele in a given sample.

[0422] High-Throughput parallel hybridizations in array format are specifically encompassed within "hybridization assays" and are described below.

[0423] Hybridization to Addressable Arrays of Oligonucleotides

[0424] Hybridization assays based on oligonucleotide arrays rely on the differences in hybridization stability of short oligonucleotides to perfectly matched and mismatched target sequence variants. Efficient access to polymorphism information is obtained through a basic structure comprising high-density arrays of oligonucleotide probes attached to a solid support (the chip) at selected positions. Each DNA chip can contain thousands to millions of individual synthetic DNA probes arranged in a grid-like pattern and miniaturized to the size of a dime.

[0425] The chip technology has already been applied with success in numerous cases. For example, the screening of mutations has been undertaken in the BRCA1 gene, in *S. cerevisiae* mutant strains, and in the protease gene of HIV-1 virus (Hacia et al., *Nature Genetics,* 14(4):441-447, 1996; Shoemaker et al., *Nature Genetics,* 14(4):450-456, 1996; Kozal et al., *Nature Medicine,* 2:753-759, 1996) the disclosures of which are incorporated herein by reference in their entirety. Chips of various formats for use in detecting biallelic polymorphisms can be produced on a customized basis by Affymetrix (GeneChip™), Hyseq (HyChip and HyGnostics), and Protogene Laboratories.

[0426] In general, these methods employ arrays of oligonucleotide probes that are complementary to target nucleic acid sequence segments from an individual which, target

sequences include a polymorphic marker. EP785280, the disclosure of which is incorporated herein by reference in its entirety, describes a tiling strategy for the detection of single nucleotide polymorphisms. Briefly, arrays may generally be "tiled" for a large number of specific polymorphisms. By "tiling" is generally meant the synthesis of a defined set of oligonucleotide probes which is made up of a sequence complementary to the target sequence of interest, as well as preselected variations of that sequence, e.g., substitution of one or more given positions with one or more members of the basis set of monomers, i.e. nucleotides. Tiling strategies are further described in PCT application No. WO 95/11995, the disclosure of which is incorporated herein by reference in its entirety. In a particular aspect, arrays are tiled for a number of specific, identified biallelic marker sequences. In particular the array is tiled to include a number of detection blocks, each detection block being specific for a specific biallelic marker or a set of biallelic markers. For example, a detection block may be tiled to include a number of probes, which span the sequence segment that includes a specific polymorphism. To ensure probes that are complementary to each allele, the probes are synthesized in pairs differing at the biallelic marker. In addition to the probes differing at the polymorphic base, monosubstituted probes are also generally tiled within the detection block. These monosubstituted probes have bases at and up to a certain number of bases in either direction from the polymorphism, substituted with the remaining nucleotides (selected from A, T, G, C and U). Typically the probes in a tiled detection block will include substitutions of the sequence positions up to and including those that are 5 bases away from the biallelic marker. The monosubstituted probes provide internal controls for the tiled array, to distinguish actual hybridization from artefactual cross-hybridization. Upon completion of hybridization with the target sequence and washing of the array, the array is scanned to determine the position on the array to which the target sequence hybridizes. The hybridization data from the scanned array is then analyzed to identify which allele or alleles of the biallelic marker are present in the sample. Hybridization and scanning may be carried out as described in PCT application No. WO 92/10092 and WO 95/11995 and U.S. Pat. No. 5,424,186, the disclosures of which are incorporated herein by reference in their entirety.

[0427] Thus, in some embodiments, the chips may comprise an array of nucleic acid sequences of fragments of about 15 nucleotides in length. In further embodiments, the chip may comprise an array including at least one of the sequences selected from the group consisting of SEQ ID No. 1-38, 40-54, 56-463, 465-487, 490-493 and the sequences complementary thereto; preferably SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, 958-977 and the sequences complementary thereto; or a fragment thereof at least about 8 consecutive nucleotides, preferably 10, 15, 20, more preferably 25, 30, 40, 47, or 50 consecutive nucleotides. In some embodiments, the chip may comprise an array of at least 2, 3, 4, 5, 6, 7, 8 or more of these polynucleotides of the invention. Solid supports and polynucleotides of the present invention attached to solid supports are further described in I. "Biallelic Markers and Polynucleotides Comprising Biallelic Markers."

[0428] Integrated Systems

[0429] Another technique, which may be used to analyze polymorphisms, includes multicomponent integrated sys-

tems, which miniaturize and compartmentalize processes such as PCR and capillary electrophoresis reactions in a single functional device. An example of such technique is disclosed in U.S. Pat. No. 5,589,136, the disclosure of which is incorporated herein by reference in its entirety, which describes the integration of PCR amplification and capillary electrophoresis in chips.

[0430] Integrated systems can be envisaged mainly when microfluidic systems are used. These systems comprise a pattern of microchannels designed onto a glass, silicon, quartz, or plastic wafer included on a microchip. The movements of the samples are controlled by electric, electroosmotic or hydrostatic forces applied across different areas of the microchip. For genotyping biallelic markers, the microfluidic system may integrate nucleic acid amplification, microsequencing, capillary electrophoresis and a detection method such as laser-induced fluorescence detection.

[0431] IV. Methods of Genetic Analysis Using the Biallelic Markers of the Present Invention

[0432] Different methods are available for the genetic analysis of complex traits (see Lander and Schork, *Science*, 265, 2037-2048, 1994), the disclosure of which is incorporated herein by reference in its entirety. The search for disease-susceptibility genes is conducted using two main methods: the linkage approach in which evidence is sought for cosegregation between a locus and a putative trait locus using family studies, and the association approach in which evidence is sought for a statistically significant association between an allele and a trait or a trait causing allele (Khoury J. et al., *Fundamentals of Genetic Epidemiology*, Oxford University Press, NY, 1993), the disclosure of which is incorporated herein by reference in its entirety. In general, the biallelic markers of the present invention find use in any method known in the art to demonstrate a statistically significant correlation between a genotype and a phenotype. The biallelic markers may be used in parametric and nonparametric linkage analysis methods. Preferably, the biallelic markers of the present invention are used to identify genes associated with detectable traits using association studies, an approach which does not require the use of affected families and which permits the identification of genes associated with complex and sporadic traits.

[0433] The genetic analysis using the biallelic markers of the present invention may be conducted on any scale. The whole set of biallelic markers of the present invention or any subset of biallelic markers of the present invention may be used. In some embodiments a subset of biallelic markers corresponding to one or several candidate genes of the present invention may be used. In other embodiments a subset of biallelic markers corresponding to candidate genes from a given metabolic pathway may be used. Such pathways include glucoronidation and glutathione conjugation. Alternatively, a subset of biallelic markers of the present invention localised on a specific chromosome segment may be used. Further, any set of genetic markers including a biallelic marker of the present invention may be used. A set of biallelic polymorphisms that, could be used as genetic markers in combination with the biallelic markers of the present invention, has been described in WO 98/20165, the disclosure of which is incorporated herein by reference in its entirety. As mentioned above, the biallelic markers of the present invention may be included in any complete or partial genetic map of the human genome. These different uses are specifically contemplated in the present invention and claims.

[0434] A. Linkage Analysis

[0435] Linkage analysis is based upon establishing a correlation between the transmission of genetic markers and that of a specific trait throughout generations within a family. Thus, the aim of linkage analysis is to detect marker loci that show cosegregation with a trait of interest in pedigrees.

[0436] Parametric Methods

[0437] When data are available from successive generations there is the opportunity to study the degree of linkage between pairs of loci. Estimates of the recombination fraction enable loci to be ordered and placed onto a genetic map. With loci that are genetic markers, a genetic map can be established, and then the strength of linkage between markers and traits can be calculated and used to indicate the relative positions of markers and genes affecting those traits (Weir, B. S., *Genetic data Analysis II. Methods for Discrete population genetic Data*, Sinauer Assoc., Inc., Sunderland, Mass., USA, 1996), the disclosure of which is incorporated herein by reference in its entirety. The classical method for linkage analysis is the logarithm of odds (lod) score method (see Morton N.E., *Am. J. Hum. Genet.*, 7:277-318, 1955; Ott J., *Analysis of Human Genetic Linkage*, John Hopkins University Press, Baltimore, 1991), the disclosures of which are incorporated herein by reference in their entirety. Calculation of lod scores requires specification of the mode of inheritance for the disease (parametric method). Generally, the length of the candidate region identified using linkage analysis is between 2 and 20 Mb. Once a candidate region is identified as described above, analysis of recombinant individuals using additional markers allows further delineation of the candidate region. Linkage analysis studies have generally relied on the use of a maximum of 5,000 microsatellite markers, thus limiting the maximum theoretical attainable resolution of linkage analysis to about 600 kb on average.

[0438] Linkage analysis has been successfully applied to map simple genetic traits that show clear Mendelian inheritance patterns and have a high penetrance (i.e., the ratio between the number of trait positive carriers of allele a and the total number of a carriers in the population). However, parametric linkage analysis suffers from a variety of drawbacks. First, it is limited by its reliance on the choice of a genetic model suitable for each studied trait. Furthermore, as already mentioned, the resolution attainable using linkage analysis is limited, and complementary studies are required to refine the analysis of the typical 2 Mb to 20 Mb regions initially identified through linkage analysis. In addition, parametric linkage analysis approaches have proven difficult when applied to complex genetic traits, such as those due to the combined action of multiple genes and/or environmental factors. It is very difficult to model these factors adequately in a lod score analysis. In such cases, too large an effort and cost are needed to recruit the adequate number of affected families required for applying linkage analysis to these situations, as recently discussed by Risch, N. and Merikangas, K. (*Science*, 273:1516-1517, 1996), the disclosure of which is incorporated herein by reference in its entirety.

[0439] Non-Parametric Methods

[0440] The advantage of the so-called non-parametric methods for linkage analysis is that they do not require specification of the mode of inheritance for the disease, they tend to be more useful for the analysis of complex traits. In non-parametric methods, one tries to prove that the inheritance pattern of a chromosomal region is not consistent with random Mendelian segregation by showing that affected relatives inherit identical copies of the region more often than expected by chance. Affected relatives should show excess "allele sharing" even in the presence of incomplete penetrance and polygenic inheritance. In non-parametric linkage analysis the degree of agreement at a marker locus in two individuals can be measured either by the number of alleles identical by state (IBS) or by the number of alleles identical by descent (IBD). Affected sib pair analysis is a well-known special case and is the simplest form of these methods.

[0441] The biallelic markers of the present invention may be used in both parametric and non-parametric linkage analysis. Preferably biallelic markers may be used in non-parametric methods which allow the mapping of genes involved in complex traits. The biallelic markers of the present invention may be used in both IBD- and IBS-methods to map genes affecting a complex trait. In such studies, taking advantage of the high density of biallelic markers, several adjacent biallelic marker loci may be pooled to achieve the efficiency attained by multi-allelic markers (Zhao et al., *Am. J. Hum. Genet.,* 63:225-240, 1998), the disclosure of which is incorporated herein by reference in its entirety.

[0442] However, both parametric and non-parametric linkage analysis methods analyse affected relatives, they tend to be of limited value in the genetic analysis of drug responses or in the analysis of side effects to treatments. This type of analysis is impractical in such cases due to the lack of availability of familial cases. In fact, the likelihood of having more than one individual in a family being exposed to the same drug at the same time is extremely low.

[0443] B. Population Association Studies

[0444] The present invention comprises methods for identifying one or several genes among a set of candidate genes that are associated with a detectable trait using the biallelic markers of the present invention. In one embodiment the present invention comprises methods to detect an association between a biallelic marker allele or a biallelic marker haplotype and a trait. Further, the invention comprises methods to identify a trait causing allele in linkage disequilibrium with any biallelic marker allele of the present invention.

[0445] As described above, alternative approaches can be employed to perform association studies: genome-wide association studies, candidate region association studies and candidate gene association studies. In a preferred embodiment, the biallelic markers of the present invention are used to perform candidate gene association studies. The candidate gene analysis clearly provides a short-cut approach to the identification of genes and gene polymorphisms related to a particular trait when some information concerning the biology of the trait is available. Further, the biallelic markers of the present invention may be incorporated in any map of

genetic markers of the human genome in order to perform genome-wide association studies. Methods to generate a high-density map of biallelic markers has been described in U.S. Provisional Patent application serial No. 60/082,614. The biallelic markers of the present invention may further be incorporated in any map of a specific candidate region of the genome (a specific chromosome or a specific chromosomal segment for example).

[0446] As mentioned above, association studies may be conducted within the general population and are not limited to studies performed on related individuals in affected families. Association studies are extremely valuable as they permit the analysis of sporadic or multifactor traits. Moreover, association studies represent a powerful method for fine-scale mapping enabling much finer mapping of trait causing alleles than linkage studies. Studies based on pedigrees often only narrow the location of the trait causing allele. Association studies using the biallelic markers of the present invention can therefore be used to refine the location of a trait causing allele in a candidate region identified by Linkage Analysis methods. Moreover, once a chromosome segment of interest has been identified, the presence of a candidate gene such as a candidate gene of the present invention, in the region of interest can provide a shortcut to the identification of the trait causing allele. Biallelic markers of the present invention can be used to demonstrate that a candidate gene is associated with a trait. Such uses are specifically contemplated in the present invention and claims.

[0447] Determining the Frequency of a Biallelic Marker Allele or of a Biallelic Marker Haplotype in a Population

[0448] Association studies explore the relationships among frequencies for sets of alleles between loci.

[0449] Determining the Frequency of an Allele in a Population

[0450] Allelic frequencies of the biallelic markers in a population can be determined using one of the methods described above under the heading "Methods for genotyping an individual for biallelic markers", or any genotyping procedure suitable for this intended purpose. Genotyping pooled samples or individual samples can determine the frequency of a biallelic marker allele in a population. One way to reduce the number of genotypings required is to use pooled samples. A major obstacle in using pooled samples is in terms of accuracy and reproducibility for determining accurate DNA concentrations in setting up the pools. Genotyping individual samples provides higher sensitivity, reproducibility and accuracy and; is the preferred method used in the present invention. Preferably, each individual is genotyped separately and simple gene counting is applied to determine the frequency of an allele of a biallelic marker or of a genotype in a given population.

[0451] Determining the Frequency of a Haplotype in a Population

[0452] The gametic phase of haplotypes is unknown when diploid individuals are heterozygous at more than one locus. Using genealogical information in families gametic phase can sometimes be inferred (Perlin et al., *Am. J. Hum. Genet.,* 55:777-787, 1994), the disclosure of which is incorporated herein by reference in its entirety. When no genealogical information is available different strategies may be used. One possibility is that the multiple-site heterozygous diploids can be eliminated from the analysis, keeping only the

homozygotes and the single-site heterozygote individuals, but this approach might lead to a possible bias in the sample composition and the underestimation of low-frequency haplotypes. Another possibility is that single chromosomes can be studied independently, for example, by asymmetric PCR amplification (see Newton et al., *Nucleic Acids Res.*, 17:2503-2516, 1989; Wu et al., *Proc. Natl. Acad. Sci. USA*, 86:2757, 1989), the disclosures of which are incorporated herein by reference in their entirety, or by isolation of single chromosome by limit dilution followed by PCR amplification (see Ruano et al., *Proc. Natl. Acad. Sci. USA*, 87:6296-6300, 1990), the disclosure of which is incorporated herein by reference in its entirety. Further, a sample may be haplotyped for sufficiently close biallelic markers by double PCR amplification of specific alleles (Sarkar, G. and Sommer S. S., *Biotechniques*, 1991), the disclosure of which is incorporated herein by reference in its entirety. These approaches are not entirely satisfying either because of their technical complexity, the additional cost they entail, their lack of generalisation at a large scale, or the possible biases they introduce. To overcome these difficulties, an algorithm to infer the phase of PCR-amplified DNA genotypes introduced by Clark A. G. (*Mol. Biol. Evol.*, 7:111-122, 1990), the disclosure of which is incorporated herein by reference in its entirety, may be used. Briefly, the principle is to start filling a preliminary list of haplotypes present in the sample by examining unambiguous individuals, that is, the complete homozygotes and the single-site heterozygotes. Then other individuals in the same sample are screened for the possible occurrence of previously recognised haplotypes. For each positive identification, the complementary haplotype is added to the list of recognised haplotypes, until the phase information for all individuals is either resolved or identified as unresolved. This method assigns a single haplotype to each multiheterozygous individual, whereas several haplotypes are possible when there are more than one heterozygous site. Alternatively, one can use methods estimating haplotype frequencies in a population without assigning haplotypes to each individual. Preferably, a method based on an expectation-maximization (EM) algorithm (Dempster et al., *J. R. Stat. Soc.*, 39B: 1-38, 1977), the disclosure of which is incorporated herein by reference in its entirety, leading to maximum-likelihood estimates of haplotype frequencies under the assumption of Hardy-Weinberg proportions (random mating) is used (see Excoffier L. and Slatkin M., *Mol. Biol. Evol.*, 12(5): 921-927, 1995), the disclosure of which is incorporated herein by reference in its entirety. The EM algorithm is a generalised iterative maximum-likelihood approach to estimation that is useful when data are ambiguous and/or incomplete. The EM algorithm is used to resolve heterozygotes into haplotypes. Haplotype estimations are further described below under the heading "Statistical methods." Any other method known in the art to determine or to estimate the frequency of a haplotype in a population may also be used.

[0453] Linkage Disequilibrium Analysis

[0454] Linkage disequilibrium is the non-random association of alleles at two or more loci and represents a powerful tool for mapping genes involved in disease traits (see Ajioka R. S. et al., *Am. J. Hum. Genet.*, 60:1439-1447, 1997), the disclosure of which is incorporated herein by reference in its entirety. Biallelic markers, because they are densely spaced in the human genome and can be genotyped in more numerous numbers than other types of genetic markers (such as RFLP or VNTR markers), are particularly useful in genetic analysis based on linkage disequilibrium. The biallelic markers of the present invention may be used in any linkage disequilibrium analysis method known in the art.

[0455] Briefly, when a disease mutation is first introduced into a population (by a new mutation or the immigration of a mutation carrier), it necessarily resides on a single chromosome and thus on a single "background" or "ancestral" haplotype of linked markers. Consequently, there is complete disequilibrium between these markers and the disease mutation: one finds the disease mutation only in the presence of a specific set of marker alleles. Through subsequent generations recombinations occur between the disease mutation and these marker polymorphisms, and the disequilibrium gradually dissipates. The pace of this dissipation is a function of the recombination frequency, so the markers closest to the disease gene will manifest higher levels of disequilibrium than those that are further away. When not broken up by recombination, "ancestral" haplotypes and linkage disequilibrium between marker alleles at different loci can be tracked not only through pedigrees but also through populations. Linkage disequilibrium is usually seen as an association between one specific allele at one locus and another specific allele at a second locus.

[0456] The pattern or curve of disequilibrium between disease and marker loci is expected to exhibit a maximum that occurs at the disease locus. Consequently, the amount of linkage disequilibrium between a disease allele and closely linked genetic markers may yield valuable information regarding the location of the disease gene. For fine-scale mapping of a disease locus, it is useful to have some knowledge of the patterns of linkage disequilibrium that exist between markers in the studied region. As mentioned above the mapping resolution achieved through the analysis of linkage disequilibrium is much higher than that of linkage studies. The high density of biallelic markers combined with linkage disequilibrium analysis provides powerful tools for fine-scale mapping. Different methods to calculate linkage disequilibrium are described below under the heading "Statistical Methods."

[0457] Population-Based Case-Control Studies of Trait-Marker Associations

[0458] As mentioned above, the occurrence of pairs of specific alleles at different loci on the same chromosome is not random and the deviation from random is called linkage disequilibrium. Association studies focus on population frequencies and rely on the phenomenon of linkage disequilibrium. If a specific allele in a given gene is directly involved in causing a particular trait, its frequency will be statistically increased in an affected (trait positive) population, when compared to the frequency in a trait negative population or in a random control population. As a consequence of the existence of linkage disequilibrium, the frequency of all other alleles present in the haplotype carrying the trait-causing allele will also be increased in trait positive individuals compared to trait negative individuals or random controls. Therefore, association between the trait and any allele (specifically a biallelic marker allele) in linkage disequilibrium with the trait-causing allele will suffice to suggest the presence of a trait-related gene in that particular region. Case-control populations can be genotyped for biallelic markers to identify associations that narrowly locate a

trait causing allele. As any marker in linkage disequilibrium with one given marker associated with a trait will be associated with the trait. Linkage disequilibrium allows the relative frequencies in case-control populations of a limited number of genetic polymorphisms (specifically biallelic markers) to be analysed as an alternative to screening all possible functional polymorphisms in order to find trait-causing alleles. Association studies compare the frequency of marker alleles in unrelated case-control populations, and represent powerful tools for the dissection of complex traits.

[0459] Case-Control Populations (Inclusion Criteria)

[0460] Population-based association studies do not concern familial inheritance but compare the prevalence of a particular genetic marker, or a set of markers, in case-control populations. They are case-control studies based on comparison of unrelated case (affected or trait positive) individuals and unrelated control (unaffected or trait negative or random) individuals. Preferably the control group is composed of unaffected or trait negative individuals. Further, the control group is ethnically matched to the case population. Moreover, the control group is preferably matched to the case-population for the main known confusion factor for the trait under study (for example age-matched for an age-dependent trait). Ideally, individuals in the two samples are paired in such a way that they are expected to differ only in their disease status. In the following "trait positive population", "case population" and "affected population" are used interchangeably.

[0461] An important step in the dissection of complex traits using association studies is the choice of case-control populations (see Lander and Schork, *Science,* 265, 2037-2048, 1994), the disclosure of which is incorporated herein by reference in its entirety. A major step in the choice of case-control populations is the clinical definition of a given trait or phenotype. Any genetic trait may be analysed by the association method proposed here by carefully selecting the individuals to be included in the trait positive and trait negative phenotypic groups. Four criteria are often useful: clinical phenotype, age at onset, family history and severity. The selection procedure for continuous or quantitative traits (such as blood pressure for example) involves selecting individuals at opposite ends of the phenotype distribution of the trait under study, so as to include in these trait positive and trait negative populations individuals with non-overlapping phenotypes. Preferably, case-control populations consist of phenotypically homogeneous populations. Trait positive and trait negative populations consist of phenotypically uniform populations of individuals representing each between 1 and 98%, preferably between 1 and 80%, more preferably between 1 and 50%, and more preferably between 1 and 30%, most preferably between 1 and 20% of the total population under study, and selected among individuals exhibiting non-overlapping phenotypes. The clearer the difference between the two trait phenotypes, the greater the probability of detecting an association with biallelic markers. The selection of those drastically different but relatively uniform phenotypes enables efficient comparisons in association studies and the possible detection of marked differences at the genetic level, provided that the sample sizes of the populations under study are significant enough.

[0462] In preferred embodiments, a first group of between 50 and 300 trait positive individuals, preferably about 100

individuals, are recruited according to their phenotypes. A similar number of trait negative individuals are included in such studies.,

[0463] In the present invention, typical examples of inclusion criteria include a disease involving the metabolic conversion of xenobiotics or the evaluation of the response to a drug or side effects to treatment with drugs.

[0464] Suitable examples of association studies using biallelic markers including the biallelic markers of the present invention, are studies involving the following populations:

[0465] a case population suffering from a disease involving the metabolic conversion of xenobiotics and a healthy unaffected control population, or

[0466] a case population treated with therapeutic agents suffering from side-effects resulting from the treatment and a control population treated with the same agents showing no side-effects, or

[0467] a case population treated with therapeutic agents showing a beneficial response and a control population treated with same agents showing no beneficial response.

[0468] In a preferred embodiment, the trait considered was a side-effect upon drug treatment, the study involved two populations derived from a clinical study of the anti-asthmatic drug zileuton. The case population was composed of asthmatic individuals treated with zileuton showing zileuton-associated hepatotoxicity monitored by the serum level of alanine aminotransferase (ALT) and the control population was composed of asthmatic individuals treated with zileuton and having no increased serum level of ALT. Inclusion criteria and association between the biallelic markers of the present invention and zileuton-associated hepatotoxicity are further described below and in Example 4.

[0469] Association Analysis

[0470] The general strategy to perform association studies using biallelic markers derived from a region carrying a candidate gene is to scan two groups of individuals (case-control populations) in order to measure and statistically compare the allele frequencies of the biallelic markers of the present invention in both groups.

[0471] If a statistically significant association with a trait is identified for at least one or more of the analysed biallelic markers, one can assume that: either the associated allele is directly responsible for causing the trait (the associated allele is the trait causing allele), or more likely the associated allele is in linkage disequilibrium with the trait causing allele. The specific characteristics of the associated allele with respect to the candidate gene function usually gives further insight into the relationship between the associated allele and the trait (causal or in linkage disequilibrium). If the evidence indicates that the associated allele within the candidate gene is most probably not the trait causing allele but is in linkage disequilibrium with the real trait causing allele, then the trait causing allele can be found by sequencing the vicinity of the associated marker.

[0472] Association studies are usually run in two successive steps. In a first phase, the frequencies of a reduced number of biallelic markers from one or several candidate genes are determined in the trait positive and trait negative

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populations. In a second phase of the analysis, the identity of the candidate gene and the position of the genetic loci responsible for the given trait is further refined using a higher density of markers from the relevant region. However, if the candidate gene under study is relatively small in length, as it is the case for many of the candidate genes analysed included in the present invention, a single phase may be sufficient to establish significant associations.

[0473] Haplotype Analysis

[0474] As described above, when a chromosome carrying a disease allele first appears in a population as a result of either mutation or migration, the mutant allele necessarily resides on a chromosome having a set of linked markers: the ancestral haplotype. This haplotype can be tracked through populations and its statistical association with a given trait can be analysed. Complementing single point (allelic) association studies with multi-point association studies also called haplotype studies increases the statistical power of association studies. Thus, a haplotype association study allows one to define the frequency and the type of the ancestral carrier haplotype. A haplotype analysis is important in that it increases the statistical power of an analysis involving individual markers.

[0475] In a first stage of a haplotype frequency analysis, the frequency of the possible haplotypes based on various combinations of the identified biallelic markers of the invention is determined. The haplotype frequency is then compared for distinct populations of trait positive and control individuals. The number of trait positive individuals, which should be, subjected to this analysis to obtain statistically significant results usually ranges between 30 and 300, with a preferred number of individuals ranging between 50 and 150. The same considerations apply to the number of unaffected individuals (or random control) used in the study. The results of this first analysis provide haplotype frequencies in case-control populations, for each evaluated haplotype frequency a p-value and an odd ratio are calculated. If a statistically significant association is found, the relative risk for an individual carrying the given haplotype of being affected with the trait under study can be approximated.

[0476] The preferred 2, 3 and 4 marker haplotypes of the invention are listed in Table 4.

[0477] The most preferred 2, 3 and 4 marker haplotypes of the invention are listed in Table 5.

[0478] Interaction Analysis

[0479] The biallelic markers of the present invention may also be used to identify patterns of biallelic markers associated with detectable traits resulting from polygenic interactions. The analysis of genetic interaction between alleles at unlinked loci requires individual genotyping using the techniques described herein. The analysis of allelic interaction among a selected set of biallelic markers with appropriate level of statistical significance can be considered as a haplotype analysis. Interaction analysis consists in stratifying the case-control populations with respect to a given haplotype for the first loci and performing a haplotype analysis with the second loci with each subpopulation.

[0480] Statistical methods used in association studies are further described below in "Statistical Methods."

[0481] Testing for Linkage in the Presence of Association

[0482] The biallelic markers of the present invention may further be used in TDT (transmission/disequilibrium test).

TDT tests for both linkage and association and is not affected by population stratification. TDT requires data for affected individuals and their parents or data from unaffected sibs instead of from parents (see Spielmann S. et al., *Am. J. Hum. Genet.,* 52:506-516, 1993; Schaid D. J. et al., *Genet. Epidemiol.,* 13:423-450, 1996, Spielmann S. and Ewens W. J., *Am. J. Hum. Genet.,* 62:450-458, 1998), the disclosures of which are incorporated herein by reference in their entirety. Such combined tests generally reduce the false-positive errors produced by separate analyses.

[0483] C. Statistical Methods

[0484] In general, any method known in the art to test whether a trait and a genotype show a statistically significant correlation may be used.

[0485] Methods in Linkage Analysis

[0486] Statistical methods and computer programs useful for linkage analysis are well known to those skilled in the art (see Terwilliger J. D. and Ott J., *Handbook of Human Genetic Linkage,* John Hopkins University Press, London, 1994; Ott J., *Analysis of Human Genetic Linkage,* John Hopkins University Press, Baltimore, 1991), the disclosures of which are incorporated herein by reference in their entirety.

[0487] Methods to Estimate Haplotype Frequencies in a Population

[0488] As described above, when genotypes are scored, it is often not possible to distinguish heterozygotes so that haplotype frequencies cannot be easily inferred. When the gametic phase is not known, haplotype frequencies can be estimated from the multilocus genotypic data. Any method known to person skilled in the art can be used to estimate haplotype frequencies (see Lange K., *Mathematical and Statistical Methods for Genetic Analysis,* Springer, New York, 1997; Weir, B. S., *Genetic data Analysis II: Methods for Discrete population genetic Data,* Sinauer Assoc., Inc., Sunderland, Mass., USA, 1996), the disclosures of which are incorporated herein by reference in their entirety. Preferably, maximum-likelihood haplotype frequencies are computed using an Expectation-Maximization (EM) algorithm (see Dempster et al., *J. R. Stat. Soc.,* 39B: 1-38, 1977; Excoffier L. and Slatkin M., *Mol. Biol. Evol.,* 12(5): 921-927, 1995)., the disclosures of which are incorporated herein by reference in their entirety. This procedure is an iterative process aiming at obtaining maximum-likelihood estimates of haplotype frequencies from multi-locus genotype data when the gametic phase is unknown. Haplotype estimations are usually performed by applying the EM algorithm using, for example, the EM-HAPLO program (Hawley M. E. et al., *Am. J. Phys. Anthropol.,* 18:104, 1994) or the Arlequin program (Schneider et al., *Arlequin: a software for population genetics data analysis,* University of Geneva, 1997), the disclosure of which is incorporated herein by reference in its entirety. The EM algorithm is a generalised iterative maximum likelihood approach to estimation and is briefly described below.

[0489] In what follows, phenotypes will refer to multi-locus genotypes with unknown haplotypic phase. Genotypes will refer to mutli-locus genotypes with known haplotypic phase.

[0490] Suppose one has a sample of N unrelated individuals typed for K markers. The data observed are the unknown-phase K-locus phenotypes that can be categorized with F different phenotypes. Further, suppose that we have H possible haplotypes (in the case of K biallelic markers, we have for the maximum number of possible haplotypes $H=2^K$)

[0491] For phenotype j with c_j possible genotypes, we have:

$$P_j = \sum_{i=1}^{c_j} P(\text{genotype}(i)) = \sum_{i=1}^{c_j} P(h_k, h_l). \qquad \text{Equation 1}$$

[0492] Here, P_j is the probability of the jth phenotype, and $P(h_k, h_l)$ is the probability of the ith genotype composed of haplotypes h_k and h_l. Under random mating (i.e. Hardy-Weinberg Equilibrium), $P(h_k h_l)$ is expressed as:

$P(h_k, h_l) = P(h_k)^2$ for $h_k = h_l$, and

$P(h_k, h_l) = 2P(h_k)P(h_l)$ for $h_k \neq h_l$ $\qquad$ Equation 2

[0493] The E-M algorithm is composed of the following steps: First, the genotype frequencies are estimated from a set of initial values of haplotype frequencies. These haplotype frequencies are denoted $P_1^{(0)}$, $P_2^{(0)}$, $P_3^{(0)}$, ..., $P_H^{(0)}$. The initial values for the haplotype frequencies may be obtained from a random number generator or in some other way well known in the art. This step is referred to the Expectation step. The next step in the method, called the Maximization step, consists of using the estimates for the genotype frequencies to re-calculate the haplotype frequencies. The first iteration haplotype frequency estimates are denoted by $P_1^{(1)}$, $P_2^{(1)}$, $P_3^{(1)}$, ..., $P_H^{(1)}$. In general, the Expectation step at the sth iteration consists of calculating the probability of placing each phenotype into the different possible genotypes based on the haplotype frequencies of the previous iteration:

$$P(h_k, h_l)^{(s)} = \frac{n_j}{N}\left[\frac{P_j(h_k, h_l)^{(s)}}{P_j}\right], \qquad \text{Equation 3}$$

[0494] where n_j is the number of individuals with the jth phenotype and $P_j(h_k, h_l)^{(s)}$ is the probability of genotype h_k, h_l in phenotype j. In the Maximization step, which is equivalent to the gene-counting method (Smith, *Ann. Hum. Genet.,* 21:254-276, 1957), the haplotype frequencies are re-estimated based on the genotype estimates:

$$P_t^{(s+1)} = \frac{1}{2}\sum_{j=1}^{F}\sum_{i=1}^{c_j}\delta_{it} P_j(h_k, h_l)^{(s)}. \qquad \text{Equation 4}$$

[0495] Here, δ_{it} is an indicator variable which counts the number of occurrences that haplotype t is present in i^{th} genotype; it takes on values 0, 1, and 2.

[0496] The E-M iterations cease when the following criterion has been reached. Using Maximum Likelihood Esti-

mation (MLE) theory, one assumes that the phenotypes j are distributed multinomially. At each iteration s, one can compute the likelihood function L. Convergence is achieved when the difference of the log-likelihood between two consecutive iterations is less than some small number, preferably 10^{-7}.

[0497] Methods to Calculate Linkage Disequilibrium Between Markers

[0498] A number of methods can be used to calculate linkage disequilibrium between any two genetic positions, in practice linkage disequilibrium is measured by applying a statistical association test to haplotype data taken from a population.

[0499] Linkage disequilibrium between any pair of biallelic markers comprising at least one of the biallelic markers of the present invention (M_i, M_j) having alleles (a_i/b_i) at marker M_i and alleles (a_j/b_j) at marker M_j can be calculated for every allele combination (a_i, a_j; a_i, b_j; b_i, a_j and b_i, b_j), according to the Piazza formula:

$\Delta_{aiaj} = \sqrt{\theta4} - \sqrt{(\theta4+\theta3)(\theta4+\theta2)}$,

[0500] where:

[0501] $\theta4 = ---=$ frequency of genotypes not having allele a_i at M_i and not having allele a_j at M_j

[0502] $\theta3 = -+=$ frequency of genotypes not having allele a_i at M_i and having allele a_j at M_j

[0503] $\theta2 = +-=$ frequency of genotypes having allele a_i at M_i and not having allele a_j at M_j

[0504] Linkage disequilibrium (LD) between pairs of biallelic markers (M_i, M_j) can also be calculated for every allele combination (ai,aj, ai,bj, b_i, a_j and b_i, b_j), according to the maximum-likelihood estimate (MLE) for delta (the composite genotypic disequilibrium coefficient), as described by Weir (Weir B. S., *Genetic Data Analysis*, Sinauer Ass. Eds, 1996). The MLE for the composite linkage disequilibrium is:

$D_{aiaj} = (2n_1+n_2+n_3+n_4/2)/N - 2(pr(a_i).pr(a_j))$

[0505] Where $n_1 = \Sigma$ phenotype (a_i/a_i, a_j/a_j), $n_2 = \Sigma$ phenotype (a_i/a_i, a_j/b_j), $n_3 = \Sigma$ phenotype (a_i/b_i, a_j/a_j), $n4 = \Sigma$ phenotype (a_i/b_i, a_j/b_j) and N is the number of individuals in the sample.

[0506] This formula allows linkage disequilibrium between alleles to be estimated when only genotype, and not haplotype, data are available.

[0507] Another means of calculating the linkage disequilibrium between markers is as follows. For a couple of biallelic markers, $M_i(a_i/b_i)$ and $M_j(a_j/b_j)$, fitting the Hardy-Weinberg equilibrium, one can estimate the four possible haplotype frequencies in a given population according to the approach described above.

[0508] The estimation of gametic disequilibrium between ai and aj is simply:

$D_{aiaj} = pr(\text{haplotype}(a_i, a_j)) - pr(a_i).pr(a_j)$.

[0509] Where $pr(a_i)$ is the probability of allele a_i and $pr(a_j)$ is the probability of allele a_j and where $pr(\text{haplotype} (a_i, a_i))$ is estimated as in Equation 3 above.

[0510] For a couple of biallelic marker only one measure of disequilibrium is necessary to describe the association between M_i and M_j.

[0511] Then a normalised value of the above is calculated as follows:

$$D'_{aiaj}=D_{aiaj}/\max(-pr(a_i).pr(a_j), \quad -pr(b_i).pr(b_j)) \quad \text{with} \quad D_{aiaj}<0$$

$$D_{aiaj}=D_{aiaj}/\max(pr(b_i).pr(a_j), \quad pr(a_i).pr(b_j)) \quad \text{with} \quad D_{aiaj}>0$$

[0512] The skilled person will readily appreciate that other LD calculation methods can be used without undue experimentation.

[0513] Linkage disequilibrium among a set of biallelic markers having an adequate heterozygosity rate can be determined by genotyping between 50 and 1000 unrelated individuals, preferably between 75 and 200, more preferably around 100.

[0514] Testing for Association

[0515] Methods for determining the statistical significance of a correlation between a phenotype and a genotype, in this case an allele at a biallelic marker or a haplotype made up of such alleles, may be determined by any statistical test known in the art and with any accepted threshold of statistical significance being required. The application of particular methods and thresholds of significance are well with in the skill of the ordinary practitioner of the art.

[0516] Testing for association is performed by determining the frequency of a biallelic marker allele in case and control populations and comparing these frequencies with a statistical test to determine if their is a statistically significant difference in frequency which would indicate a correlation between the trait and the biallelic marker allele under study. Similarly, a haplotype analysis is performed by estimating the frequencies of all possible haplotypes for a given set of biallelic markers in case and control populations, and comparing these frequencies with a statistical test to determine if their is a statistically significant correlation between the haplotype and the phenotype (trait) under study. Any statistical tool useful to test for a statistically significant association between a genotype and a phenotype may be used. Preferably the statistical test employed is a chi-square test with one degree of freedom. A P-value is calculated (the P-value is the probability that a statistic as large or larger than the observed one would occur by chance).

[0517] Statistical Significance

[0518] In preferred embodiments, significance for diagnosis purposes, either as a positive basis for further diagnostic tests or as a preliminary starting point for early preventive therapy, the p value related to a biallelic marker association is preferably about $1\times10{-}2$ or less, more preferably about $1\times10{-}4$ or less, for a single biallelic marker analysis and about $1\times10{-}3$ or less, still more preferably $1\times10{-}6$ or less and most preferably of about $1\times10{-}8$ or less, for a haplotype analysis involving several markers. These values are believed to be applicable to any association studies involving single or multiple marker combinations.

[0519] The skilled person can use the range of values set forth above as a starting point in order to carry out association studies with biallelic markers of the present invention. In doing so, significant associations between the biallelic

markers of the present invention and responses to drugs or side effects upon treatment with drugs or diseases involving the metabolic conversion of xenobiotics can be revealed and used for diagnosis and drug screening purposes.

[0520] Phenotypic Permutation

[0521] In order to confirm the statistical significance of the first stage haplotype analysis described above, it might be suitable to perform further analyses in which genotyping data from case-control individuals are pooled and randomised with respect to the trait phenotype. Each individual genotyping data is randomly allocated to two groups, which contain the same number of individuals as the case-control populations used to compile the data obtained in the first stage. A second stage haplotype analysis is preferably run on these artificial groups, preferably for the markers included in the haplotype of the first stage analysis showing the highest relative risk coefficient. This experiment is reiterated preferably at least between 100 and 10000 times. The repeated iterations allow the determination of the percentage of obtained haplotypes with a significant p-value level.

[0522] Assessment of Statistical Association

[0523] To address the problem of false positives similar analysis may be performed with the same case-control populations in random genomic regions. Results in random regions and the candidate region are compared as described in US Provisional Patent Application entitled "Methods, software and apparati for identifying genomic regions harbouring a gene associated with a detectable trait".

[0524] Evaluation of Risk Factors

[0525] The association between a risk factor (in genetic epidemiology the risk factor is the presence or the absence of a certain allele or haplotype at marker loci) and a disease is measured by the odds ratio (OR) and by the relative risk (RR). If $P(R^+)$ is the probability of developing the disease for individuals with R and $P(R^-)$ is the probability for individuals without the risk factor, then the relative risk is simply the ratio of the two probabilities, that is:

$$RR=P(R^+)/P(R^-)$$

[0526] In case-control studies, direct measures of the relative risk cannot be obtained because of the sampling design. However, the odds ratio allows a good approximation of the relative risk for low-incidence diseases and can be calculated:

$$OR = \left[\frac{F^+}{1-F^+}\right] \bigg/ \left[\frac{F^-}{(1-F^-)}\right]$$

[0527] F^+ is the frequency of the exposure to the risk factor in cases and F^- is the frequency of the exposure to the risk factor in controls. F^+ and F^- are calculated using the allelic or haplotype frequencies of the study and further depend on the underlying genetic model (dominant, recessive, additive . . .).

[0528] One can further estimate the attributable risk (AR) which describes the proportion of individuals in a population exhibiting a trait due to a given risk factor. This measure is important in quantitating the role of a specific factor in disease etiology and in terms of the public health impact of

a risk factor. The public health relevance of this measure lies in estimating the proportion of cases of disease in the population that could be prevented if the exposure of interest were absent. AR is determined as follows:

$$AR=P_E(RR-1)/(P_E(RR-1)+1)$$

[0529] AR is the risk attributable to a biallelic marker allele or a biallelic marker haplotype. P_E is the frequency of exposure to an allele or a haplotype within the population at large; and RR is the relative risk which, is approximated with the odds ratio when the trait under study has a relatively low incidence in the general population.

[0530] D. Identification of Biallelic Markers in Linkage Disequilibrium with the Biallelic Markers of the Invention

[0531] Once a first biallelic marker has been identified in a genomic region of interest, the practitioner of ordinary skill in the art, using the teachings of the present invention, can easily identify additional biallelic markers in linkage disequilibrium with this first marker. As mentioned before any marker in linkage disequilibrium with a first marker associated with a trait will be associated with the trait. Therefore, once an association has been demonstrated between a given biallelic marker and a trait, the discovery of additional biallelic markers associated with this trait is of great interest in order to increase the density of biallelic markers in this particular region. The causal gene or mutation will be found in the vicinity of the marker or set of markers showing the highest correlation with the trait.

[0532] Identification of additional markers in linkage disequilibrium with a given marker involves: (a) amplifying a genomic fragment comprising a first biallelic marker from a plurality of individuals; (b) identifying of second biallelic markers in the genomic region harboring said first biallelic marker; (c) conducting a linkage disequilibrium analysis between said first biallelic marker and second biallelic markers; and (d) selecting said second biallelic markers as being in linkage disequilibrium with said first marker. Subcombinations comprising steps (b) and (c) are also contemplated.

[0533] Methods to identify biallelic markers and to conduct linkage disequilibrium analysis are described herein and can be carried out by the skilled person without undue experimentation. The present invention then also concerns biallelic markers which are in linkage disequilibrium with the specific biallelic markers shown in Table 11(A-B) and which are expected to present similar characteristics in terms of their respective association with a given trait.

[0534] E. Identification of Functional Mutations

[0535] Once a positive association is confirmed with a biallelic marker of the present invention, the associated candidate gene can be scanned for mutations by comparing the sequences of a selected number of trait positive and trait negative or control individuals. In a preferred embodiment, functional regions such as exons and splice sites, promoters and other regulatory regions of the candidate gene are scanned for mutations. Preferably, trait positive individuals carry the haplotype shown to be associated with the trait and trait negative individuals do not carry the haplotype or allele associated with the trait. The mutation detection procedure is essentially similar to that used for biallelic site identification.

[0536] The method used to detect such mutations generally comprises the following steps: (a) amplification of a region of the candidate gene comprising a biallelic marker or a group of biallelic markers associated with the trait from DNA samples of trait positive patients and trait negative controls; (b) sequencing of the amplified region; (c) comparison of DNA sequences from trait-positive patients and trait-negative controls; and (d) determination of mutations specific to trait-positive patients. Subcombinations which comprise steps (b) and (c) are specifically contemplated.

[0537] It is preferred that candidate polymorphisms be then verified by screening a larger population of cases and controls by means of any genotyping procedure such as those described herein, preferably using a microsequencing technique in an individual test format. Polymorphisms are considered as candidate mutations when present in cases and controls at frequencies compatible with the expected association results.

[0538] Identification of mutations and low frequency polymorphisms in exons 3-5, in the 5'UTR region and in the 3' flanking region of the MGST-II gene is further described in Example 5. Eight polymorphisms were identified in the region of the MGST-II gene that was scanned. Three mutations were identified in the 3'UTR region. One mutation in exon 4 causes an amino acid substitution (Tyr→His) at the polypeptide level. A mutation in exon 5 introduces a stop codon into the ORF leading to the expression of a truncated MGST-II polypeptide. These mutations modify the specificity, activity and function of the MGST-II enzyme and therefore represent functional mutations of the MGST-II gene. Candidate polymorphisms and mutations suspected of being responsible for the detectable phenotype, such as hepatotoxicity to zileuton or asthma, can be confirmed by screening a larger population of affected and unaffected individuals using any of the genotyping procedures described herein. Preferably the microsequencing technique is used. In a preferred embodiment trait positive and trait negative populations are genotyped for the candidate polymorphisms identified in Example 5 (10-286-289, 10-286-345, 10-286-375, 10-523-232, 10-289-201, 10-290-37, 10-290-326 and 10-290-328) most preferably for the mutations in exons 4 and 5 (10-289-201 and 10-290-37). Polymorphisms are considered as candidate "trait-causing" mutations when they exhibit a statistically significant correlation with the detectable phenotype.

[0539] V. Biallelic Markers of the Invention in Methods of Genetic Diagnostics

[0540] The biallelic markers of the present invention can also be used to develop diagnostics tests capable of identifying individuals who express a detectable trait as the result of a specific genotype or individuals whose genotype places them at risk of developing a detectable trait at a subsequent time. The trait analyzed using the present diagnostics may be any detectable trait, including a response to a drug or side effects to a drug upon treatment or a disease involving the metabolic conversion of xenobiotics.

[0541] The diagnostic techniques of the present invention may employ a variety of methodologies to determine whether a test subject has a biallelic marker pattern associated with an increased risk of developing a detectable trait or whether the individual suffers from a detectable trait as a result of a particular mutation, including methods which

enable the analysis of individual chromosomes for haplotyping, such as family studies, single sperm DNA analysis or somatic hybrids.

[0542] The present invention provides diagnostic methods to determine whether an individual is at risk of developing a disease or suffers from a disease resulting from a mutation or a polymorphism in a candidate gene of the present invention. The present invention also provides methods to determine whether an individual is likely to respond positively to a therapeutic agent or whether an individual is at risk of developing an adverse side effect to a therapeutic agent.

[0543] These methods involve obtaining a nucleic acid sample from the individual and, determining, whether the nucleic acid sample contains at least one allele or at least one biallelic marker haplotype, indicative of a risk of developing the trait or indicative that the individual expresses the trait as a result of possessing a particular candidate gene polymorphism or mutation (trait-causing allele).

[0544] Preferably, in such diagnostic methods, a nucleic acid sample is obtained from the individual and this sample is genotyped using methods described herein in "Methods of Genotyping an Individual for Biallelic Markers." The diagnostics may be based on a single biallelic marker or on a group of biallelic markers.

[0545] In each of these methods, a nucleic acid sample is obtained from the test subject and the biallelic marker pattern of one or more of the biallelic markers listed in Table 11(A-B) is determined.

[0546] In one embodiment, a PCR amplification is conducted on the nucleic acid sample to amplify regions in which polymorphisms associated with a detectable phenotype have been identified. The amplification products are sequenced to determine whether the individual possesses one or more polymorphisms associated with a detectable phenotype. The primers used to generate amplification products may comprise the primers listed in Table 17. Alternatively, the nucleic acid sample is subjected to microsequencing reactions as described above to determine whether the individual possesses one or more polymorphisms associated with a detectable phenotype resulting from a mutation or a polymorphism in a candidate gene. The primers used in the microsequencing reactions may include the primers listed in Table 16. In another embodiment, the nucleic acid sample is contacted with one or more allele specific oligonucleotide probes which, specifically hybridize to one or more candidate gene alleles associated with a detectable phenotype. The probes used in the hybridization assay may include the probes listed in Table 18. Diagnostic kits comprising polynucleotides of the present invention are further described in section I.

[0547] These diagnostic methods are extremely valuable as they can, in certain circumstances, be used to initiate preventive treatments or to allow an individual carrying a significant haplotype to foresee warning signs such as minor symptoms. For diseases in which attacks may be extremely violent and sometimes fatal if not treated on time, the knowledge of a potential predisposition, even if this predisposition is not absolute, might contribute in a very significant manner to treatment efficacy. Similarly, a diagnosed predisposition to a potential side effect could immediately direct the physician toward a treatment for which such side effects have not been observed during clinical trials.

[0548] Diagnostics, which analyze and predict response to a drug or side effects to a drug, may be used to determine whether an individual should be treated with a particular drug. For example, if the diagnostic indicates a likelihood that an individual will respond positively to treatment with a particular drug, the drug may be administered to the individual. Conversely, if the diagnostic indicates that an individual is likely to respond negatively to treatment with a particular drug, an alternative course of treatment may be prescribed. A negative response may be defined as either the absence of an efficacious response or the presence of toxic side effects.

[0549] Clinical drug trials represent another application for the markers of the present invention. One or more markers indicative of response to a drug or to side effects to a drug may be identified using the methods described above. Thereafter, potential participants in clinical trials of such an agent may be screened to identify those individuals most likely to respond favorably to the drug and exclude those likely to experience side effects. In that way, the effectiveness of drug treatment may be measured in individuals who respond positively to the drug, without lowering the measurement as a result of the inclusion of individuals who are unlikely to respond positively in the study and without risking undesirable safety problems.

[0550] In a preferred embodiment the identity of the nucleotide present at, at least one, biallelic marker selected from the group consisting of 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440 and 10-420-284 is determined, and optionally wherein the detectable trait is asthma, or optionally the detectable trait is hepatotoxicity to the anti-asthmatic drug zileuton. In another preferred embodiment the identity of the nucleotide present at, at least one of the polymorphic sites selected from the group consisting of 12-447-58, 12-455-326, 12-461-299, 12-453-429, 12-424-198, 12-454-363, 12-716-295, 10-428-219, 12-720-80, 10-420-248, 12-721-440, 12-653-423, 10-470-25, 10-471-84, 10-471-85, 12-637-219 and 12-652-203 is determined, and optionally wherein the detectable trait is asthma. In another preferred embodiment the identity of the nucleotide present at, at least one of the polymorphic sites selected from the group consisting of 12-453-429, 12-455-326, 12-453-429, 12-454-363, 12-441-233, 12-461-299, 12-426-154, 12-424-198, 12-716-295, 10-428-219, 12-720-80, 12-156-91, 12-140-134, 12-653-423, 10-471-84, 10-471-85, 10-470-25, 12-652-203, 12-637-219, 12-721-440, and 10-420-284 is determined, and optionally wherein the detectable trait is hepatotoxicity to the anti-asthmatic drug zileuton. Diagnostic kits comprising polynucleotides of the present invention are further described in "Biallelic Markers and Polynucleotides Comprising Biallelic Markers."

[0551] VI. Association of Biallelic Markers of the Invention With Asthma

[0552] In the context of the present invention, an association between the MGST-II, ME1, UGT1A7 and UGT2B4 genes and asthma was established. See Example 3.

[0553] Asthma affects over 5% of the population in industrialized countries. It is increasing in prevalence and severity and has a rising mortality (Rang H. P., Ritter J. M. and Dale M. M.; *Pharmacology*; Churchill Livingstone, NY, 1995). Bronchial asthma is a multifactorial syndrome rather than a single disease, defined as airway obstruction characterized by inflammatory changes in the airways and bronchial hyper-responsiveness. In addition to the evidenced impact of environmental factors on the development of asthma, patterns of clustering and segregation in asthmatic families have suggested a genetic component to asthma. However the lack of a defined and specific asthma phenotype and of suitable markers for genetic analysis is proving to be a major hurdle for reliably identifying genes associated with asthma. The identification of genes implicated in asthma would represent a major step towards the identification of new molecular targets for the development of anti-asthma drugs. Moreover there is no straightforward physiological or biological blood test for the asthmatic state. As a result, adequate asthma treatment is often delayed, thereby allowing the inflammation process to better establish itself. Thus, there is a need for the identification of asthma susceptibility genes in order to develop an efficient and reliable asthma diagnostic test.

[0554] As mentioned above, products of arachidonic acid metabolism are important inflammatory mediators and have been involved in a number of inflammatory diseases, including asthma. More specifically, prostaglandins and leukotrienes are thought to play a major role in the inflammatory process observed in asthma patients.

[0555] In order to investigate and identify a genetic origin to asthma, a candidate gene scan was conducted. This approach comprised:

[0556] a) selecting candidate genes potentially involved in the pathological pathway of interest, in this case arachidonic acid metabolism, and

[0557] b) identifying biallelic markers in those genes, and finally

[0558] c) conducting association studies to identify biallelic marker alleles or haplotypes associated with asthma.

[0559] Further details concerning this association study are provided in Example 3, results are briefly summarized below.

[0560] Two groups of independent individuals were used in this association study in accordance with the invention: the case-control populations. The two groups corresponded to 297 asthmatic individuals and 178 control individuals. The trait positive asthma population was mostly composed of individuals from Caucasian ethnic background (>90%). The control population was composed of individuals from a random US Caucasian population.

[0561] In the association study described in Example 3, several biallelic marker haplotypes were shown to be significantly associated with asthma. A preferred haplotype consisting of three biallelic markers (12-455-326, 12-453-429 and 12-424-198) presented a p-value of 3.2 10^{-5}. Another preferred haplotype consisting of four biallelic markers (12-455-326, 12-453-429, 12-424-198 and 12-454-363) had a p-value of 1.2 10^{-6}. Phenotypic permutation tests

confirmed the statistical significance of these results. These haplotypes can therefore be considered to be significantly associated with asthma.

[0562] This information is extremely valuable. The knowledge of a potential genetic predisposition to asthma, even if this predisposition is not absolute, might contribute in a very significant manner to treatment efficacy of asthma patients and to the development of new therapeutic and diagnostic tools.

[0563] VII. Association of Biallelic Markers of the Invention with Hepatotoxicity to Anti-Asthma Drug Zileuton (Zyflo™)

[0564] In the context of the present invention, an association between the the MGST-II, ME1, UGT1A7 and UGT2B4 genes and side effects related to treatment with the anti-asthmatic drug zileuton was discovered.

[0565] As mentioned above, bronchial asthma is a multifactorial syndrome rather than a single disease, defined as airway obstruction characterized by inflammatory changes in the airways and bronchial hyper-responsiveness. Although initially reversible with bronchiodilators, airway obstruction becomes increasingly irreversible if treated poorly. Asthma management therefore relies on early and regular use of drugs that control the disease. As a consequence, there is a strong need for efficient and safe therapeutic opportunities for patients with asthma. There are two main categories of anti-asthmatic drugs—bronchodilators and anti-inflammatory agents. There is now general agreement on the need to implement early anti-inflammatory treatment rather than relying on symptomatic treatment with bronchiodilators alone. The leukotrienes, a family of proinflammatory mediators arising via arachidonic acid metabolism, have been implicated in the inflammatory cascade that occurs in asthmatic airways. Of great relevance to the pathogenesis of asthma are the 5-lipoxygenase and the 5-lipoxygenase activating protein (FLAP), which catalyze the initial steps in the biosynthesis of leukotrienes from arachidonic acid. Given the significant role of the inflammatory process in asthma, pharmacological agents, such as leukotriene antagonists, FLAP inhibitors and 5-lipoxygenase inhibitors have been developed.

[0566] Zileuton (Zyflo™) is an active inhibitor of 5-lipoxygenase, the enzyme that catalyzes the formation of leukotrienes from arachidonic acid, indicated for prophylaxis and chronic treatment of asthma. A minority of zileuton-treated patients develop liver function abnormalities as close monitoring revealed that elevations of liver function tests may occur during treatment with zileuton. In the present invention the ALT test (serum level of alanine aminotransferase) was used, which is considered the most sensitive indicator of liver injury.

[0567] In order to investigate and identify a genetic origin to zileuton-associated hepatotoxicity, a candidate gene scan was conducted. This approach comprised:

[0568] a) selecting candidate genes potentially involved in the pathological pathway of interest or in the metabolism of zileuton, and

[0569] b) identifying biallelic markers in those genes, and finally

[0570]   c) conducting association studies to identify biallelic marker alleles or haplotypes associated with elevations of liver function tests upon treatment with zileuton.

[0571]   Further details concerning this association study are provided in Example 4, results are briefly summarized below.

[0572]   Two groups of unrelated individuals were used in this association study in accordance with the invention: the case-control populations. The case population was composed of 89 asthmatic individuals treated with zileuton showing zileuton-associated hepatotoxicity monitored by the serum level of alanine aminotransferase (ALT) and the control population was composed of 208 asthmatic individuals treated with zileuton and having no increased serum ALT level.

[0573]   The association study conducted with the biallelic markers derived from the MGST-II locus showed that several haplotypes were significantly associated with zileuton-associated hepatotoxicity. A preferred haplotype consisting of three biallelic markers (12-441-233, 12-461-299 and 12-453-429) presented a p-value of $1.5 \ 10^{-5}$ and an odd ratio of 3.63. A second preferred haplotype consisting of four biallelic markers (12-441-233, 12-461-299, 12-453-429 and 12-426-154) had a p-value of $5.2 \ 10^{-7}$ and an odd ratio of 5.75.

[0574]   This information is extremely valuable. The knowledge of a potential genetic predisposition to hepatotoxicity upon treatment with zileuton, even if this predisposition is not absolute, might contribute in a very significant manner to the safety of asthma treatment and to the development of diagnostic tools.

[0575]   Similar association studies, with different case-control populations, can be routinely carried out by the skilled technician using the biallelic markers of the present invention in order to identify other association between traits and DME and MGST-II-related biallelic marker alleles or haplotypes.

[0576]   VI. Computer-Related Embodiments

[0577]   As used herein the term "nucleic acid codes of the invention" encompass the nucleotide sequences comprising, consisting essentially of, or consisting of any one of the following: a) a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500 or 1000 nucleotides, to the extent that a polynucleotide of these lengths is consistent with the lengths of the particular Sequence ID, of a sequence selected from the group consisting of the sequences described in Table 12, and the complements thereof; b) a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500 or 1000 nucleotides, to the extent that a polynucleotide of these lengths is consistent with the lengths of the particular Sequence ID, of a sequence selected from the group consisting of the sequences described in Table 13, and the complements thereof, c) a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 500 nucleotides, to the extent that a polynucleotide of these lengths is consistent with the lengths of the particular Sequence ID, of a sequence selected from the group consisting of the sequences described in Table 16, more preferably a set of markers or sequences consisting of those markers or sequences found in SEQ ID Nos. 3, 5, 9, 13-15, 25, 31, 33, 37, 38, 41, 323, 345, 351-353, 357, 377, and 380, and the complements thereof, wherein said span includes an DME-related biallelic marker, preferably an DME-related biallelic marker described in Table 11(A-B), preferably the biallelic markers found in Tables 19, 20, 21 and 22; or more preferably the biallelic markers found in SEQ ID Nos. 3, 5, 9, 13-15, 25, 31, 33, 37, 38, 41, 323, 345, 351-353, 357, 377, 380, in said sequence with the alternative allele present at said biallelic marker.

[0578]   The "nucleic acid codes of the invention" further encompass nucleotide sequences homologous to a contiguous span of at least 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500 or 1000 nucleotides, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular Sequence ID, of a sequence selected from the group consisting of the sequences described in Tables 12, 13 and 16 and the complements thereof. Homologous sequences refer to a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% homology to these contiguous spans. Homology may be determined using any method described herein, including BLAST2N with the default parameters or with any modified parameters. Homologous sequences also may include RNA sequences in which uridines replace the thymines in the nucleic acid codes of the invention. It will be appreciated that the nucleic acid codes of the invention can be represented in the traditional single character format (See the inside back cover of Stryer, Lubert. *Biochemistry*, 3rd edition. W. H Freeman & Co., New York.) or in any other format or code which records the identity of the nucleotides in a sequence.

[0579]   It will be appreciated by those skilled m the art that the nucleic acid codes of the invention, one or more of the polypeptide codes of SEQ ID Nos. 488 and 489 can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any of the presently known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the nucleic acid codes of the invention and one or more of the polypeptide codes of SEQ ID Nos. 488 and 489. Another aspect of the present invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, 20, 25, 30, or 50 nucleic acid codes of the invention, and the complements thereof. Another aspect of the present invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, 20, 25, 30, or 50 polypeptide codes of SEQ ID Nos. 488 and 489.

[0580]   Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

[0581]   Embodiments of the present invention include systems, particularly computer systems which store and manipulate the sequence information described herein. As used herein, "a computer system" refers to the hardware

components, software components, and data storage components used to analyze the nucleotide sequences of the nucleic acid codes of the invention, or the amino acid sequences of the polypeptide codes of SEQ ID Nos. 488 and 489. In one embodiment, the computer system is a Sun Enterprise 1000 server (Sun Microsystems, Palo Alto, Calif.). The computer system preferably includes a processor for processing, accessing and manipulating the sequence data. The processor can be any well-known type of central processing unit, such as the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq or International Business Machines. Preferably, the computer system is a general-purpose system that comprises the processor and one or more internal data storage components for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable. In one particular embodiment, the computer system includes a processor connected to a bus which is connected to a main memory (preferably implemented as RAM) and one or more internal data storage devices, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system further includes one or more data-retrieving device for reading the data stored on the internal data storage devices. The data-retrieving device may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, etc. In some embodiments, the internal data storage device is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data-retrieving device. The computer system includes a display which is used to display output to a computer user. It should also be noted that the computer system can be linked to other computer systems in a network or wide area network to provide centralized access to the computer system. Software for accessing and processing the nucleotide sequences of the nucleic acid codes of the invention, or the amino acid sequences of the polypeptide codes of SEQ ID Nos. 488 and 489 (such as search tools, compare tools, and modeling tools etc.) may reside in main memory during execution. In some embodiments, the computer system may further comprise a sequence comparer for comparing the above-described nucleic acid codes of the invention or polypeptide codes of SEQ ID Nos. 488 and 489 stored on a computer readable medium to reference nucleotide or polypeptide sequences stored on a computer readable medium. A "sequence comparer" refers to one or more programs which are implemented on the computer system to compare a nucleotide or polypeptide sequence with other nucleotide or polypeptide sequences and/or compounds including but not limited to peptides, peptidomyimetics, and chemicals stored within the data storage means. For example, the sequence comparer may compare the nucleotide sequences of the nucleic acid codes of the invention, or the amino acid sequences of the polypeptide codes of SEQ ID Nos. 488 and 489 stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies, motifs implicated in biological function, or structural motifs. The various

sequence comparer programs identified elsewhere in this patent specification are particularly contemplated for use in this aspect of the invention.

[0582] One embodiment is a process for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system, or a public database such as GENBANK, PIR OR SWISSPROT that is available through the Internet.

[0583] The process begins at a start state and then moves to a state wherein the new sequence to be compared is stored to a memory in a computer system. As discussed above, the memory could be any type of memory, including RAM or an internal storage device. The process then moves to a state wherein a database of sequences is opened for analysis and comparison. The process then moves to a state wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system. Once a comparison of the two sequences has been performed at the state, a determination is made at a decision state whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process. If a determination is made that the two sequences are the same, the process moves to a state wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process moves to a decision state wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process terminates at an end state. However, if more sequences do exist in the database, then the process moves to a state wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database. It should be noted that if a determination had been made at the decision state that the sequences were not homologous, then the process would move immediately to the decision state in order to determine if any other sequences were available in the database for comparison. Accordingly, one aspect of the present invention is a computer system comprising a processor, a data storage device having stored thereon a nucleic acid code of the invention or a polypeptide code of SEQ ID Nos. 488 and 489, a data storage device having retrievably stored thereon reference nucleotide sequences or polypeptide sequences to be compared to the nucleic acid code of the invention or polypeptide code of SEQ ID Nos. 488 and 489 and a sequence comparer for conducting the comparison. The

sequence comparer may indicate a homology level between the sequences compared or identify structural motifs in the above described nucleic acid code of the invention and polypeptide codes of SEQ ID Nos. 488 and 489 or it may identify structural motifs in sequences which are compared to these nucleic acid codes and polypeptide codes. In some embodiments, the data storage device may have stored thereon the sequences of at least 2, 5, 10, 15, 20, 25, 30, or 50 of the nucleic acid codes of the invention or polypeptide codes of SEQ ID Nos. 488 and 489.

[0584] Another aspect of the present invention is a method for determining the level of homology between a nucleic acid code of the invention and a reference nucleotide sequence, comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through the use of a computer program which determines homology levels and determining homology between the nucleic acid code and the reference nucleotide sequence with the computer program. The computer program may be any of a number of computer programs for determining homology levels, including those specifically enumerated herein, including BLAST2N with the default parameters or with any modified parameters. The method may be implemented using the computer systems described above. The method may also be performed by reading 2, 5, 10, 15, 20, 25, 30, or 50 of the above described nucleic acid codes of the invention through use of the computer program and determining homology between the nucleic acid codes and reference nucleotide sequences.

[0585] One embodiment is a process in a computer for determining whether two sequences are homologous. The process begins at a start state and then moves to a state wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state. The process then moves to a state wherein the first character in the first sequence is read and then to a state wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it should be in the single letter amino acid code so that the first and sequence sequences can be easily compared.

[0586] A determination is then made at a decision state whether the two characters are the same. If they are the same, then the process moves to a state wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process moves to a decision state to determine whether there are any more characters either sequence to read. If there aren't any more characters to read, then the process moves to a state wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%. Alternatively, the computer program may be a computer program which compares the nucleotide

sequences of the nucleic acid codes of the present invention, to reference nucleotide sequences in order to determine whether the nucleic acid code of SEQ ID Nos. 1-977 differs from a reference nucleic acid sequence at one or more positions. Optionally such a program records the length and identity of inserted, deleted or substituted nucleotides with respect to the sequence of either the reference polynucleotide or the nucleic acid code of SEQ ID Nos. 1-977. In one embodiment, the computer program may be a program which determines whether the nucleotide sequences of the nucleic acid codes of the invention contain a biallelic marker or single nucleotide polymorphism (SNP) with respect to a reference nucleotide sequence. This single nucleotide polymorphism may comprise a single base substitution, insertion, or deletion, while this biallelic marker may comprise about one to ten consecutive bases substituted, inserted or deleted.

[0587] Another aspect of the present invention is a method for determining the level of homology between a polypeptide code of SEQ ID Nos. 488 and 489 and a reference polypeptide sequence, comprising the steps of reading the polypeptide code of SEQ ID Nos. 488 and 489 and the reference polypeptide sequence through use of a computer program which determines homology levels and determining homology between the polypeptide code and the reference polypeptide sequence using the computer program.

[0588] Accordingly, another aspect of the present invention is a method for determining whether a nucleic acid code of the invention differs at one or more nucleotides from a reference nucleotide sequence comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through use of a computer program which identifies differences between nucleic acid sequences and identifying differences between the nucleic acid code and the reference nucleotide sequence with the computer program. In some embodiments, the computer program is a program which identifies single nucleotide polymorphisms. The method may be implemented by the computer systems described above. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30, or 50 of the nucleic acid codes of the invention and the reference nucleotide sequences through the use of the computer program and identifying differences between the nucleic acid codes and the reference nucleotide sequences with the computer program. In other embodiments the computer based system may further comprise an identifier for identifying features within the nucleotide sequences of the nucleic acid codes of the invention or the amino acid sequences of the polypeptide codes of SEQ ID Nos. 488 and 489. An "identifier" refers to one or more programs which identifies certain features within the above-described nucleotide sequences of the nucleic acid codes of the invention or the amino acid sequences of the polypeptide codes of SEQ ID Nos. 488 and 489. In one embodiment, the identifier may comprise a program which identifies an open reading frame in the cDNAs codes of SEQ ID Nos. 486 and 487.

[0589] One embodiment is an identifier process for detecting the presence of a feature in a sequence. The process begins at a start state and then moves to a state wherein a first sequence that is to be checked for features is stored to a memory in the computer system. The process then moves to a state wherein a database of sequence features is opened.

Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG." Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group (world wide web site: gcg.com). Once the database of features is opened at the state, the process moves to a state wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state. A determination is then made at a decision state whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process moves to a state wherein the name of the found feature is displayed to the user. The process then moves to a decision state wherein a determination is made whether move features exist in the database. If no more features do exist, then the process terminates at an end state. However, if more features do exist in the database, then the process reads the next sequence feature at a state and loops back to the state wherein the attribute of the next feature is compared against the first sequence. It should be noted, that if the feature attribute is not found in the first sequence at the decision state, the process moves directly to the decision state in order to determine if any more features exist in the database. In another embodiment, the identifier may comprise a molecular modeling program which determines the 3-dimensional structure of the polypeptides codes of SEQ ID Nos. 488 and 489. In some embodiments, the molecular modeling program identifies target sequences that are most compatible with profiles representing the structural environments of the residues in known three-dimensional protein structures. (See, e.g., Eisenberg et al., U.S. Pat. No. 5,436, 850 issued Jul. 25, 1995, the disclosure of which is incorporated herein by reference in its entirety). In another technique, the known three-dimensional structures of proteins in a given family are superimposed to define the structurally conserved regions in that family. This protein modeling technique also uses the known three-dimensional structure of a homologous protein to approximate the structure of the polypeptide codes of SEQ ID Nos. 488 and 489. (See e.g., Srinivasan, et al., U.S. Pat. No. 5,557,535 issued Sep. 17, 1996, the disclosure of which is incorporated herein by reference in its entirety). Conventional homology modeling techniques have been used routinely to build models of proteases and antibodies. (Sowdhamini et al., Protein Engineering 10:207, 215 (1997), the disclosure of which is incorporated herein by reference in its entirety). Comparative approaches can also be used to develop three-dimensional protein models when the protein of interest has poor sequence identity to template proteins. In some cases, proteins fold into similar three-dimensional structures despite having very weak sequence identities. For example, the three-dimensional structures of a number of helical cytokines fold in similar three-dimensional topology in spite of weak sequence homology. The recent development of threading methods now enables the identification of likely folding patterns in a number of situations where the structural relatedness between target and template(s) is not detectable at the sequence level. Hybrid methods, in which fold recognition is performed using Multiple Sequence Threading (MST), structural equivalencies are deduced from the threading output using a distance geometry program DRAGON to construct a low resolution model, and a full-atom representation is constructed using a molecular modeling package such as QUANTA.

[0590] According to this 3-step approach, candidate templates are first identified by using the novel fold recognition algorithm MST, which is capable of performing simultaneous threading of multiple aligned sequences onto one or more 3-D structures. In a second step, the structural equivalencies obtained from the MST output are converted into interresidue distance restraints and fed into the distance geometry program DRAGON, together with auxiliary information obtained from secondary structure predictions. The program combines the restraints in an unbiased manner and rapidly generates a large number of low resolution model confirmations. In a third step, these low resolution model confirmations are converted into full-atom models and subjected to energy minimization using the molecular modeling package QUANTA. (See e.g., Aszódi et al., Proteins: Structure, Function, and Genetics, Supplement 1:3842 (1997), the disclosure of which is incorporated herein by reference in its entirety).

[0591] The results of the molecular modeling analysis may then be used in rational drug design techniques to identify agents which modulate the activity of the polypeptide codes of SEQ ID Nos. 488 and 489. Accordingly, another aspect of the present invention is a method of identifying a feature within the nucleic acid codes of the invention or the polypeptide codes of SEQ ID Nos. 488 and 489 comprising reading the nucleic acid code(s) or the polypeptide code(s) through the use of a computer program which identifies features therein and identifying features within the nucleic acid code(s) or polypeptide code(s) with the computer program. In one embodiment, computer program comprises a computer program which identifies open reading frames. In a further embodiment, the computer program identifies structural motifs in a polypeptide sequence. In another embodiment, the computer program comprises a molecular modeling program. The method may be performed by reading a single sequence or at least 2, 5, 10, 15, 20, 25, 30, or 50 of the nucleic acid codes of the invention or the polypeptide codes of SEQ ID Nos. 488 and 489 through the use of the computer program and identifying features within the nucleic acid codes or polypeptide codes with the computer program. The nucleic acid codes of the invention or the polypeptide codes of SEQ ID Nos. 488 and 489 may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, the nucleic acid codes of the invention or the polypeptide codes of SEQ ID Nos. 488 and 489 may be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparers, identifiers, or sources of reference nucleotide or polypeptide sequences to be compared to the nucleic acid codes of the invention or the polypeptide codes of SEQ ID Nos. 488 and 489. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the nucleic acid codes of the invention or the polypeptide codes of SEQ ID No. 488 and 489. The programs and databases which may be used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applica-

tions Group), MacLook (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, *J. Mol. Biol.* 215: 403 (1990), the disclosure of which is incorporated herein by reference in its entirety), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA,* 85: 2444 (1988), the disclosure of which is incorporated herein by reference in its entirety), FASTDB (Brutlag et al. Comp. App. Biosci. 6:237-245, 1990, the disclosure of which is incorporated herein by reference in its entirety), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius^2.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the EMBL/Swissprotein database, the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwents's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and data bases would be apparent to one of skill in the art given the present disclosure. Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

[0592] It should be noted that the nucleic acid codes of the invention further encompass all of the polynucleotides disclosed, described or claimed in the present invention. Also, it should be noted that the polypeptide codes of SEQ ID Nos. 488 and 489 further encompass all of the polypeptides disclosed, described or claimed in the present invention. Moreover, the present invention specifically contemplates the storage of such codes on computer readable media and computer systems individually or in combination, as well as the use of such codes and combinations in the methods of section "VI. Computer-Related Embodiments."

[0593] Throughout this application, various publications, patents, and published patent applications are cited. The disclosures of the publications, patents, and published patent specifications referenced in this application are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

[0594] VII. DNA Typing Methods and Systems

[0595] The present invention also encompasses a DNA typing system having a much higher discriminatory power than currently available typing systems. The systems and associated methods are particularly applicable in the identification of individuals for forensic science and paternity determinations. These applications have become increasingly important; in forensic science, for example, the identification of individuals by polymorphism analysis has become widely accepted by courts as evidence.

[0596] While forensic geneticists have developed many techniques to compare homologous segments of DNA to determine if the segments are identical or if they differ in one or more nucleotides, each technique still has certain disadvantages. In particular, the techniques vary widely in terms of expense of analysis, time required to carry out an analysis and statistical power.

[0597] RFLP Analysis Methods

[0598] The best known and most widespread method in forensic DNA typing is the restriction fragment length polymorphism (RFLP) analysis. In RFLP testing, a repetitive DNA sequence referred to as a variable number tandem repeat (VNTR) which varies between individuals is analyzed. The core repeat is typically a sequence of about 15 base pairs in length, and highly polymorphic VNTR loci can have an average of about 20 alleles. DNA restriction sites located on either site of the VNTR are exploited to create DNA fragments from about 0.5 Kb to less than 10 Kb which are then separated by electrophoresis, indicating the number of repeats found in the individual at the particular loci. RFLP methods generally consist of (1) extraction and isolation of DNA, (2) restriction endonuclease digestion; (3) separation of DNA fragments by electrophoresis; (4) capillary transfer; (5) hybridization with radiolabelled probes; (6) autoradiography; and (7) interpretation of results (Lee, H. C. et al., Am. J. Forensic. Med. Pathol. 15(4): 269-282 (1994)). RFLP methods generally combine analysis at about 5 loci and have much higher discriminate potential than other available test due the highly polymorphic nature of the VNTRs. However, autoradiography is costly and time consuming and an analysis generally takes weeks or months for turnaround. Additionally, a large amount of sample DNA is required, which is often not available at a crime scene. Furthermore, the reliability of the system and its credibility as evidence is decreased because the analysis of tightly spaced bands on electrophoresis results in a high rate of error.

[0599] PCR Methods

[0600] PCR based methods offer an alternative to RFLP methods. In a first method called AmpFLP, DNA fragments containing VNTRs are amplified and then separated electrophoretically, without the restriction step of RFLP method. While this method allows small quantities of sample DNA to be used, decreases analysis time by avoiding autoradiography, and retains high discriminatory potential, it nevertheless requires electrophoretic separation which takes substantial time and introduces an significant error rate. In another AmpFLP method, short tandem repeats (STRs) of 2 to 8 base pairs are analyzed. STRs are more suitable to analysis of degraded DNA samples since they require smaller amplified fragments but have the disadvantage of requiring separation of the amplified fragments. While STRs are far less informative than longer repeats, similar discriminatory potential can be achieved if enough STRs are used in a single analysis.

[0601] Other methods include sequencing of mitochondrial DNA, which is especially suitable for situations where sample DNA is very degraded or in small quantities. However, only a small region of 1 Kb of the mitochondrial DNA

referred to as the D-Loop locus has been found useful for typing because of its polymorphic nature, resulting in lower discriminatory potential than with RFLP or AmpFLP methods. Furthermore, DNA sequencing is expensive to carry out on a large number of samples.

[0602] Further available methods include dot-blot methods, which involve using allele specific oligonucleotide probes which hybridize sequence specifically to one allele of a polymorphic site. Systems include the HLA DQ-alpha kit developed by Cetus Corp. which has a discriminatory value of about 1 in 20, and a dot-blot strip referred to as the Polymarker strip combining five genetic loci for a discriminatory value of about one in a few thousand. (Weedn, V., Clinics in Lab. Med. 16(1): 187-196 (1996)).

[0603] In addition to difficulties in analysis and time consuming laboratory procedures, it remains desirable for all DNA typing systems to have a higher discriminatory power. Several applications exist in which even the most discriminating tests need improvement in order to remove the considerable remaining doubt resulting from such analyses. Table 6 below lists characteristics of currently available forensic testing systems (Weedn, (1996)) and compares them with the method of the invention.

[0604] Applications

[0605] As described above, an important application of DNA typing tests is to determine whether a DNA sample (e.g. from a crime scene) originated from an individual suspected of leaving said DNA sample.

[0606] There are several applications for DNA typing which require a particularly powerful genotyping system. In a first application, a high powered typing system is advantageous when for example a suspect is identified by searching a DNA profile database such as that maintained by the U.S. Federal Bureau of Investigation. Since databases may contain large numbers of data entries that are expected to increase consistently, currently used forensic systems can be expected to identify several matching DNA profiles due to their relative lack of power. While database searches generally reinforce the evidence by excluding other possible suspects, low powered typing systems resulting in the identification of several individuals may often tend to diminish the overall case against a defendant.

[0607] In another application, a target population is systematically tested to identify an individual having the same DNA profile as that of a DNA sample. In such a situation, a defendant is chosen at random based on DNA profile from a large population of innocent individuals. Since the population tested can often be large enough that at least one positive match is identified, and it is usually not possible to exhaustively test a population, the usefulness of the evidence will depend on the level of significance of the forensic test. In order to render such an application useful as a sole or primary source of evidence, DNA typing systems of extremely high discriminatory potential are required.

[0608] In yet another application, it is desirable to be able to discriminate between related individuals. Because related individuals will be expected to share a large portion of alleles at polymorphic sites, a very high powered DNA typing assay would be required to discriminate between them. This can have important effects if a sample is found to match the defendant's DNA profile and no evidence that the perpetrator is a relative can be found.

[0609] Accordingly, there is a need in this art for a rapid, simple, inexpensive and accurate technique having a very high resolution value to determine relationships between individuals and differences in degree of relationships. Also, there is a need in the art for a very accurate genetic relationship test procedure which uses very small amounts of an original DNA sample, yet produces very accurate results.

[0610] The present invention thus involves methods for the identification of individuals comprising determining the identity of the nucleotides at set of genetic markers in a biological sample, wherein said set of genetic markers comprises at least one DME-related biallelic marker. The present invention provides an extensive set of biallelic markers allowing a higher discriminatory potential than the genetic markers used in current forensic typing systems. Also, biallelic markers can be genotyped in individuals with much higher efficiency and accuracy than the genetic markers used in current forensic typing systems. In preferred embodiments, the invention comprises determining the identity of a nucleotide at a DME-related biallelic marker by single nucleotide primer extension, which does not require electrophoresis as in techniques described above and results in lower rate of experimental error. As shown in Table 6, above, in comparison with PCR based VNTR based methods which allow discriminatory potential of thousands to millions, and RFLP based methods which allow discriminatory potential of merely millions to billions under optimal assumptions, the biallelic marker based method of the present invention provides a radical increase in discriminatory potential.

[0611] Any suitable set of genetic markers and biallelic markers of the invention may be used, and may be selected according to the discriminatory power desired. Biallelic markers, sets of biallelic markers, probes, primers, and methods for determining the identity of said biallelic markers are further described herein.

[0612] Discriminatory Potential of Biallelic Marker Typing

[0613] Calculating Discriminatory Potential

[0614] The discriminatory potential of the forensic test can be determined in terms of the profile frequency, also referred to as the random match probability, by applying the product rule. The product rule involves multiplying the allelic frequencies of all the individual alleles tested, and multiplying by an additional factor of 2 for each heterozygous locus.

[0615] In one example discussed below, the discriminatory potential of biallelic marker typing can be considered in the context of forensic science. In order to determine the discriminatory potential with respect to the numbers of biallelic markers to be used in a genetic typing system, the formulas and calculations below assume that (1) the population under study is sufficiently large (so that we can assume no consanguinity); (2) all markers chosen are not correlated, so that the product rule (Lander and Budlowle (1992)) can be applied; and (3) the ceiling rule can be applied or that the allelic frequencies of markers in the population under study are known with sufficient accuracy.

[0616] As noted in Weir, B. S., *Genetic data Analysis II: Methods for Discrete population genetic Data*, Sinauer Assoc., Inc., Sunderland, Mass., USA, 1996, the example assumes a crime has been committed and a sample of DNA from the perpetrator (P) is available for analysis. The genotype of this DNA sample can be determined for several genetic markers, and the profile A of the perpetrator can thereby be determined.

[0617] In this example, one suspect (S) is available for typing. The same set of genetic markers, such as the biallelic markers of the invention, are typed and the same profile A is obtained for (S) and (P). Two hypotheses are thus presented as follows:

[0618] (1) either S is P (event C)

[0619] (2) either S is not P (event C).

[0620] The ratio L of both probabilities can then be calculated using the following equation:

$$L = \frac{pr(S = A, P = A/C)}{pr(S = A, P = A/\overline{C})}$$

[0621] L can then further be calculated by the following equation:

$$L = \frac{1}{pr(P = A/S = A,\overline{C})} \quad (1) \qquad \text{Equation 1}$$

[0622] These probabilities as well as L can be calculated in several settings, notably for different kinship coefficients between P and S for a genetic marker (see Weir, (1996)).

[0623] Assuming that all genetic markers chosen are independent of each other, the global ratio L for a set of genetic markers will be the product over each genetic marker of all L.

[0624] It is further possible to estimate the mean number of biallelic markers or VNTRs required to have a ratio L equal to 10^8 or 10^6 by calculating the expectancy of the random variable L using the following equation:

$$E(L) = \prod_{i=1}^{N} E(L_i) \quad \text{where } N \text{ is the number of loci}$$

[0625] where N is the number of loci

$$E(L_i) = \sum_{j=1}^{G_i} pr(P = A_{ij}/S = A_{ij}, \overline{C}) \cdot L_{ij},$$

[0626] where A_{ij} is the genotype j at the ith marker, L_{ij} the ratio associated with such genotype, G_i being the number of genotypes at locus i. From equation 1, it can easily be

derived that the expectancy of L_i is G_i, the number of possible genotypes of this marker.

[0627] The general expectancy for a set of genetic markers can then be expressed by the following equation:

$$E(L) = \prod_{i=1}^{N} G_i \quad (2) \qquad \text{Equation 2}$$

[0628] Biallelic Marker-Based DNA Typing Systems

[0629] Using the equations described above, it is possible to select biallelic marker-based DNA typing systems having a desired discriminatory potential.

[0630] Using biallelic markers, E(L) can thus be expressed as 3^N. When using VNTR-based DNA typing systems, assuming the VNTRs have 10 alleles, E(L) can be expressed as 55^N. Based on these results, the number of biallelic markers or VNTRs needed to obtain, in mean, a ratio of at least 10^6 or 10^8 can calculated, and are set forth below in Table 7.

[0631] Thus, in a first embodiment, DNA typing systems and methods of the invention may comprise genotyping a set of at least 13 or at least 17 biallelic markers to obtain a ratio of at least 10^6 or 10^8, assuming a flat distribution of L across the biallelic markers. In preferred embodiments, a greater number of biallelic markers is genotyped to obtain a higher L value. Preferably at least 1, 2, 3, 4, 5, 10, 13, 15, 17, 20, 25, 30, 40, 50, 70, 85, 100, 150, or all of the DME-related biallelic markers are genotyped. Said DNA typing systems of the invention would result in L values as listed in Table 8 below as an indication of the discriminate potential of the systems of the invention.

[0632] In situations where the distribution of L is not flat, such as in the worst case when the perpetrator is homozygous for the major allele at each genetic locus and L thus takes the lowest value, a larger number of biallelic markers is required for the same discriminatory potential. Therefore, in preferred embodiments, DNA typing systems and methods of the invention using a larger number of biallelic markers allow for uneven distributions of L across the biallelic markers. For example, assuming unrelated individuals, a set of independent markers having an allelic frequency of 0.1/0.9, and the genetic profile of a homozygote at each genetic loci for the major allele, 66 biallelic markers are required to obtain a ratio of 10^6, and 88 biallelic markers are required to obtain a ratio of 10^8. Thus, in preferred embodiments based on the use of markers having a major allele of sufficiently high frequency, this is a first estimation of the upper bound of markers required in a DNA typing system.

[0633] In further embodiments, it is also desirable to have the ability to discriminate between relatives. Although unrelated individuals have a low probability of sharing genetic profiles, the probability is greatly increased for relatives. For example, the DNA profile of a suspect matches the DNA profile of a sample at a crime scene, and the probability of obtaining the same DNA profile if left by an untyped relative is required. Table 9 below (Weir (1996)) lists probabilities for several different types of relationships, assuming alleles

A$_i$ and A$_j$, and population frequencies p$_i$ and p$_j$, and lists likelihood ratios assuming genetic loci having allele frequencies of 0.1.

[0634] In one example, where the suspect is the full brother of the perpetrator, the number of required biallelic markers will be 187 assuming the profile is that of a homozygote for the major allele at each biallelic marker.

[0635] In yet further embodiments, the DNA typing systems and methods of the present invention may further take into account effects of subpopulations on the discriminatory potential. In embodiments described above for example, DNA typing systems consider close familial relationships, but do not take into account membership in the same population. While population membership is expected to have little effect, the invention may further comprise genotyping a larger set of biallelic markers to achieve higher discriminatory potential. Alternatively, a larger set of biallelic markers may be optimized for typing selected populations; alternatively, the ceiling principle may be used to study allele frequencies from individuals in various populations of interest, taking for any particular genotype the maximum allele frequency found among the populations.

[0636] The invention thus encompasses methods for genotyping comprising determining the identity of a nucleotide at least 13, 15, 17, 20, 25, 30, 40, 50, 66, 70, 85, 88, 100, 187, 200, 300, 500, 700, 1000 or 2000 biallelic markers in a biological sample, wherein at least 1, 2, 3, 4, 5, 10, 13, 17, 20, 25, 30, 40, 50, 70, 85, 100, 150, 200, 300, 400 or all of said biallelic markers are DME-related biallelic markers selected from the group consisting of SEQ ID Nos. 485-487, 494-531, 533-547, 549-846, 848-956, 958-977.

[0637] Any markers known in the art may be used with the DME-related biallelic markers of the present invention in the DNA typing methods and systems described herein, for example in anyone of the following web sites offering collections of SNPs and information about those SNPs:

[0638] The Genetic Annotation Initiative (web site: cgap-.nci.nih.gov/GAI/). An NIH run site which contains information on candidate SNPs thought to be related to cancer and tumorigenesis generally.

[0639] dbSNP Polymorphism Repository (world wide web site: ncbi.nlm.nih.gov/SNP/). A more comprehensive NIH-run database containing information on SNPs with broad applicability in biomedical research.

[0640] HUGO Mutation Database Initiative (web site: ariel.ucs.unimelb.edu.au:80/~cotton/mdi.htm). A database meant to provide systematic access to information about human mutations including SNPs. This site is maintained by the Human Genome Organisation (HUGO).

[0641] Human SNP Database (world wide web site:—genome.wi.niit.edu/SNP/human/index.html). Managed by the Whitehead Institute for Biomedical Research Genome Institute, this site contains information about SNPs resulting from the many Whitehead research projects on mapping and sequencing.

[0642] SNPs in the Human-Genome SNP database (world wide web site: ibc.wustl.edu/SNP). This website provides access to SNPs that have been organized by chromosomes and cytogenetic location. The site is run by Washington University.

[0643] HGBase (web site: hgbase.cgr.ke.se/). HGBASE is an attempt to summarize all known sequence variations in the human genome, to facilitate research into how genotypes affect common diseases, drug responses, and other complex phenotypes, and is run by the Karolinska Institute of Sweden.

[0644] The SNP Consortium Database (web site: snp.c-shl.org/db/snp/map). A collection of SNPs and related information resulting from the collaborative effort of a number of large pharmaceutical and information processing companies.

[0645] GeneSNPs (world wide web site: genome.uta-h.edu/genesnps/). Run by the University of Utah, this site contains information about SNPs resulting from the U.S. National Institute of Environmental Health's initiative to understand the relationship between genetic variation and response to environmental stimuli and xenobiotics.

[0646] In addition, biallelic markers provided in the following patents and patent applications may also be used with the map-realted biallelic markers of the invention in the DNA typing methods and systems described above: U.S. Ser. No. 60/206,615, filed Mar. 24, 2000; U.S. Ser. No. 60/216,745, filed Jun. 30, 2000; WIPO Serial No. PCT/IB00/00184, filed Feb. 11, 2000; WIPO Serial No. PCT/IB98/01193, filed Jul. 17, 1998; PCT Publication No. WO 99/54500, filed Apr. 21, 1999; and WIPO Serial No. PCT/IB00/00403, filed Mar. 24, 2000.

[0647] Biallelic markers, sets of biallelic markers, probes, primers, and methods for determining the identity of a nucleotide at said biallelic markers are also encompassed and are further described herein, and may encompass any further limitation described in this disclosure, alone or in any combination.

[0648] Forensic matching by microsequencing is further described in Example 7 below.

EXAMPLES

[0649] Several of the methods of the present invention are described in the following examples, which are offered by way of illustration and not by way of limitation. Many other modifications and variations of the invention as herein set forth can be made without departing from the spirit and scope thereof and therefore only such limitations should be imposed as are indicated by the appended claims. All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Example 1

De Novo Identification of Biallelic Markers

[0650] The biallelic markers set forth in this application were isolated from human genomic sequences. To identify biallelic markers, genomic fragments were amplified, sequenced and compared in a plurality of individuals.

[0651] DNA Samples

[0652] Donors were unrelated and healthy. They represented a sufficiently diverse group for being representative of a French heterogeneous population. The DNA from 100 individuals was extracted and tested for the de novo identification of biallelic markers.

[0653] DNA samples were prepared from peripheral venous blood as follows. Thirty ml of peripheral venous blood were taken from each donor in the presence of EDTA. Cells (pellet) were collected after centrifugation for 10 minutes at 2000 rpm. Red cells were lysed in a lysis solution (50 ml final volume: 10 mM Tris pH 7.6; 5 mM $MgCl_2$; 10 mM NaCl). The solution was centrifuged (10 minutes, 2000 rpm) as many times as necessary to eliminate the residual red cells present in the supernatant, after resuspension of the pellet in the lysis solution. The pellet of white cells was lysed overnight at 42° C. with 3.7 ml of lysis solution composed of: (a) 3 ml TE 10-2 (Tris-HCl 10 mM, EDTA 2 mM)/NaCl 0.4 M; (b) 200 μl SDS 10%; and (c) 500 μl proteinase K (2 mg proteinase K in TE 10-2/NaCl 0.4 M).

[0654] For the extraction of proteins, 1 ml saturated NaCl (6M) (1/3.5 v/v) was added. After vigorous agitation, the solution was centrifuged for 20 minutes at 10000 rpm. For the precipitation of DNA, 2 to 3 volumes of 100% ethanol were added to the previous supernatant, and the solution was centrifuged for 30 minutes at 2000 rpm. The DNA solution was rinsed three times with 70% ethanol to eliminate salts, and centrifuged for 20 minutes at 2000 rpm. The pellet was dried at 37° C., and resuspended in 1 ml TE 10-1 or 1 ml water. The DNA concentration was evaluated by measuring the OD at 260 nm (1 unit OD=50 μg/ml DNA). To determine the presence of proteins in the DNA solution, the OD 260/OD 280 ratio was determined. Only DNA preparations having a OD 260/OD 280 ratio between 1.8 and 2 were used in the subsequent examples described below. DNA pools were constituted by mixing equivalent quantities of DNA from each individual.

[0655] Amplification of Genomic DNA by PCR

[0656] Amplification of specific genomic sequences was carried out on pooled DNA samples obtained as described above.

[0657] Amplification Primers

[0658] The primers used for the amplification of human genomic DNA fragments were defined with the OSP software (Hillier & Green, 1991). Preferably, primers included, upstream of the specific bases targeted for amplification, a common oligonucleotide tail useful for sequencing. Primers PU contain the following additional PU 5' sequence: TGTAAAACGACGGCCAGT; primers RP contain the following RP 5' sequence: CAGGAAACAGCTATGACC. Primers are listed in Table 17.

[0659] Amplification

[0660] PCR assays were performed using the following protocol:

Final volume	25	μl
DNA	21	ng/μ
$MgCl_2$	2	mM
dNTP (each)	200	μM
primer (each)	2.9	ng/μl
Ampli Taq Gold DNA polymerase	0.05	unit/μl

[0661] PCR buffer (10×=0.1 M Tris HCl pH 8.3 0.5M KCl) 1×

[0662] DNA amplification was performed on a Genius II thermocycler. After heating at 94° C. for 10 min, 40 cycles were performed. Cycling times and temperatures were: 30 sec at 94° C., 55° C. for 1 min and 30 sec at 72° C. Holding for 7 min at 72° C. allowed final elongation. The quantities of the amplification products obtained were determined on 96-well microtiter plates, using a fluorometer and Picogreen as intercalant agent (Molecular Probes).

[0663] Sequencing of Amplified Genomic DNA and Identification of Biallelic Polymorphisms

[0664] Sequencing of the amplified DNA was carried out on ABI 377 sequencers. The sequences of the amplification products were determined using automated dideoxy terminator sequencing reactions with a dye terminator cycle sequencing protocol. The products of the sequencing reactions were run on sequencing gels and the sequences were determined using gel image analysis (ABI Prism DNA Sequencing Analysis software 2.1.2 version).

[0665] The sequence data were further evaluated to detect the presence of biallelic markers within the amplified fragments. The polymorphism search was based on the presence of superimposed peaks in the electrophoresis pattern resulting from different bases occurring at the same position. However, the presence of two peaks can be an artifact due to background noise. To exclude such an artifact, the two DNA strands were sequenced and a comparison between the two strands was carried out. In order to be registered as a polymorphic sequence, the polymorphism had to be detected on both strands. Further, some biallelic single nucleotide polymorphisms were confirmed by microsequencing as described below.

[0666] Biallelic markers were identified in the analyzed fragments, and are shown in Table 11(A-B) and Table 3(A-D).

Example 2

Genotyping of Biallelic Markers

[0667] The biallelic markers identified as described above were further confirmed and their respective frequencies were determined through microsequencing. Microsequencing was carried out on individual DNA samples obtained as described herein.

[0668] Microsequencing Primers

[0669] Amplification of genomic DNA fragments from individual DNA samples was performed as described in Example 1 using the same set of PCR primers (See Table 17). Microsequencing was carried out on the amplified fragments using specific primers (See Table 16). The preferred primers used in microsequencing had about 19 nucleotides in length and hybridized just upstream of the considered polymorphic base.

[0670] The microsequencing reactions were performed as follows: 5 μl of PCR products were added to 5 μl purification mix (2U SAP (Shrimp alkaline phosphate) (Amersham E70092X)); 2U Exonuclease I (Amersham E70073Z); and 1 μl SAP buffer (200 mM Tris-HCl pH 8, 100 mM $MgCl_2$) in a microtiter plate. The reaction mixture was incubated 30 minutes at 37° C., and denatured 10 minutes at 94° C. afterwards. To each well was then added 20 μl of microsequencing reaction mixture containing: 10 pmol microsequencing oligonucleotide (19mers, GENSET, crude synthesis, 5 OD), 1 U Thermosequenase (Amersham E79000G), 1.25 μl Thermosequenase buffer (260 mM Tris HCl pH 9.5, 65 mM $MgCl_2$), and the two appropriate fluorescent ddNTPs complementary to the nucleotides at the polymorphic site

corresponding to both polymorphic bases (11.25 nM TAMRA-ddTTP; 16.25 nM ROX-ddCTP; 1.675 nM REG-ddATP; 1.25 nM RHO-ddGTP; Perkin Elmer, Dye Terminator Set 401095). After 4 minutes at 94° C., 20 PCR cycles of 15 sec at 55° C., 5 sec at 72° C., and 10 sec at 94° C. were carried out in a Tetrad PTC-225 thermocycler (MJ Research). The microtiter plate was centrifuged 10 sec at 1500 rpm. The unincorporated dye terminators were removed by precipitation with 19 μl MgCl$_2$ 2 mM and 55 μl 100% ethanol. After 15 minute incubation at room temperature, the microtiter plate was centrifuged at 3300 rpm 15 minutes at 4° C. After discarding the supernatants, the microplate was evaporated to dryness under reduced pressure (Speed Vac). Samples were resuspended in 2.5 μl formamide EDTA loading buffer and heated for 2 min at 95° C. 0.8 μl microsequencing reaction were loaded on a 10% (19:1) polyacrylanude sequencing gel. The data were collected by an ABI PRISM 377 DNA sequencer and processed using the GENESCAN software (Perkin Elmer).

[0671] Frequency of Biallelic Markers

[0672] Frequencies are reported for the less common allele only and are shown in Table 11(A-B). The frequencies for both alleles are shown for preferred MGST-II, ME1, UGT1A7 and UGT2B4 markers in Tables 19, 20, 21 and 22, respectively.

Example 3

Association Between Asthma and the Biallelic Markers of the MGST-II Gene

[0673] Collection of DNA Samples from Trait Positive and Control Individuals

[0674] The disease trait followed in this association study was asthma, a disease involving the leukotriene pathway. The asthmatic population corresponded to 298 individuals that took part in a clinical study for the evaluation of the anti-asthmatic drug zileuton. More than 90% of these 297 asthmatic individuals had a Caucasian ethnic background. The control population corresponded to 178 individuals from a random US Caucasian population.

[0675] Genotyping of Affected and Control Individuals

[0676] The general strategy to perform the association studies was to individually scan the DNA samples from all individuals in each of the populations described above in order to establish the allele frequencies of the above described biallelic markers in each of these populations.

[0677] Allelic frequencies of the above-described biallelic marker alleles in each population were determined by performing microsequencing reactions on amplified fragments obtained by genomic PCR performed on the DNA samples from each individual. Genomic PCR and microsequencing were performed as detailed above in Examples 1 and 2 using the described PCR and microsequencing primers.

[0678] Haplotype Frequency Analysis

[0679] None of the single marker alleles showed a significant association with asthma however, significant results were obtained in haplotype studies. Allelic frequencies were useful to check that the markers used in the haplotype studies meet the Hardy-Weinberg proportions (random mating).

[0680] The results of the haplotype analysis using 13 biallelic markers (12-421-135, 12-421-140, 12-430-80,

12-441-233, 12-442-133, 12-447-58, 12-455-326, 12-461-299, 12-45-3429, 12-424-198, 12-454-363, 12-458-196 and 12-426-154) are shown in Table 23. Haplotype analysis for association of MGST-II biallelic markers and asthma was performed by estimating the frequencies of all possible 2, 3 and 4 marker haplotypes in the asthmatic and control populations described above. Haplotype estimations were performed by applying the Expectation-Maximization (EM) algorithm (Excoffier and Slatkin, *Mol. Biol. Evol.,* 12:921-927, 1995), using the EM-HAPLO program (Hawley et al., *Am. J. Phys. Anthropol.,* 18:104, 1994) as described above. Estimated haplotype frequencies in the asthmatic and control population were compared by means of a chi-square statistical test (one degree of freedom).

[0681] Table 23 shows the most significant haplotypes obtained. Haplotype No. 6 consisting of three biallelic markers (12-455-326, 12-453-429 and 12-424-198) had a p-value of $3.2e10^{-5}$ and an odds ratio of 12.22. Estimated haplotype frequencies were 11.8% in the cases and 1.1% in the controls. Haplotype No. 18 consisting of four biallelic markers (12-455-326, 12-453-429, 12-424-198 and 12-454-363) had a p-value of $1.2e10^{-6}$ and an odds ratio of 100.00. Both haplotypes are related as three out of four biallelic marker alleles (G at 12-455-326; C at 12-453-429 and T and 12-454-363) are common to both haplotypes. Haplotype No. 19 consisting of four biallelic markers (12-447-58, 12-455-326, 12-461-299 and 12-453-429) had a p-value of $8.2e10^{-6}$ and an odds ratio of 100.00. Markers 12-455-326 and 12-453-429 are common in all three significant haplotypes; therefore, they represent preferred markers for the diagnosis of asthma. Haplotypes Nos. 6, 18 and 19 are strongly associated with asthma. Haplotypes Nos. 7-17 and 20-30 also showed very significant association (see Table 23).

[0682] The statistical significance of the results obtained for the haplotype analysis was evaluated by a phenotypic permutation test reiterated 1000 or 10,000. For this computer simulation, data from the asthmatic and control individuals were pooled and randomly allocated to two groups which contained the same number of individuals as the case-control populations used to produce the data summarized in Table 23. A haplotype analysis was then run on these artificial groups for the 3 markers included in haplotype No. 6 (haplotype GCT), the 4 markers included in haplotype No. 18 (haplotype GCTG) and the 4 markers included in haplotype No. 19 (haplotype CATT), all of which showed a strong association with asthma. This experiment was reiterated 1000 and 10,000 times and the results are shown in Table 24. These results demonstrate that among 1000 iterations only 3 and among 10,000 iterations only 31 of the obtained haplotypes had a p-value comparable to the one obtained for haplotype No. 6 (haplotype GCT). These results demonstrate that among 1000 iterations 0 and among 10,000 iterations only 5 of the obtained haplotypes had a p-value comparable to the one obtained for haplotype No. 18 (haplotype GCTG). These results further demonstrate that among 1000 iterations only 12 and among 10,000 iterations only 76 of the obtained haplotypes had a p-value comparable to the one obtained for haplotype No. 19 (haplotype CATT). These results clearly validate the statistical significance of the association between the haplotypes shown in Table 23 and asthma.

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Example 4

Association Between Side Effects Upon Treatment With the Anti-Asthmatic Drug Zileuton (Zyflo™) and the Biallelic Markers of the Invention

[0683] Collection of DNA Samples From Trait Positive and Control Individuals

[0684] The side effect examined in this study was the hepatotoxicity experienced by asthmatic individuals as a result of their treatment with Zileuton as part of a clinical study. Asthmatic individuals were unrelated and more than 90% of the individuals had a Caucasian ethnic background. Hepatotoxicity was monitored by measuring the serum levels of alanine aminotransferase (ALT), which is a sensitive indicator of liver cell damage.

[0685] More than 90% of the asthmatic individuals participating in this study did not experience Zileuton-associated ALT increase compared to their ALT levels prior to zileuton intake. As mentioned above, an association study is more informative if the populations considered present extreme phenotypes. Therefore, the asthmatic individuals, which were selected for the side effect positive trait (ALT+), corresponded to 89 individuals that presented at least 3 times the upper limit of normal (ULN) level of ALT. On the other side, the asthmatic individuals that were selected for the side effect negative trait (ALT−) corresponded to 208 individuals that presented less than 1×ULN of ALT. ALT+ and ALT− populations corresponded to 4% and 35% respectively of the total asthmatic individuals that participated in this study.

[0686] Genotyping of Affected and Control Individuals

[0687] The general strategy to perform the Association studies was to individually scan the DNA samples from all individuals in each of the populations described above in order to establish the allele frequencies of the above described biallelic markers in each of these populations.

[0688] Allelic frequencies of the above-described biallelic marker alleles in each population were determined by performing microsequencing reactions on amplified fragments obtained by genomic PCR performed on the DNA samples from each individual. Genomic PCR and microsequencing were performed as detailed above in Examples 1 and 2 using the described PCR and microsequencing primers.

[0689] Allelic Frequency Analysis

[0690] None of the single marker alleles showed a significant association with hepatotoxicity to zileuton however, significant results were obtained in haplotype studies. See Tables 25, 28, and 31 for the allelic frequency analysis of MGST-II, UGT1A7, and UGT2B4, respectively.

[0691] Haplotype Frequency Analysis of the MGST-II-Related Biallelic Markers

[0692] The results of the haplotype analysis using 13 MGST-II-related biallelic markers (12-421-135, 12-421-140, 12-430-80, 12-441-233, 12-442-133, 12-447-58, 12-455-326, 12-461-299, 12-453-429, 12-424-198, 12-454-363, 12-458-196 and 12-426-154) are shown in Table 26. Haplotype analysis for association of MGST-II biallelic markers and asthma was performed by estimating the frequencies of all possible 2, 3 and 4 marker haplotypes in the ALT+ and ALT− populations described above. Haplotype estimations were performed by applying the Expectation-Maximization (EM) algorithm (Excoffier and Slatkin, *Mol. Biol. Evol.*, 12:921-927, 1995), using the EM-HAPLO pro-

gram (Hawley et al., *Am. J. Phys. Anthropol.*, 18:104, 1994) as described above. Estimated haplotype frequencies in the ALT+ and ALT− populations were compared by means of a chi-square statistical test (one degree of freedom).

[0693] Table 26 shows the most significant haplotypes obtained. Haplotype No. 6 consisting of three biallelic markers (12-441-233, 12-461-299 and 12-453-429) presented a p-value of 1.5×10^{-5} and an odd-ratio of 3.63. Estimated haplotype frequencies were 15.7% in the cases and 4.9% in the controls. Haplotype No. 19 consisting of four biallelic markers (12-441-233, 12-461-299, 12-453-429 and 12-426-154) had a p-value of 5.2×10^{-7} and an odd ratio of 5.75. Estimated haplotype frequencies were 14.1% in the cases and 2.8% in the controls. Both haplotypes showed strong association with elevated serum ALT level upon treatment with zileuton. Both haplotypes are related as three out of four biallelic marker alleles (C at 12-441-233, T at 12-461-299 and T at 12-453-429) are common to both haplotypes. Haplotypes Nos. 7-18 and 20-31 of Table 25 also showed very significant association.

[0694] The statistical significance of the results obtained for the haplotype analysis was evaluated by a phenotypic permutation test reiterated 1000 or 10,000 times on a computer. For this computer simulation, data from the ALT+ and ALT− populations were pooled and randomly allocated to two groups which contained the same number of individuals as the ALT+ and ALT− populations used to produce the data summarized in Table 26. A haplotype analysis was then run on these artificial groups for the 3 markers included in haplotype No. 6 (haplotype CTT) and for the 4 markers included in haplotype No. 19 (haplotype CTTA) which, showed the strongest association with secondary effects to zileuton. This experiment was reiterated 1000 and 10,000 times and the results are shown in Table 27. These results demonstrate that among 1000 iterations only 1 and among 10,000 iterations only 12 of the obtained haplotypes had a p-value comparable to the one obtained for haplotype No. 6 (haplotype CTT). These results further demonstrate that among 1000 iterations only 1 and among 10,000 iterations only 7 of the obtained haplotypes had a p-value comparable to the one obtained for haplotype No. 19 (haplotype CTTA). These results clearly validate the statistical significance of the association between hepatotoxicity to Zyflo™ and the haplotypes shown in Table 26.

[0695] Haplotype Frequency Analysis of the UGT1A7-Related Biallelic Markers

[0696] The results of the haplotype analysis using 7 UGT1A7-related biallelic markers (12-121-326, 12-128-225, 12-148-311, 12-156-91, 12-139-380, 12-140-134, 12-142-321) are shown in Table 29. Haplotype analysis for association of UGT1A7 biallelic markers and asthma was performed by estimating the frequencies of all possible 2, 3 and 4 marker haplotypes in the ALT+ and ALT− populations described above. Haplotype estimations were performed by applying the Expectation-Maximization (EM) algorithm (Excoffier and Slatkin, *Mol. Biol. Evol.*, 12:921-927, 1995), using the EM-HAPLO program (Hawley et al., *Am. J. Phys. Anthropol.*, 18:104, 1994) as described above. Estimated haplotype frequencies in the ALT+ and ALT− populations were compared by means of a chi-square statistical test (one degree of freedom).

[0697] Table 29 shows the most significant haplotypes obtained. Haplotype No. 8 consisting of three biallelic markers (12-121-326, 12-156-91 and 12-142-321) presented a p-value of 7.0×10^{-4} and an odd-ratio of 2.98. Estimated

haplotype frequencies were 14.1% in the cases and 5.2% in the controls. Haplotype No. 12 consisting of four biallelic markers (12-128-225, 12-156-91, 12-139-380, 12-140-134) had a p-value of 7.7 10^{-3} and an odd ratio of 1.85. Estimated haplotype frequencies were 27.4% in the cases and 17.0% in the controls.

[0698] The statistical significance of the results obtained for the haplotype analysis was evaluated by a phenotypic permutation test reiterated 1000 or 10,000 times on a computer. For this computer simulation, data from the ALT+ and ALT– populations were pooled and randomly allocated to two groups which contained the same number of individuals as the ALT+ and ALT– populations used to produce the data summarized in Table 29. A haplotype analysis was then run on these artificial groups for the 4 markers included in haplotype No. 12 (haplotype AAGT), which showed the strongest association with secondary effects to zileuton. This experiment was reiterated 1000 and 10,000 times and the results are shown in Table 30.

[0699] Haplotype Frequency Analysis of the UGT2B4-Related Biallelic Markers

[0700] The results of the haplotype analysis using 8 UGT2B4-related biallelic markers (12-653-423, 10-470-25, 10-471-84, 10-471-85, 12-637-219, 12-639-95, 12-652-203, 12-462-417) are shown in Tables 32A-C. Haplotype analysis for association of UGT2B4 biallelic markers and asthma was performed by estimating the frequencies of all possible 2, 3 and 4 marker haplotypes in the ALT+ and ALT– populations described above. Haplotype estimations were performed by applying the Expectation-Maximization (EM) algorithm (Excoffier and Slatkin, *Mol. Biol. Evol.,* 12:921-927, 1995), using the EM-HAPLO program (Hawley et al., *Am. J. Phys. Anthropol.,* 18:104, 1994) as described above. Estimated haplotype frequencies in the ALT+ and ALT– populations were compared by means of a chi-square statistical test (one degree of freedom).

[0701] Tables 32A-C show the most significant haplotypes obtained. Haplotype No. 1 consisting of two biallelic markers (10-470-25 and 12-652-203) presented a p-value of 3.8 10^{-3} and an odd-ratio of 1.83. Estimated haplotype frequencies were 30.3% in the cases and 19.2% in the controls. Haplotype No. 35 consisting of four biallelic markers (12-653-423, 10-470-25, 12-639-95, 12-652-203) had a p-value of 1.8 10^{-4} and an odd ratio of 2.45. Estimated haplotype frequencies were 25.5% in the cases and 12.3% in the controls.

[0702] The statistical significance of the results obtained for the haplotype analysis was evaluated by a phenotypic permutation test reiterated 1000 or 10,000 times on a computer. For this computer simulation, data from the ALT+ and ALT– populations were pooled and randomly allocated to two groups which contained the same number of individuals as the ALT+ and ALT– populations used to produce the data summarized in Tables 32A-C. A haplotype analysis was then run on these artificial groups for the 2 markers included in haplotype No. 1 (haplotype TC), which showed the strongest association with secondary effects to zileuton. This experiment was reiterated 1000 and 10,000 times and the results are shown in Table 33.

Example 5

Identification of Mutations and of Low Frequency Alleles of the MGST-II Gene

[0703] Exons 3-5, the 5'UTR region and the 3'region of the MGST-II gene were screened for mutations by comparing their sequence in individuals exhibiting elevated ALT levels upon treatment with zileuton (ALT+) and in individuals showing normal ALT levels upon treatment with zileuton (ALT–). ALT+ and ALT– individuals are further described in Example 4. Intron sequences immediately flanking the exons were also screened.

[0704] To identify mutations, fragments of the MGST-II gene were amplified, sequenced and compared in ALT+ and ALT– individuals. DNA samples from each individual were processed separately.

[0705] DNA Samples

[0706] Individual DNA samples were obtained as described in Example 1.

[0707] Amplification of the MGST-II Gene

[0708] Amplification primers are described in Table 17 and PCR assays were performed as described in Example 1.

[0709] Sequencing of Amplified Genomic DNA: Identification of Mutations and of Low Frequency Polymorphisms

[0710] Sequencing of the amplified DNA was carried out on ABI 377 sequencers. The sequences of the amplification products were determined using automated dideoxy terminator sequencing reactions with a dye terminator cycle sequencing protocol. The products of the sequencing reactions were run on sequencing gels and the sequences were determined using gel image analysis (ABI Prism DNA Sequencing Analysis software 2.1.2 version).

[0711] The sequence data was further analyzed to detect the presence of mutations and of low frequency alleles. The sequences obtained with 79 ALT+ individuals and 105 ALT– individuals were compared. New polymorphisms/mutations were detected and the genotype of each individual for these markers was determined. Results are shown below:

[0712] Eight polymorphisms were identified in the region of the MGST-II gene that was scanned. Three mutations were identified in the 3'UTR region. One mutation in exon 4 causes an amino acid substitution (Tyr→His) at the polypeptide level. A mutation in exon 5 introduces a stop codon into the ORF leading to the expression of a truncated MGST-II polypeptide. These mutations modify the specificity, activity and function of the MGST-II enzyme and therefore represent functional mutations of the MGST-II gene.

Example 6

Preparation of Antibody Compositions to MGST-II Variants

[0713] Preferably antibody compositions, specifically binding the 93-His variant or the SEQ ID No. 489 variant of MGST-II, are prepared.

[0714] Substantially pure protein or polypeptide is isolated from transfected or transformed cells containing an expression vector encoding the MGST-II protein or a portion thereof. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms per ml. Monoclonal or polyclonal antibodies to the protein can then be prepared as follows:

[0715] Monoclonal Antibody Production by Hybridoma Fusion

[0716] Monoclonal antibody to epitopes in the MGST-II protein or a portion thereof can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (*Nature,* 256:495, 1975) or derivative methods

thereof (see Harlow and Lane, *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, pp. 53-242, 1988).

[0717] Briefly, a mouse is repetitively inoculated with a few micrograms of the MGST-II protein or a portion thereof over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and derivative methods thereof Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, N.Y. Section 21-2.

[0718] Polyclonal Antibody Production by Immunization

[0719] Polyclonal antiserum containing antibodies to heterogeneous epitopes in the MGST-II protein or a portion thereof can be prepared by immunizing suitable non-human animal with the MGST-II protein or a portion thereof, which can be unmodified or modified to enhance immunogenicity. A suitable non-human animal is preferably a non-human mammal is selected, usually a mouse, rat, rabbit, goat, or horse. Alternatively, a crude preparation which, has been enriched for MGST-II concentration can be used to generate antibodies. Such proteins, fragments or preparations are introduced into the non-human mammal in the presence of an appropriate adjuvant (e.g. aluminum hydroxide, RIBI, etc.) which is known in the art. In addition the protein, fragment or preparation can be pretreated with an agent which will increase antigenicity, such agents are known in the art and include, for example, methylated bovine serum albumin (mBSA), bovine serum albumin (BSA), Hepatitis B surface antigen, and keyhole limpet hemocyanin (KLH). Serum from the immunized animal is collected, treated and tested according to known procedures. If the serum contains polyclonal antibodies to undesired epitopes, the polyclonal antibodies can be purified by immunoaffinity chromatography.

[0720] Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker (1987). An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. J. *Clin. Endocrinol. Metab.* 33:988-991 (1971).

[0721] Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. (See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell, 1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum. Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., (Chap. 42: *Manual of Clinical Immunology,* 2d Ed. Rose and Friedman, Eds., *Amer. Soc. For Microbiol.,* Washington, D.C., 1980).

[0722] Antibody preparations prepared according to either the monoclonal or the polyclonal protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

Example 7

Forensic Matching by Microsequencing

[0723] DNA samples are isolated from forensic specimens of, for example, hair, semen, blood or skin cells by conventional methods. A panel of PCR primers based on a number of the sequences of the invention is then utilized according to the methods described herein to amplify DNA of approximately 500 bases in length from the forensic specimen. The alleles present at each of the selected biallelic markers site according to biallelic markers of the invention are then identified according Examples discussed herein. A simple database comparison of the analysis results determines the differences, if any, between the sequences from a subject individual or from a database and those from the forensic sample. In a preferred method, statistically significant differences between the suspect's DNA sequences and those from the sample conclusively prove a lack of identity. This lack of identity can be proven, for example, with only one sequence. Identity, on the other hand, should be demonstrated with a large number of sequences, all matching. Preferably, a minimum of 13, 17, 20, 25, 30, 40, 50, 66, 70, 85, 88, 100, 187, 200 or 500 biallelic markers are used to test identity between the suspect and the sample.

[0724] It should be noted that in the accompanying Sequence Listing, all instances of the symbol "n" in the nucleic acid sequences mean that the nucleotide can be adenine, guanine, cytosine or thymine.

[0725] In some instances, the polymorphic bases of the biallelic markers alter the identity of amino acids in the encoded polypeptide. This is indicated in the accompanying Sequence Listing by use of the feature VARIANT, placement of a Xaa at the position of the polymorphic amino acid, and definition of Xaa as the two alternative amino acids. For example, if one allele of a biallelic marker is the codon CAC, which encodes histidine, while the other allele of the biallelic marker is CAA, which encodes glutamine, the Sequence Listing for the encoded polypeptide will contain an Xaa at the location of the polymorphic amino acid. In this instance, Xaa would be defined as being histidine or glutamine.

[0726] In other instances, Xaa may indicate an amino acid whose identity is unknown because of nucleotide sequence ambiguity. In this instance, the feature UNSURE is used, Xaa is placed at the position of the unknown amino acid, and Xaa is defined as being any of the 20 amino acids or a limited number of amino acids suggested by the genetic code.

[0727] The Sequence Listing for this application is on duplicate compact discs labeled "Copy 1" and "Copy 2." Copy 1 and Copy 2 each contain only one file named "SEQLIST4filing.TXT" which was created on Nov. 6, 2002, and is 990 KB. The entire contents of each of the computer discs are incorporated herein by reference in their entireties.

TABLE 1

BIALLELIC MARKER ID	SEQ ID NO. IN TABLE 11A	BIALLELIC MARKER POSITION IN SEQ ID NO.	SEQ ID NO. IN TABLE 11B	BIALLELIC MARKER POSITION IN SEQ ID NO.
12-421-140	1	501	494	24
12-424-192	2	501	495	24
12-424-198	3	501	496	24
12-425-57	4	501	497	24
12-426-154	5	461	498	24

[0728]

TABLE 2

Candidate Gene	Abbreviation
Microsomal glutathione S-transferase II	MGST2 or MGST II
Malate decarboxylase enzyme	DME or ME1
Cytochrome P450 1A2	CYP1A2
Cytochrome P450 2C8	CYP2C8
Cytochrome P450 2C9	CYP2C9
Cytochrome P450 2C18	CYP2C18
Cytochrome P450 3A4–3A7	CYP3A4–CYP3A7
Cytochrome P450 3A7	CYP3A7
Flavin-containing monooxygenases	FMO
Glutathione reductase	GSHR
Glutathione synthase	GSHS
γ-glutamylcysteine synthetase	GLCL

TABLE 2-continued

Candidate Gene	Abbreviation
γ-glutamyltransferase 5	GGT5
Dipeptidase	DP
Glucose 6-phosphate dehydrogenase	G6PDH
Phosphogluconate dehydrogenase	PGDH
Uridine diphosphateglucoronosyl transferase 1A7	UGT1A7
Uridine diphosphateglucoronosyl transferase B4	UGT2B4
Uridine diphosphateglucoronosyl transferase B7	UGT2B7
Uridine diphosphateglucoronosyl transferase B10	UGT2B10
Uridine diphosphateglucoronosyl transferase B15	UGT2B15

[0729]

TABLE 3A

NON-GENOMIC BIALLELIC MARKERS

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID (FIG. 1A)		POSITION OF BIALLELIC MARKER IN SEQ ID (FIG. 1B)	
		SEQ ID No.	Position	SEQ ID No	Position
12-424-192	A/G	2	501	495	47
12-424-198	G/T	3	501	496	47
12-426-154	G/A	5	461	498	47
12-429-198	C/T	6	501	499	47
12-430-80	C/T	7	501	500	47
12-433-215	A/G	8	501	501	47
12-441-233	A/G	9	501	502	47
12-441-343	G/A	10	501	503	47
12-442-221	T/C	11	501	504	47
12-447-58	G/C	12	501	505	47
12-449-300	T/C	472	501	965	47
12-453-429	C/T	13	501	506	47
12-454-363	A/G	14	501	507	47
12-455-326	T/C	15	501	508	47
12-455-383	G/A	16	501	509	47
12-456-269	A/G	17	501	510	47
12-456-380	G/T	18	501	511	47
12-457-204	A/G	19	501	512	47
12-457-206	C/T	20	501	513	47
12-458-196	A/T	21	501	514	47
12-458-438	T/C	22	501	515	47
12-460-274	A/G	23	501	516	47
12-461-124	A/C	24	501	517	47
12-461-299	C/T	25	501	518	47
12-461-465	C/T	26	501	519	47
12-462-280	C/T	27	501	520	47
12-464-66	G/T	28	501	521	47
12-465-234	G/T	30	501	523	47
12-465-26	C/T	29	501	522	47
12-442-133	Deletion G	437	501	930	47

TABLE 3A-continued

NON-GENOMIC BIALLELIC MARKERS

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID (FIG. 1A)		POSITION OF BIALLELIC MARKER IN SEQ ID (FIG. 1B)	
		SEQ ID No.	Position	SEQ ID No	Position
12-449-63	Insertion AT	438	501	931	47
10-454-242	Deletion AT	439	501	932	47
12-463-230	Deletion CAT	440	501	933	47
12-426-199	Deletion	441	501	934	47

[0730]

TABLE 3B

BIALLELIC MARKERS IN GENOMIC SEQUENCE (SEQ ID No. 485)

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID
10-286-289	G/C	7564
10-286-345	A/T	7619
10-286-375	A/G	7649
12-425-57	G/A	17258
12-421-135	insertion of a T	21590
12-421-140	A/G	21595
10-523-232	C/T	36971
10-289-201	C/T	45214
10-290-37	C/T	45741
10-290-326	A/G	46029
10-290-328	G/T	46032

[0731]

TABLE 3C

BIALLELIC MARKERS IN MGST-II cDNA (SEQ ID No 486)

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID
10-286-289	G/C	98
10-286-345	A/T	153
10-286-375	A/G	183
10-289-201	C/T	478
10-290-37	C/T	526

[0732]

TABLE 3D

BIALLELIC MARKERS IN MGST-II cDNA (SEQ ID No 487)

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID
10-286-289	G/C	98
10-286-345	A/T	153

TABLE 3D-continued

BIALLELIC MARKERS IN MGST-II cDNA (SEQ ID No 487)

BIALLELIC MARKER ID	ALLELES	POSITION OF BIALLELIC MARKER IN SEQ ID
10-286-375	A/G	183
10-289-201	C/T	378
10-290-37	C/T	426

[0733]

TABLE 4

GENE	MARKER 1	MARKER 2	MARKER 3	MARKER 4
MGST-II	12-455-326	12-453-429	12-424-198	
MGST-II	12-455-326	12-453-429	12-424-198	12-454-363
MGST-II	12-447-58	12-455-326	12-461-299	12-453-429
MGST-II	12-441-233	12-461-299	12-453-429	
MGST-II	12-441-233	12-461-299	12-453-429	12-426-154
MGST-II	12-426-154	12-424-198		
MGST-II	12-426-154	12-461-299	12-424-198	
ME1	10-428-219	12-724-225		
UGT1A7	12-128-225	12-156-91	12-139-380	12-140-134
UGT1A7	12-148-311	12-156-91	12-139-380	12-140-134
UGT2B4	10-470-25	12-652-203		
UGT2B4	10-470-25	12-637-219	12-652-203.	

[0734]

TABLE 5

GENE	MARKER 1	MARKER 2	MARKER 3	MARKER 4
MGST-II	12-455-326	12-453-429	12-424-198	
MGST-II	12-455-326	12-453-429	12-424-198	12-454-363
MGST-II	12-447-58	12-455-326	12-461-299	12-453-429
MGST-II	12-426-154	12-424-198		
ME1	10-428-219	12-724-225		
UGT1A7	12-128-225	12-156-91	12-139-380	12-140-134
UGT2B4	10-470-25	12-652-203		

[0735]

TABLE 6

Test type	Technology	Turnaround time	Discrimina-tory potential	Sensitivity (amount DNA)	Sample
RFLP	VNTR (auto-radiography)	Weeks or months	10^6 to 10^9	10 ng	Highly intact DNA
AmpFLP	VNTR (PCR based)	Days	10^3 to 10^6	100 pg	Moderate degradation
Dot blot (ex HLADQA1)	Sequence specific oligo-nucleotide probes	Days	10^1 to 10^3	1 ng	Moderate degradation
Mito-chondrial DNA	D-loop sequence (PCR based)	Days	10^2	1 pg	Severe degradation
Present marker set	Biallelic Markers (set of 13, set of 100, set of 500)	Hours to Days (through-put dependent)	10^6, 10^{47}, 10^{484}	100 pg	Moderate degradation

[0736]

TABLE 7

Marker sets	$L = 10^6$	$L = 10^8$
Biallelic	13	17
5-allele markers (e.g. VNTR)	5	7
10-allele markers (e.g. VNTR)	4	5

[0737]

TABLE 8

Number of Biallelic Markers	L
50	$7.2 * 10^{23}$
100	$5*10^{47}$
484	$3\hat{~}484$

[0738]

TABLE 9

Genotype	Relationship	Pr(p = A|S = A)	L
A_iA_j	Full brothers	$(1 + p_i + p_j + 2p_i p_j)/4$	3.3
	Father and son	$(p_i + p_j)/2$	10.0
	Half brothers	$(p_i + p_j + 4p_i p_j)/4$	16.7
	Uncle and nephew	$(1 + p_i + p_j + 2p_i p_j)/4$	16.7
	First cousins	$(1 + p_i + p_j + 12p_i p_j)/8$	25.0
	Unrelated	$2p_i p_j$	50.0
A_jA_j	Full brothers	$(1 + p_i)^2/4$	3.3
	Father and son	p_i	10.0
	Half brothers	$p_i(1 + p_i)/2$	18.2
	Uncle and nephew	$p_i(1 + p_i)/2$	18.2
	First cousins	$p_i(1 + 3p_i)/4$	30.8
	Unrelated	p_i^2	100.0

[0739]

TABLE 10

Marker ID	Position in MGST-II gene	Least common allele/ mutation	Original allele	Genotype of ALT+ and ALT− individuals
10-286-289	5'UTR	G	C	79 ALT+ C/C
				104 ALT− C/C
				1 ALT− GC
10-286-345	5'UTR	T	A	65 ALT+ A/A
				12 ALT+ A/T
				2 ALT+ T/T
				82 ALT− A/A
				19 ALT− A/T
				3 ALT− T/T
10-286-375	5'UTR	G	A	78 ALT+ A/A
				1 ALT+ A/G
				104 ALT− A/A
10-523-232	Exon 3	T	C	75 ALT+ C/C
				100 ALT− C/C
				1 ALT− C/T
				76 ALT+ T/T
				2 ALT+ C/T
				100 ALT− T/T
10-289-201	Exon 4	C	T	1 ALT− C/T
10-290-37	Exon 5	T	C	78 ALT+ C/C
				1 ALT+ C/T
				104 ALT− C/C
10-290-326	3' region	A	G	79 ALT+ G/G
				103 ALT− G/G
				1 ALT− A/G
10-290-328	3' region	deletion	—	1 ALT+ deletion
				78 ALT+ —
				104 ALT− —

[0740]

TABLE 11A

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
MGST2	12-421-140	1	501	N		
MGST2	12-424-192	2	501	N		
MGST2	12-424-198	3	501	N		
MGST2	12-425-57	4	501	N		
MGST2	12-426-154	5	461	N		
MGST2	12-429-198	6	501	N		
MGST2	12-430-80	7	501	Y	T	5.38
MGST2	12-433-215	8	501	N		
MGST2	12-441-233	9	501	Y	G	34.48
MGST2	12-441-343	10	501	N		
MGST2	12-442-221	11	501	N		
MGST2	12-447-58	12	501	Y	G	34.27
MGST2	12-453-429	13	501	Y	T	42.55
MGST2	12-454-363	14	501	N		
MGST2	12-455-326	15	501	Y	C	41.94
MGST2	12-455-383	16	501	N		
MGST2	12-456-269	17	501	N		
MGST2	12-456-380	18	501	N		
MGST2	12-457-204	19	501	N		
MGST2	12-457-206	20	501	N		
MGST2	12-458-196	21	501	N		
MGST2	12-458-438	22	501	N		
MGST2	12-460-274	23	501	N		
MGST2	12-461-124	24	501	N		
MGST2	12-461-299	25	501	Y	C	42.39
MGST2	12-461-465	26	501	N		
MGST2	12-462-280	27	501	N		
MGST2	12-464-66	28	501	N		
MGST2	12-465-26	29	501	N		
MGST2	12-465-234	30	501	N		
ME1	10-428-219	31	501	N		
ME1	10-429-84	32	501	N		
ME1	10-420-284	33	501	N		
ME1	10-423-411	34	501	N		
ME1	12-713-95	35	232	Y	C	31.18
ME1	12-713-149	36	286	N		
ME1	12-716-295	37	500	Y	T	32.8
ME1	12-720-80	38	480	Y	C	33.69
ME1	12-721-281	40	426	N		
ME1	12-721-440	41	501	Y	A	1.6
ME1	12-723-293	42	503	Y	T	0.54
ME1	12-724-195	43	501	N		
ME1	12-724-225	44	501	Y	T	25.86
CYP1A2	10-153-329	45	330	Y	G	2.69
CYP1A2	10-95-342	46	342	Y	A	0.54
CYP1A2	10-100-277	47	277	Y	C	2.33
CYP1A2	10-102-294	48	297	Y	C	30.85
CYP2C8	10-413-394	49	501	Y	A	38.07
CYP2C8	10-414-243	50	501	N		
CYP2C8	10-416-273	51	501	Y	T	39.25
CYP2C8	10-418-177	52	501	Y	G	11.41
CYP2C8	12-665-315	53	501	Y	T	22.58
CYP2C8	12-666-324	54	501	Y	C	10.87
CYP2C9	10-76-177	56	178	N		
CYP2C9	10-76-217	57	218	N		
CYP2C9	10-76-333	58	334	Y	G	13.04
CYP2C9	10-77-316	59	501	N		
CYP2C9	10-155-78	60	78	N		
CYP2C9	10-155-104	61	104	N		
CYP2C9	10-156-52	62	52	Y	A	4.79
CYP2C9	10-157-39	63	39	Y	C	30.65
CYP2C9	10-157-131	64	131	Y		
CYP2C9	10-157-166	65	166	N		
CYP2C9	10-157-246	66	246	N		
CYP2C9	10-159-161	67	161	Y	A	11.29
CYP2C9	10-159-162	68	162	N		
CYP2C18	10-83-169	69	169	Y	T	12.9
CYP2C18	10-84-152	70	152	N		
CYP2C18	10-84-243	71	243	Y	T	32.56

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
CYP2C18	10-84-277	72	277	N		
CYP2C18	10-84-295	73	295	Y		
CYP2C18	10-85-43	74	43	Y		
CYP2C18	10-85-117	75	117	Y	T	12.35
CYP2C18	10-85-320	76	320	N		
CYP2C18	10-86-121	77	121	Y		
CYP3A4–CYP3A7	12-244-275	78	501	N		
CYP3A4–CYP3A7	12-251-153	79	450	Y		
CYP3A4–CYP3A7	12-254-115	80	246	N		
CYP3A4–CYP3A7	12-254-180	81	311	Y		
CYP3A4–CYP3A7	12-265-300	82	499	N		
CYP3A4–CYP3A7	12-271-118	83	501	N		
CYP3A4–CYP3A7	12-272-112	84	501	Y		
CYP3A7	10-216-182	85	503	Y	A	6.59
CYP3A7	10-217-91	86	501	Y	T	12.64
CYP3A7	10-213-292	87	503	Y	G	12.9
CYP3A7	10-214-279	88	501	Y	C	13.3
CYP3A7	10-214-380	89	501	Y	G	13.44
FMO	2-1-216	90	216	N		
FMO	2-1-397	91	397	N		
FMO	2-3-232	92	232	N		
FMO	2-4-51	93	51	N		
FMO	2-4-126	94	126	N		
FMO	2-5-202	95	202	N		
FMO	2-5-275	96	275	N		
FMO	2-5-346	97	346	N		
FMO	2-8-171	98	171	N		
FMO	2-9-188	99	188	N		
FMO	2-9-223	100	223	N		
FMO	2-10-107	101	107	N		
FMO	2-10-378	102	377	N		
FMO	2-11-284	103	284	N		
FMO	2-11-156	104	156	N		
FMO	2-11-379	105	379	N		
FMO	2-12-223	106	223	N		
FMO	2-14-239	107	239	N		
FMO	2-27-185	118	185	N		
FMO	2-27-378	119	378	N		
FMO	2-29-142	120	142	N		
FMO	2-29-166	121	166	N		
FMO	2-29-205	122	204	N		
FMO	2-29-206	123	205	N		
FMO	2-29-314	124	313	N		
FMO	2-32-68	125	68	N		
FMO	2-35-357	126	357	N		
FMO	2-36-256	127	256	N		
FMO	2-36-354	128	353	N		
FMO	2-42-236	129	236	N		
FMO	2-43-139	130	139	N		
FMO	2-44-215	131	213	N		
FMO	2-45-38	132	38	N		
FMO	2-45-183	133	183	N		
FMO	2-45-335	134	335	N		
FMO	2-45-394	135	394	N		
FMO	2-48-39	136	39	N		
FMO	2-48-72	137	72	N		
FMO	2-48-156	138	156	N		
FMO	2-48-285	139	285	N		
FMO	2-49-167	140	167	N		
GSHR	10-436-43	141	501	Y	C	19.44
GSHR	10-436-376	142	501	Y	A	32.76
GSHR	10-431-51	143	501	Y	A	20.21
GSHR	10-432-93	144	477	Y		
GSHR	12-631-208	145	433	Y	G	17.39

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
GSHS	10-260-282	146	501	N		
GSHS	10-263-26	147	503	N		
GSHS	10-258-408	148	501	N		
GSHS	12-317-259	149	501	N		
GSHS	12-323-385	150	501	Y	T	47.85
GSHS	12-324-219	151	357	N		
GSHS	12-324-335	152	473	Y	C	35.16
GSHS	12-324-380	153	501	N		
GSHS	12-325-30	154	501	N		
GSHS	12-327-31	155	479	Y	G	47.87
GSHS	12-327-415	156	500	N		
GSHS	12-331-270	157	501	N		
GSHS	12-331-275	158	501	N		
GSHS	12-334-320	159	503	N		
GSHS	12-334-391	160	503	N		
GSHS	12-335-417	161	501	N		
GSHS	12-337-189	162	501	N		
GSHS	12-340-130	163	381	N		
GSHS	12-340-210	164	461	Y	T	46.81
GSHS	12-340-222	165	473	N		
GSHS	12-340-240	166	491	N		
GSHS	12-341-99	167	501	Y	A	17.2
GSHS	12-342-32	168	501	N		
GSHS	12-344-349	169	501	Y	G	43.75
GSHS	12-345-453	170	501	N		
GSHS	12-346-204	171	501	N		
GLCL	10-364-55	172	501	N		
GLCL	10-364-108	173	501	N		
GLCL	10-364-267	174	501	N		
GLCL	10-367-20	175	497	N		
GLCL	10-351-389	176	501	N		
GLCL	10-353-102	177	501	N		
GLCL	12-474-346	178	501	Y	A	25.28
GLCL	10-354-320	179	501	N		
GLCL	10-354-360	180	501	N		
GLCL	10-355-87	181	501	N		
GLCL	10-358-60	182	501	N		
GLCL	12-468-63	183	501	Y	C	46.24
GLCL	12-468-388	184	501	N		
GLCL	12-468-491	185	501	N		
GLCL	12-469-132	186	501	N		
GLCL	12-469-245	187	501	N		
GLCL	12-472-435	188	501	N		
GLCL	12-473-311	189	501	N		
GLCL	12-473-483	190	501	N		
GLCL	12-475-85	191	501	N		
GLCL	12-475-446	192	485	N		
GLCL	12-477-100	193	501	N		
GLCL	12-477-331	194	501	N		
GLCL	12-477-332	195	501	N		
GLCL	12-477-44	196	501	N		
GLCL	12-478-223	197	501	N		
GLCL	12-478-320	198	501	N		
GLCL	12-479-289	199	501	N		
GLCL	12-482-237	200	501	N		
GLCL	12-482-285	201	501	N		
GLCL	12-482-482	202	501	N		
GLCL	12-483-322	203	499	N		
GLCL	12-484-46	204	501	N		
GLCL	12-490-312	205	501	N		
GLCL	12-491-295	206	501	N		
GLCL	12-493-417	207	501	N		
GLCL	12-494-373	208	501	N		
GLCL	12-495-166	209	501	N		
GLCL	12-495-272	210	501	N		
GLCL	12-495-424	211	501	N		
GLCL	12-500-220	212	501	N		
GLCL	12-501-155	213	501	N		
GLCL	12-503-52	214	501	N		
GLCL	12-503-62	215	501	N		
GLCL	12-504-54	216	499	N		

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
GLCL	12-504-96	217	501	N		
GLCL	12-504-428	218	501	Y	C	47.22
GLCL	12-507-53	219	474	Y	C	47.78
GLCL	12-507-92	220	501	N		
GLCL	12-507-159	221	500	N		
GLCL	12-507-177	222	501	N		
GLCL	12-508-29	223	501	N		
GLCL	12-509-42	224	501	N		
GLCL	12-509-126	225	501	N		
GLCL	12-510-59	226	501	N		
GLCL	12-511-74	227	501	Y	C	11.45
GGT5	10-325-311	228	501	N		
GGT5	10-327-120	229	501	N		
GGT5	10-331-179	230	501	N		
GGT5	10-331-357	231	501	N		
GGT5	10-334-263	232	501	N		
GGT5	10-321-226	233	501	N		
GGT5	12-183-98	234	501	N		
GGT5	12-185-78	235	501	N		
GGT5	12-186-154	236	501	Y	A	6.99
GGT5	12-186-397	237	501	Y	C	28.08
GGT5	12-187-65	238	501	Y	C	23.37
GGT5	12-187-66	239	501	N		
GGT5	12-189-348	240	501	Y	A	26.09
GGT5	12-192-63	241	501	N		
GGT5	12-192-64	242	502	N		
GGT5	12-192-268	243	501	N		
GGT5	12-192-334	244	501	N		
GGT5	12-192-352	245	501	N		
GGT5	12-194-135	246	501	N		
GGT5	12-194-325	247	501	N		
GGT5	12-194-337	248	501	N		
GGT5	12-194-479	249	501	N		
DP	10-442-133	250	501	Y	C	7.06
DP	10-444-248	251	484	Y	G	42.78
DP	10-445-281	252	501	Y	T	13.3
DP	12-668-362	253	501	N		
DP	12-670-48	254	501	N		
DP	12-670-91	255	501	N		
DP	12-670-157	256	501	Y	T	47.83
DP	12-671-148	257	501	Y	C	41.49
DP	12-679-245	258	253	N		
DP	12-679-371	259	379	N		
DP	12-679-426	260	434	N		
DP	12-680-331	261	503	N		
G6PDH	10-151-154	262	154	Y	A	0.54
G6PDH	10-138-206	263	205	Y	T	8.51
G6PDH	10-138-352	264	351	Y	C	14.04
PGDH	12-586-414	265	501	Y	A	28.33
PGDH	12-587-379	266	499	N		
PGDH	12-588-103	267	501	Y	A	48.88
PGDH	12-589-152	268	501	N		
PGDH	12-592-118	269	501	Y	A	49.46
PGDH	12-593-174	270	501	Y	C	34.71
PGDH	12-596-124	271	501	Y	A	26.7
PGDH	12-602-196	272	501	Y	C	31.72
PGDH	12-602-350	273	501	N		
PGDH	12-603-191	274	501	Y	C	27.42
PGDH	12-783-73	275	501	N		
PGDH	12-783-421	276	501	N		
PGDH	12-785-200	277	501	N		
PGDH	12-785-393	278	501	N		
PGDH	12-787-103	279	501	N		
PGDH	12-790-396	280	501	N		
PGDH	12-791-211	281	501	N		
PGDH	12-792-233	282	501	N		
PGDH	12-793-383	283	505	N		
PGDH	12-803-125	284	501	N		
PGDH	12-805-115	285	501	N		
PGDH	12-808-52	286	501	N		
PGDH	12-808-75	287	501	N		

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
PGDH	12-809-119	288	501	N		
PGDH	12-810-77	289	501	N		
PGDH	10-265-178	290	501	N		
PGDH	10-266-203	291	503	N		
UGT1A7	10-403-312	292	501	N		
UGT1A7	10-405-54	293	501	N		
UGT1A7	10-408-356	294	501	N		
UGT1A7	10-409-148	295	501	N		
UGT1A7	10-409-249	296	501	N		
UGT1A7	10-410-274	297	501	N		
UGT1A7	10-410-280	298	501	N		
UGT1A7	10-410-337	299	501	N		
UGT1A7	12-121-326	300	501	Y	A	26.88
UGT1A7	12-122-341	301	501	N		
UGT1A7	12-122-381	302	501	N		
UGT1A7	12-124-169	303	501	N		
UGT1A7	12-124-194	304	501	Y	C	19.41
UGT1A7	12-124-300	305	501	N		
UGT1A7	12-124-58	306	501	N		
UGT1A7	12-126-222	307	501	N		
UGT1A7	12-126-297	308	501	N		
UGT1A7	12-128-225	309	501	Y	G	47.44
UGT1A7	12-129-176	310	501	N		
UGT1A7	12-130-203	311	501	N		
UGT1A7	12-130-260	312	501	N		
UGT1A7	12-131-112	313	501	N		
UGT1A7	12-132-157	314	255	N		
UGT1A7	12-132-437	315	501	N		
UGT1A7	12-133-153	316	666	N		
UGT1A7	12-133-318	317	501	N		
UGT1A7	12-136-238	318	249	N		
UGT1A7	12-138-141	319	501	N		
UGT1A7	12-138-42	320	501	N		
UGT1A7	12-138-67	321	501	N		
UGT1A7	12-139-380	322	501	Y	A	21.24
UGT1A7	12-140-134	323	501	Y	T	41.01
UGT1A7	12-140-329	324	501	N		
UGT1A7	12-140-385	325	501	N		
UGT1A7	12-141-159	326	501	Y	T	37.5
UGT1A7	12-141-392	327	501	N		
UGT1A7	12-142-315	328	501	N		
UGT1A7	12-142-321	329	501	Y	G	45.56
UGT1A7	12-143-453	330	501	Y	G	49.39
UGT1A7	12-144-169	331	501	N		
UGT1A7	12-144-33	332	501	N		
UGT1A7	12-146-174	333	501	N		
UGT1A7	12-146-47	334	501	N		
UGT1A7	12-148-283	335	501	N		
UGT1A7	12-148-311	336	501	Y	T	43.17
UGT1A7	12-149-320	337	501	N		
UGT1A7	12-151-174	338	501	N		
UGT1A7	12-151-196	339	501	N		
UGT1A7	12-151-270	340	501	N		
UGT1A7	12-152-453	341	501	N		
UGT1A7	12-153-116	342	501	Y	T	37.78
UGT1A7	12-154-480	343	501	N		
UGT1A7	12-155-403	344	501	N		
UGT1A7	12-156-91	345	501	Y	A	50
UGT1A7	12-157-437	346	501	N		
UGT1A7	12-158-213	347	501	N		
UGT1A7	12-158-450	348	480	N		
UGT1A7	12-161-157	349	501	N		
UGT1A7	12-162-21	350	498	N		
UGT2B4	10-470-25	351	503	Y	T	44.44
UGT2B4	10-471-84	352	503	Y	A	27.17
UGT2B4	10-471-85	353	503	Y		
UGT2B4	10-473-333	355	503	N		
UGT2B4	10-494-284	356	503	N		
UGT2B4	12-637-219	357	499	Y	G	36.17
UGT2B4	12-639-95	358	499	Y	G	34.95
UGT2B4	12-639-241	359	499	N		

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
UGT2B4	12-640-151	360	499	N		
UGT2B4	12-640-296	361	499	N		
UGT2B4	12-640-325	362	499	Y		
UGT2B4	12-640-413	363	499	N		
UGT2B4	12-641-120	364	499	N		
UGT2B4	12-641-122	365	499	N		
UGT2B4	12-641-223	366	432	N		
UGT2B4	12-641-267	367	388	N		
UGT2B4	12-642-387	368	503	N		
UGT2B4	12-642-417	369	503	Y	G	26.51
UGT2B4	12-646-429	370	434	N		
UGT2B4	12-646-433	371	438	N		
UGT2B4	12-647-145	372	503	N		
UGT2B4	12-648-123	373	251	N		
UGT2B4	12-648-300	374	428	Y	T	37.91
UGT2B4	12-648-402	375	501	N		
UGT2B4	12-652-115	376	298	N		
UGT2B4	12-652-203	377	386	Y	C	42.47
UGT2B4	12-652-274	378	457	N		
UGT2B4	12-652-371	379	501	N		
UGT2B4	12-653-423	380	499	N		
UGT2B4	12-654-115	381	499	N		
UGT2B4	12-654-207	382	499	N		
UGT2B4	12-657-396	383	503	N		
UGT2B4	12-658-120	384	503	N		
UGT2B4	12-659-382	385	501	N		
UGT2B4	12-660-134	386	306	N		
UGT2B4	12-662-80	387	497	N		
UGT2B7	12-906-149	388	501	Y		
UGT2B7	12-906-154	389	501	N		
UGT2B7	12-906-251	390	501	N		
UGT2B7	12-906-451	391	501	N		
UGT2B7	12-907-199	392	244	Y	G	3.23
UGT2B7	12-907-482	393	501	N		
UGT2B7	12-909-36	394	53	N		
UGT2B7	12-909-176	395	193	N		
UGT2B7	12-909-484	396	501	N		
UGT2B7	12-910-76	397	347	Y	A	23.91
UGT2B7	12-910-295	398	503	N		
UGT2B7	12-911-22	399	240	Y	C	43.96
UGT2B7	12-912-65	400	501	N		
UGT2B7	12-914-106	401	384	Y	C	44.62
UGT2B7	12-914-252	402	503	N		
UGT2B10	10-448-266	403	503	N		
UGT2B10	10-453-330	404	503	N		
UGT2B10	10-455-367	405	503	N		
UGT2B10	12-5-158	406	503	Y	T	11.8
UGT2B10	12-9-367	407	499	Y	A	14.67
UGT2B10	12-10-303	408	501	Y	T	14.67
UGT2B10	12-14-264	409	501	Y	T	14.29
UGT2B10	12-17-86	410	499	Y	A	44.02
UGT2B10	12-19-163	411	501	Y	G	29.89
UGT2B15	10-457-284	412	503	N		
UGT2B15	10-460-221	413	503	N		
UGT2B15	10-460-232	414	503	N		
UGT2B15	10-460-235	415	503	N		
UGT2B15	10-460-236	416	503	N		
UGT2B15	10-460-285	417	503	N		
UGT2B15	12-605-58	418	501	Y	T	47.67
UGT2B15	12-607-207	419	501	N		
UGT2B15	12-609-119	420	499	Y	T	45.11
UGT2B15	12-609-180	421	499	N		
UGT2B15	12-609-233	422	499	N		
UGT2B15	12-611-294	423	501	Y	A	43.26
UGT2B15	12-612-41	424	501	Y	C	28.65
UGT2B15	12-613-302	425	499	N		
UGT2B15	12-614-471	426	501	Y	T	39.44
UGT2B15	12-620-192	427	503	Y	T	30.36
UGT2B15	12-621-49	428	503	N		
UGT2B15	12-622-325	429	364	N		
UGT2B15	12-624-82	430	501	N		

TABLE 11A-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
UGT2B15	12-624-83	431	501	N		
UGT2B15	12-624-107	432	489	N		
UGT2B15	12-624-146	433	501	N		
UGT2B15	12-624-288	434	501	N		
UGT2B15	12-624-293	435	501	N		
MGST2	12-421-135	436	501	N		
MGST2	12-442-133	437	501	Y	C	5.85
MGST2	12-449-63	438	501	N		
MGST2	12-454-242	439	501	N		
MGST2	12-463-250	440	501	N		
MGST2	12-462-199	441	501	N		
DME	10-430-287	442	501	N		
DME	12-718-432	443	501	N		
CYP3A4–CYP3A7	12-269-301	444	501	Y		
FMO	2-13-398	445	501	N		
FMO	2-28-132	446	501	N		
FMO	2-39-27	447	501	N		
FMO	2-45-155	448	501	N		
FMO	2-4-391	449	501	N		
GSHS	12-345-410	450	501	N		
GLCL	10-358-353	451	501	N		
GLCL	10-360-190	452	501	N		
GLCL	10-365-374	453	501	N		
GLCL	10-367-58	454	501	N		
GLCL	12-468-424	455	501	N		
GLCL	12-481-293	456	501	N		
GLCL	12-499-86	457	501	N		
GLCL	12-500-217	458	501	N		
GLCL	12-511-101	459	501	N		
6PGD	12-586-443	460	501	N		
6PGD	12-593-287	461	501	N		
6PGD	12-795-383	462	501	N		
UGT2B4	10-494-332	463	501	N		
UGT2B4	12-659-251	465	429	N		
UGT2B7	12-912-419	466	501	N		
UGT2B7	12-914-28	467	306	N		
UGT2B15	12-624-307	468	501	N		
MGST2	10-290-326	469	501	N		
MGST2	10-290-37	470	501	N		
MGST2	10-523-232	471	501	N		
MGST2	12-449-300	472	501	N		
G6PDH	10-186-212	473	212	N		
MGST2	10-286-289	474	501	N		
MGST2	10-286-345	475	501	N		
MGST2	10-286-375	476	501	N		
MGST2	10-289-201	477	501	N		
GGT5	10-321-28	478	501	N		
CYP1A2	10-98-265	479	265	N		
UGT1A7	12-157-115	480	501	N		
GLCL	12-472-48	481	501	N		
GLCL	12-477-151	482	501	N		
GLCL	12-479-214	483	501	N		
MGST2	10-290-328	484	501	N		

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TABLE 11B

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
MGST2	12-421-140	494	24	N		
MGST2	12-424-192	495	24	N		
MGST2	12-424-198	496	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
MGST2	12-425-57	497	24	N		
MGST2	12-426-154	498	24	N		
MGST2	12-429-198	499	24	N		
MGST2	12-430-80	500	24	Y	T	5.38
MGST2	12-433-215	501	24	N		
MGST2	12-441-233	502	24	Y	G	34.48
MGST2	12-441-343	503	24	N		
MGST2	12-442-221	504	24	N		
MGST2	12-447-58	505	24	Y	G	34.27
MGST2	12-453-429	506	24	Y	T	42.55
MGST2	12-454-363	507	24	N		
MGST2	12-455-326	508	24	Y	C	41.94
MGST2	12-455-383	509	24	N		
MGST2	12-456-269	510	24	N		
MGST2	12-456-380	511	24	N		
MGST2	12-457-204	512	24	N		
MGST2	12-457-206	513	24	N		
MGST2	12-458-196	514	24	N		
MGST2	12-458-438	515	24	N		
MGST2	12-460-274	516	24	N		
MGST2	12-461-124	517	24	N		
MGST2	12-461-299	518	24	Y	C	42.39
MGST2	12-461-465	519	24	N		
MGST2	12-462-280	520	24	N		
MGST2	12-464-66	521	24	N		
MGST2	12-465-26	522	24	N		
MGST2	12-465-234	523	24	N		
ME1	10-428-219	524	24	N		
ME1	10-429-84	525	24	N		
ME1	10-420-284	526	24	N		
ME1	10-423-411	527	24	N		
ME1	12-713-95	528	24	Y	C	31.18
ME1	12-713-149	529	24	N		
ME1	12-716-295	530	24	Y	T	32.8
ME1	12-720-80	531	24	Y	C	33.69
ME1	12-721-281	533	24	N		
ME1	12-721-440	534	24	Y	A	1.6
ME1	12-723-293	535	24	Y	T	0.54
ME1	12-724-195	536	24	N		
ME1	12-724-225	537	24	Y	T	25.86
CYP1A2	10-153-329	538	24	Y	G	2.69
CYP1A2	10-95-342	539	24	Y	A	0.54
CYP1A2	10-100-277	540	24	Y	C	2.33
CYP1A2	10-102-294	541	24	Y	C	30.85
CYP2C8	10-413-394	542	24	Y	A	38.07
CYP2C8	10-414-243	543	24	N		
CYP2C8	10-416-273	544	24	Y	T	39.25
CYP2C8	10-418-177	545	24	Y	G	11.41
CYP2C8	12-665-315	546	24	Y	T	22.58
CYP2C8	12-666-324	547	24	Y	C	10.87
CYP2C9	10-76-177	549	24	N		
CYP2C9	10-76-217	550	24	N		
CYP2C9	10-76-333	551	24	Y	G	13.04
CYP2C9	10-77-316	552	24	N		
CYP2C9	10-155-78	553	24	N		
CYP2C9	10-155-104	554	24	N		
CYP2C9	10-156-52	555	24	Y	A	4.79
CYP2C9	10-157-39	556	24	Y	C	30.65
CYP2C9	10-157-131	557	24	Y		
CYP2C9	10-157-166	558	24	N		
CYP2C9	10-157-246	559	24	N		
CYP2C9	10-159-161	560	24	Y	A	11.29
CYP2C9	10-159-162	561	24	N		
CYP2C18	10-83-169	562	24	Y	T	12.9
CYP2C18	10-84-152	563	24	N		
CYP2C18	10-84-243	564	24	Y	T	32.56
CYP2C18	10-84-277	565	24	N		
CYP2C18	10-84-295	566	24	Y		
CYP2C18	10-85-43	567	24	Y		
CYP2C18	10-85-117	568	24	Y	T	12.35
CYP2C18	10-85-320	569	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
CYP2C18	10-86-121	570	24	Y		
CYP3A4–CYP3A7	12-244-275	571	24	N		
CYP3A4–CYP3A7	12-251-153	572	24	Y		
CYP3A4–CYP3A7	12-254-115	573	24	N		
CYP3A4–CYP3A7	12-254-180	574	24	Y		
CYP3A4–CYP3A7	12-265-300	575	24	N		
CYP3A4–CYP3A7	12-271-118	576	24	N		
CYP3A4–CYP3A7	12-272-112	577	24	Y		
CYP3A7	10-216-182	578	24	Y	A	6.59
CYP3A7	10-217-91	579	24	Y	T	12.64
CYP3A7	10-213-292	580	24	Y	G	12.9
CYP3A7	10-214-279	581	24	Y	C	13.3
CYP3A7	10-214-380	582	24	Y	G	13.44
FMO	2-1-216	583	24	N		
FMO	2-1-397	584	24	N		
FMO	2-3-232	585	24	N		
FMO	2-4-51	586	24	N		
FMO	2-4-126	587	24	N		
FMO	2-5-202	588	24	N		
FMO	2-5-275	589	24	N		
FMO	2-5-346	590	24	N		
FMO	2-8-171	591	24	N		
FMO	2-9-188	592	24	N		
FMO	2-9-223	593	24	N		
FMO	2-10-107	594	24	N		
FMO	2-10-378	595	24	N		
FMO	2-11-284	596	24	N		
FMO	2-11-156	597	24	N		
FMO	2-11-379	598	24	N		
FMO	2-12-223	599	24	N		
FMO	2-14-239	600	24	N		
FMO	2-14-370	601	24	N		
FMO	2-17-104	602	24	N		
FMO	2-17-396	603	24	N		
FMO	2-22-43	604	24	N		
FMO	2-22-138	605	24	N		
FMO	2-23-82	606	24	N		
FMO	2-23-166	607	24	N		
FMO	2-23-244	608	24	N		
FMO	2-24-115	609	24	N		
FMO	2-25-36	610	24	N		
FMO	2-27-185	611	24	N		
FMO	2-27-378	612	24	N		
FMO	2-29-142	613	24	N		
FMO	2-29-166	614	24	N		
FMO	2-29-205	615	24	N		
FMO	2-29-206	616	24	N		
FMO	2-29-314	617	24	N		
FMO	2-32-68	618	24	N		
FMO	2-35-357	619	24	N		
FMO	2-36-256	620	24	N		
FMO	2-36-354	621	24	N		
FMO	2-42-236	622	24	N		
FMO	2-43-139	623	24	N		
FMO	2-44-215	624	24	N		
FMO	2-45-38	625	24	N		
FMO	2-45-183	626	24	N		
FMO	2-45-335	627	24	N		
FMO	2-45-394	628	24	N		
FMO	2-48-39	629	24	N		
FMO	2-48-72	630	24	N		
FMO	2-48-156	631	24	N		
FMO	2-48-285	632	24	N		
FMO	2-49-167	633	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
GSHR	10-436-43	634	24	Y	C	19.44
GSHR	10-436-376	635	24	Y	A	32.76
GSHR	10-431-51	636	24	Y	A	20.21
GSHR	10-432-93	637	24	Y		
GSHR	12-631-208	638	24	Y	G	17.39
GSHS	10-260-282	639	24	N		
GSHS	10-263-26	640	24	N		
GSHS	10-258-408	641	24	N		
GSHS	12-317-259	642	24	N		
GSHS	12-323-385	643	24	Y	T	47.85
GSHS	12-324-219	644	24	N		
GSHS	12-324-335	645	24	Y	C	35.16
GSHS	12-324-380	646	24	N		
GSHS	12-325-30	647	24	N		
GSHS	12-327-31	648	24	Y	G	47.87
GSHS	12-327-415	649	24	N		
GSHS	12-331-270	650	24	N		
GSHS	12-331-275	651	24	N		
GSHS	12-334-320	652	24	N		
GSHS	12-334-391	653	24	N		
GSHS	12-335-417	654	24	N		
GSHS	12-337-189	655	24	N		
GSHS	12-340-130	656	24	N		
GSHS	12-340-210	657	24	Y	T	46.81
GSHS	12-340-222	658	24	N		
GSHS	12-340-240	659	24	N		
GSHS	12-341-99	660	24	Y	A	17.2
GSHS	12-342-32	661	24	N		
GSHS	12-344-349	662	24	Y	G	43.75
GSHS	12-345-453	663	24	N		
GSHS	12-346-204	664	24	N		
GLCL	10-364-55	665	24	N		
GLCL	10-364-108	666	24	N		
GLCL	10-364-267	667	24	N		
GLCL	10-367-20	668	24	N		
GLCL	10-351-389	669	24	N		
GLCL	10-353-102	670	24	N		
GLCL	12-474-346	671	24	Y	A	25.28
GLCL	10-354-320	672	24	N		
GLCL	10-354-360	673	24	N		
GLCL	10-355-87	674	24	N		
GLCL	10-358-60	675	24	N		
GLCL	12-468-63	676	24	Y	C	46.24
GLCL	12-468-388	677	24	N		
GLCL	12-468-491	678	24	N		
GLCL	12-469-132	679	24	N		
GLCL	12-469-245	680	24	N		
GLCL	12-472-435	681	24	N		
GLCL	12-473-311	682	24	N		
GLCL	12-473-483	683	24	N		
GLCL	12-475-85	684	24	N		
GLCL	12-475-446	685	24	N		
GLCL	12-477-100	686	24	N		
GLCL	12-477-331	687	24	N		
GLCL	12-477-332	688	24	N		
GLCL	12-477-44	689	24	N		
GLCL	12-478-223	690	24	N		
GLCL	12-478-320	691	24	N		
GLCL	12-479-289	692	24	N		
GLCL	12-482-237	693	24	N		
GLCL	12-482-285	694	24	N		
GLCL	12-482-482	695	24	N		
GLCL	12-483-322	696	24	N		
GLCL	12-484-46	697	24	N		
GLCL	12-490-312	698	24	N		
GLCL	12-491-295	699	24	N		
GLCL	12-493-417	700	24	N		
GLCL	12-494-373	701	24	N		
GLCL	12-495-166	702	24	N		
GLCL	12-495-272	703	24	N		
GLCL	12-495-424	704	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO- SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
GLCL	12-500-220	705	24	N		
GLCL	12-501-155	706	24	N		
GLCL	12-503-52	707	24	N		
GLCL	12-503-62	708	24	N		
GLCL	12-504-54	709	24	N		
GLCL	12-504-96	710	24	N		
GLCL	12-504-428	711	24	Y	C	47.22
GLCL	12-507-53	712	24	Y	C	47.78
GLCL	12-507-92	713	24	N		
GLCL	12-507-159	714	24	N		
GLCL	12-507-177	715	24	N		
GLCL	12-508-29	716	24	N		
GLCL	12-509-42	717	24	N		
GLCL	12-509-126	718	24	N		
GLCL	12-510-59	719	24	N		
GLCL	12-511-74	720	24	Y	C	11.45
GGT5	10-325-311	721	24	N		
GGT5	10-327-120	722	24	N		
GGT5	10-331-179	723	24	N		
GGT5	10-331-357	724	24	N		
GGT5	10-334-263	725	24	N		
GGT5	10-321-226	726	24	N		
GGT5	12-183-98	727	24	N		
GGT5	12-185-78	728	24	N		
GGT5	12-186-154	729	24	Y	A	6.99
GGT5	12-186-397	730	24	Y	C	28.08
GGT5	12-187-65	731	24	Y	C	23.37
GGT5	12-187-66	732	24	N		
GGT5	12-189-348	733	24	Y	A	26.09
GGT5	12-192-63	734	24	N		
GGT5	12-192-64	735	24	N		
GGT5	12-192-268	736	24	N		
GGT5	12-192-334	737	24	N		
GGT5	12-192-352	738	24	N		
GGT5	12-194-135	739	24	N		
GGT5	12-194-325	740	24	N		
GGT5	12-194-337	741	24	N		
GGT5	12-194-479	742	24	N		
DP	10-442-133	743	24	Y	C	7.06
DP	10-444-248	744	24	Y	G	42.78
DP	10-445-281	745	24	Y	T	13.3
DP	12-668-362	746	24	N		
DP	12-670-48	747	24	N		
DP	12-670-91	748	24	N		
DP	12-670-157	749	24	Y	T	47.83
DP	12-671-148	750	24	Y	C	41.49
DP	12-679-245	751	24	N		
DP	12-679-371	752	24	N		
DP	12-679-426	753	24	N		
DP	12-680-331	754	24	N		
G6PDH	10-151-154	755	24	Y	A	0.54
G6PDH	10-138-206	756	24	Y	T	8.51
G6PDH	10-138-352	757	24	Y	C	14.04
PGDH	12-586-414	758	24	Y	A	28.33
PGDH	12-587-379	759	24	N		
PGDH	12-588-103	760	24	Y	A	48.88
PGDH	12-589-152	761	24	N		
PGDH	12-592-118	762	24	Y	A	49.46
PGDH	12-593-174	763	24	Y	C	34.71
PGDH	12-596-124	764	24	Y	A	26.7
PGDH	12-602-196	765	24	Y	C	31.72
PGDH	12-602-350	766	24	N		
PGDH	12-603-191	767	24	Y	C	27.42
PGDH	12-783-73	768	24	N		
PGDH	12-783-421	769	24	N		
PGDH	12-785-200	770	24	N		
PGDH	12-785-393	771	24	N		
PGDH	12-787-103	772	24	N		
PGDH	12-790-396	773	24	N		
PGDH	12-791-211	774	24	N		
PGDH	12-792-233	775	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
PGDH	12-793-383	776	24	N		
PGDH	12-803-125	777	24	N		
PGDH	12-805-115	778	24	N		
PGDH	12-808-52	779	24	N		
PGDH	12-808-75	780	24	N		
PGDH	12-809-119	781	24	N		
PGDH	12-810-77	782	24	N		
PGDH	10-265-178	783	24	N		
PGDH	10-266-203	784	24	N		
UGT1A7	10-403-312	785	24	N		
UGT1A7	10-405-54	786	24	N		
UGT1A7	10-408-356	787	24	N		
UGT1A7	10-409-148	788	24	N		
UGT1A7	10-409-249	789	24	N		
UGT1A7	10-410-274	790	24	N		
UGT1A7	10-410-280	791	24	N		
UGT1A7	10-410-337	792	24	N		
UGT1A7	12-121-326	793	24	Y	A	26.88
UGT1A7	12-122-341	794	24	N		
UGT1A7	12-122-381	795	24	N		
UGT1A7	12-124-169	796	24	N		
UGT1A7	12-124-194	797	24	Y	C	19.41
UGT1A7	12-124-300	798	24	N		
UGT1A7	12-124-58	799	24	N		
UGT1A7	12-126-222	800	24	N		
UGT1A7	12-126-297	801	24	N		
UGT1A7	12-128-225	802	24	Y	G	47.44
UGT1A7	12-129-176	803	24	N		
UGT1A7	12-130-203	804	24	N		
UGT1A7	12-130-260	805	24	N		
UGT1A7	12-131-112	806	24	N		
UGT1A7	12-132-157	807	24	N		
UGT1A7	12-132-437	808	24	N		
UGT1A7	12-133-153	809	24	N		
UGT1A7	12-133-318	810	24	N		
UGT1A7	12-136-238	811	24	N		
UGT1A7	12-138-141	812	24	N		
UGT1A7	12-138-42	813	24	N		
UGT1A7	12-138-67	814	24	N		
UGT1A7	12-139-380	815	24	Y	A	21.24
UGT1A7	12-140-134	816	24	Y	T	41.01
UGT1A7	12-140-329	817	24	N		
UGT1A7	12-140-385	818	24	N		
UGT1A7	12-141-159	819	24	Y	T	37.5
UGT1A7	12-141-392	820	24	N		
UGT1A7	12-142-315	821	24	N		
UGT1A7	12-142-321	822	24	Y	G	45.56
UGT1A7	12-143-453	823	24	Y	G	49.39
UGT1A7	12-144-169	824	24	N		
UGT1A7	12-144-33	825	24	N		
UGT1A7	12-146-174	826	24	N		
UGT1A7	12-146-47	827	24	N		
UGT1A7	12-148-283	828	24	N		
UGT1A7	12-148-311	829	24	Y	T	43.17
UGT1A7	12-149-320	830	24	N		
UGT1A7	12-151-174	831	24	N		
UGT1A7	12-151-196	832	24	N		
UGT1A7	12-151-270	833	24	N		
UGT1A7	12-152-453	834	24	N		
UGT1A7	12-153-116	835	24	Y	T	37.78
UGT1A7	12-154-480	836	24	N		
UGT1A7	12-155-403	837	24	N		
UGT1A7	12-156-91	838	24	Y	A	50
UGT1A7	12-157-437	839	24	N		
UGT1A7	12-158-213	840	24	N		
UGT1A7	12-158-450	841	24	N		
UGT1A7	12-161-157	842	24	N		
UGT1A7	12-162-21	843	24	N		
UGT2B4	10-470-25	844	24	Y	T	44.44
UGT2B4	10-471-84	845	24	Y	A	27.17
UGT2B4	10-471-85	846	24	Y		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
UGT2B4	10-473-333	848	24	N		
UGT2B4	10-494-284	849	24	N		
UGT2B4	12-637-219	850	24	Y	G	36.17
UGT2B4	12-639-95	851	24	Y	G	34.95
UGT2B4	12-639-241	852	24	N		
UGT2B4	12-640-151	853	24	N		
UGT2B4	12-640-296	854	24	N		
UGT2B4	12-640-325	855	24	Y		
UGT2B4	12-640-413	856	24	N		
UGT2B4	12-641-120	857	24	N		
UGT2B4	12-641-122	858	24	N		
UGT2B4	12-641-223	859	24	N		
UGT2B4	12-641-267	860	24	N		
UGT2B4	12-642-387	861	24	N		
UGT2B4	12-642-417	862	24	Y	G	26.51
UGT2B4	12-646-429	863	24	N		
UGT2B4	12-646-433	864	24	N		
UGT2B4	12-647-145	865	24	N		
UGT2B4	12-648-123	866	24	N		
UGT2B4	12-648-300	867	24	Y	T	37.91
UGT2B4	12-648-402	868	24	N		
UGT2B4	12-652-115	869	24	N		
UGT2B4	12-652-203	870	24	Y	C	42.47
UGT2B4	12-652-274	871	24	N		
UGT2B4	12-652-371	872	24	N		
UGT2B4	12-653-423	873	24	N		
UGT2B4	12-654-115	874	24	N		
UGT2B4	12-654-207	875	24	N		
UGT2B4	12-657-396	876	24	N		
UGT2B4	12-658-120	877	24	N		
UGT2B4	12-659-382	878	24	N		
UGT2B4	12-660-134	879	24	N		
UGT2B4	12-662-80	880	24	N		
UGT2B7	12-906-149	881	24	Y		
UGT2B7	12-906-154	882	24	N		
UGT2B7	12-906-251	883	24	N		
UGT2B7	12-906-451	884	24	N		
UGT2B7	12-907-199	885	24	Y	G	3.23
UGT2B7	12-907-482	886	24	N		
UGT2B7	12-909-36	887	24	N		
UGT2B7	12-909-176	888	24	N		
UGT2B7	12-909-484	889	24	N		
UGT2B7	12-910-76	890	24	Y	A	23.91
UGT2B7	12-910-295	891	24	N		
UGT2B7	12-911-22	892	24	Y	C	43.96
UGT2B7	12-912-65	893	24	N		
UGT2B7	12-914-106	894	24	Y	C	44.62
UGT2B7	12-914-252	895	24	N		
UGT2B10	10-448-266	896	24	N		
UGT2B10	10-453-330	897	24	N		
UGT2B10	10-455-367	898	24	N		
UGT2B10	12-5-158	899	24	Y	T	11.8
UGT2B10	12-9-367	900	24	Y	A	14.67
UGT2B10	12-10-303	901	24	Y	T	14.67
UGT2B10	12-14-264	902	24	Y	T	14.29
UGT2B10	12-17-86	903	24	Y	A	44.02
UGT2B10	12-19-163	904	24	Y	G	29.89
UGT2B15	10-457-284	905	24	N		
UGT2B15	10-460-221	906	24	N		
UGT2B15	10-460-232	907	24	N		
UGT2B15	10-460-235	908	24	N		
UGT2B15	10-460-236	909	24	N		
UGT2B15	10-460-285	910	24	N		
UGT2B15	12-605-58	911	24	Y	T	47.67
UGT2B15	12-607-207	912	24	N		
UGT2B15	12-609-119	913	24	Y	T	45.11
UGT2B15	12-609-180	914	24	N		
UGT2B15	12-609-233	915	24	N		
UGT2B15	12-611-294	916	24	Y	A	43.26
UGT2B15	12-612-41	917	24	Y	C	28.65
UGT2B15	12-613-302	918	24	N		

TABLE 11B-continued

GENE	BIALLELIC MARKER ID	SEQ ID NO.	BIALLELIC MARKER POSITION IN SEQ ID NO.	VALIDATION MICRO-SEQUENCING	GENOTYPING LEAST COMMON ALLELE	FREQUENCY %
UGT2B15	12-614-471	919	24	Y	T	39.44
UGT2B15	12-620-192	920	24	Y	T	30.36
UGT2B15	12-621-49	921	24	N		
UGT2B15	12-622-325	922	24	N		
UGT2B15	12-624-82	923	24	N		
UGT2B15	12-624-83	924	24	N		
UGT2B15	12-624-107	925	24	N		
UGT2B15	12-624-146	926	24	N		
UGT2B15	12-624-288	927	24	N		
UGT2B15	12-624-293	928	24	N		
MGST2	12-421-135	929	24	N		
MGST2	12-442-133	930	24	Y	C	5.85
MGST2	12-449-63	931	24	N		
MGST2	12-454-242	932	24	N		
MGST2	12-463-250	933	24	N		
MGST2	12-462-199	934	24	N		
DME	10-430-287	935	24	N		
DME	12-718-432	936	24	N		
CYP3A4–CYP3A7	12-269-301	937	24	Y		
FMO	2-13-398	938	24	N		
FMO	2-28-132	939	24	N		
FMO	2-39-27	940	24	N		
FMO	2-45-155	941	24	N		
FMO	2-4-391	942	24	N		
GSHS	12-345-410	943	24	N		
GLCL	10-358-353	944	24	N		
GLCL	10-360-190	945	24	N		
GLCL	10-365-374	946	24	N		
GLCL	10-367-58	947	24	N		
GLCL	12-468-424	948	24	N		
GLCL	12-481-293	949	24	N		
GLCL	12-499-86	950	24	N		
GLCL	12-500-217	951	24	N		
GLCL	12-511-101	952	24	N		
6PGD	12-586-443	953	24	N		
6PGD	12-593-287	954	24	N		
6PGD	12-795-383	955	24	N		
UGT2B4	10-494-332	956	24	N		
UGT2B4	12-659-251	958	24	N		
UGT2B7	12-912-419	959	24	N		
UGT2B7	12-914-28	960	24	N		
UGT2B15	12-624-307	961	24	N		
MGST2	10-290-326	962	24	N		
MGST2	10-290-37	963	24	N		
MGST2	10-523-232	964	24	N		
MGST2	12-449-300	965	24	N		
G6PDH	10-186-212	966	24	N		
MGST2	10-286-289	967	24	N		
MGST2	10-286-345	968	24	N		
MGST2	10-286-375	969	24	N		
MGST2	10-289-201	970	24	N		
GGT5	10-321-28	971	24	N		
CYP1A2	10-98-265	972	24	N		
UGT1A7	12-157-115	973	24	N		
GLCL	12-472-48	974	24	N		
GLCL	12-477-151	975	24	N		
GLCL	12-479-214	976	24	N		
MGST2	10-290-328	977	24	N		

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TABLE 12

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE	POSITION RANGE OF PREFERRED SEQUENCES
1	12-421-140	A	G	1–1001
2	12-424-192	A	G	190–801; 865–999
3	12-424-198	G	T	184–795; 859–993
4	12-425-57	G	A	208–225; 266–478
5	12-426-154	A	G	152–961
6	12-429-198	C	T	260–784; 822–1001
7	12-430-80	T	C	1–996
8	12-433-215	A	G	1–1001
12	12-447-58	G	C	1–36; 390–914
13	12-453-429	C	T	1–1001
14	12-454-363	A	G	1–315; 377–466; 598–619
15	12-455-326	T	C	1–357; 391–594; 760–827
16	12-455-383	G	A	1–414; 448–651; 817–884
17	12-456-269	A	G	1–536
19	12-457-204	A	G	437–527; 761–1001
20	12-457-206	C	T	435–525; 759–1001
21	12-458-196	T	A	1–727
22	12-458-438	T	C	1–21; 298–1001
23	12-460-274	A	G	1–499; 563–1001
24	12-461-124	A	C	1–203; 259–644; 687–773; 807–833
25	12-461-299	C	T	1–28; 84–469; 512–591
26	12-461-465	C	T	1–303; 346–432
27	12-462-280	C	T	1–1001
28	12-464-66	G	T	1–215; 261–1001
29	12-465-26	C	T	1–61; 99–1001
30	12-465-234	G	T	1–1001
31	10-428-219	A	G	1–398; 506–1000
33	10-420-284	C	T	1–302; 472–772
34	10-423-411	C	T	1–227; 333–821
35	12-713-95	C	T	1–668
36	12-713-149	G	C	1–668
37	12-716-295	C	T	1–902
38	12-720-80	G	C	1–982
40	12-721-281	A	C	1–926
41	12-721-440	A	G	1–1000
42	12-723-293	C	T	1–1001
43	12-724-195	C	T	277–1001
44	12-724-225	C	T	245–1001
49	10-413-394	A	G	1–139; 831–1001
51	10-416-273	A	T	1–258; 855–1001
53	12-665-315	T	C	180–1001
54	12-666-324	A	C	338–375; 414–501; 581–611
78	12-244-275	A	G	
79	12-251-153	A	C	1–111; 158–950
82	12-265-300	T	C	1–999
84	12-272-112	A	C	396–416
85	10-216-182	A	G	77–151; 186–235; 320–488; 733–761
86	10-217-91	C	T	1–189; 434–462; 810–871; 925–1001
101	2-10-107	C	T	260–450
102	2-10-378	A	G	260–450
104	2-11-156	A	C	369–387
105	2-11-379	A	G	369–387
121	2-29-166	C	T	
141	10-436-43	G	C	1–630; 794–1001
142	10-436-376	A	G	1–297; 461–642; 977–1001
143	10-431-51	A	C	329–534; 682–860
144	10-432-93	A	G	1–40; 374–559; 705–885
146	10-260-282	G	T	1–24; 95–324; 482–1001
147	10-263-26	A	C	1–632; 754–1001
148	10-258-408	A	G	337–668

TABLE 12-continued

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE	POSITION RANGE OF PREFERRED SEQUENCES
149	12-317-259	G	A	1–44; 116–150; 181–241; 281–673; 968–1001
150	12-323-385	T	C	340–1001
152	12-324-335	G	C	1–176; 395–906
153	12-324-380	A	G	1–159; 378–889
154	12-325-30	C	T	272–837; 977–1001
155	12-327-31	G	T	1–570; 655–821; 875–922; 973–999
156	12-327-415	A	G	1–208; 293–459; 513–1001
157	12-331-270	G	A	1–531; 834–1001
158	12-331-275	T	G	125–536; 839–1001
159	12-334-320	A	G	68–705
160	12-334-391	A	G	1–634; 982–1001
161	12-335-417	G	C	239–1001
162	12-337-189	A	G	1–1001
163	12-340-130	A	G	196–881
164	12-340-210	C	T	196–961
165	12-340-222	G	T	196–973
166	12-340-240	C	T	196–975
167	12-341-99	A	G	1–1001
168	12-342-32	T	C	238–397; 859–960
169	12-344-349	G	T	365–1001
170	12-345-453	G	C	1–606
192	12-475-446	G	A	
228	10-325-311	A	G	41–312; 421–891
231	10-331-357	G	T	76–218; 373–730; 938–1001
232	10-334-263	A	G	1–359; 481–604; 781–893
235	12-185-78	C	T	1–1001
236	12-186-154	A	G	335–876
237	12-186-397	C	T	92–633; 967–1001
238	12-187-65	C	T	345–846
239	12-187-66	A	G	344–845
240	12-189-348	G	A	281–358; 407–1001
246	12-194-135	G	T	543–1001
247	12-194-325	A	G	351–1001
248	12-194-337	A	G	339–1001
249	12-194-479	C	T	197–1001
251	10-444-248	A	G	
254	12-670-48	G	C	1–961
255	12-670-91	C	T	1–918
256	12-670-157	C	T	1–852
257	12-671-148	C	T	1–908
258	12-679-245	A	G	96–465
259	12-679-371	A	G	96–465
260	12-679-426	C	T	96–465
265	12-586-414	A	G	1–71; 149–929
268	12-589-152	T	G	164–1001
269	12-592-118	A	T	353–1001
270	12-593-174	T	C	342–815
271	12-596-124	A	G	1–742
272	12-602-196	C	T	1–240; 436–641
274	12-603-191	T	C	1–709
275	12-783-73	G	C	1–769; 981–1001
277	12-785-200	C	T	351–510
279	12-787-103	G	A	1–47; 232–324; 401–1001
280	12-790-396	G	A	47–64; 393–1001
281	12-791-211	A	G	1–379; 467–818
284	12-803-125	T	A	125–1001
285	12-805-115	G	A	1–66; 278–838; 959–1001
286	12-808-52	A	G	400–1001
287	12-808-75	G	C	377–1001
289	12-810-77	G	A	99–1001
293	10-405-54	C	T	1–492
297	10-410-274	A	C	643–805
301	12-122-341	C	T	1–23; 150–282; 324–435; 593–620

TABLE 12-continued

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE	POSITION RANGE OF PREFERRED SEQUENCES
302	12-122-381	A	C	110–242; 284–395; 553–580
303	12-124-169	G	T	1–727; 788–1001
304	12-124-194	C	T	1–702; 763–1001
305	12-124-300	G	T	1–596; 657–1001
306	12-124-58	A	C	1–837; 898–1001
308	12-126-297	T	C	1–508; 944–1001
310	12-129-176	G	T	163–1001
313	12-131-112	T	C	254–422
315	12-132-437	A	C	258–991
316	12-133-153	T	C	1–515; 607–666; 775–918; 976–1001
317	12-133-318	T	A	1–515; 607–666; 775–918; 976–1001
318	12-136-238	A	G	1–643
319	12-138-141	G	A	1–393; 521–651
320	12-138-42	T	G	1–294; 422–552
321	12-138-67	G	A	1–319; 447–577
322	12-139-380	A	G	94–157; 276–663
323	12-140-134	G	T	1–142; 212–951
324	12-140-329	C	T	1–756
325	12-140-385	G	C	1–700
326	12-141-159	T	C	1–1001
327	12-141-392	C	A	1–1001
328	12-142-315	A	G	1–1001
329	12-142-321	A	G	1–1001
330	12-143-453	A	G	1–1001
331	12-144-169	G	A	97–1001
332	12-144-33	G	A	1–1001
333	12-146-174	T	C	1–529; 765–805
336	12-148-311	T	C	1–251; 684–1001
337	12-149-320	T	C	202–1001
338	12-151-174	G	T	313–1001
339	12-151-196	C	T	291–1001
340	12-151-270	A	G	217–1001
341	12-152-453	C	T	1–239; 526–576; 623–1001
342	12-153-116	C	T	1–819; 866–1001
346	12-157-437	A	C	1–865
347	12-158-213	T	C	1–1001
348	12-158-450	T	G	206–980
350	12-162-21	A	G	1–998
351	10-470-25	A	T	1–339; 413–539; 730–1001
355	10-473-333	C	T	461–744
356	10-494-284	C	T	89–349; 450–513
359	12-639-241	T	G	1–199; 652–667; 742–763
360	12-640-151	G	A	1–806; 943–1001
361	12-640-296	T	G	1–862
362	12-640-325	T	C	1–1001
363	12-640-413	C	G	1–1001
364	12-641-120	G	A	98–1001
365	12-641-122	T	G	100–1001
366	12-641-223	G	A	1–15; 134–1001
367	12-641-267	T	C	1–15; 134–1001
368	12-642-387	A	G	1–224; 292–1001
369	12-642-417	A	G	1–194; 262–1001
372	12-647-145	A	G	1–898
373	12-648-123	A	G	1–751
374	12-648-300	C	T	1–839
375	12-648-402	G	C	1–810; 909–1001
376	12-652-115	C	T	1–30; 191–798
377	12-652-203	A	C	1–30; 191–886
378	12-652-274	G	T	1–30; 191–957
379	12-652-371	C	T	138–1001
380	12-653-423	T	A	1–603
381	12-654-115	T	C	161–1001
382	12-654-207	G	A	1–95; 253–1001
383	12-657-396	A	G	329–1001
384	12-658-120	A	T	1–1001
385	12-659-382	A	G	1–1001
386	12-660-134	A	G	291–608; 886–983

TABLE 12-continued

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE	POSITION RANGE OF PREFERRED SEQUENCES
387	12-662-80	G	C	480–1001
391	12-906-451	A	C	447–532; 865–1001
392	12-907-199	G	T	
394	12-909-36	A	G	
397	12-910-76	A	G	1–785; 854–870
398	12-910-295	C	T	1–1001
399	12-911-22	G	C	1–740
400	12-912-65	C	T	1–183; 290–1001
401	12-914-106	C	T	1–1001
402	12-914-252	A	T	1–1001
404	10-453-330	C	T	1–207; 761–1001
406	12-5-158	C	T	672–1001
407	12-9-367	G	A	1–15; 249–283; 400–425; 493–666; 722–1001
409	12-14-264	C	T	1–1001
410	12-17-86	T	A	1–1001
411	12-19-163	A	G	230–388; 525–709; 824–866
414	10-460-232	A	G	1–349; 521–1001
415	10-460-235	C	T	1–346; 518–1001
416	10-460-236	A	G	1–345; 517–1001
417	10-460-285	A	T	1–296; 468–1001
418	12-605-58	G	T	1–1001
419	12-607-207	G	A	1–746; 885–902
420	12-609-119	T	G	1–234; 294–1001
421	12-609-180	T	G	40–295; 355–1001
422	12-609-233	G	A	138–348; 404–1001
423	12-611-294	G	A	1–617; 895–967
424	12-612-41	C	T	1–56; 398–708
425	12-613-302	C	G	1–86; 336–1001
426	12-614-471	T	A	1–1001
427	12-620-192	G	T	318–1001
428	12-621-49	A	G	1–242; 451–815
429	12-622-325	C	T	
430	12-624-82	T	C	1–1001
431	12-624-83	G	A	1–1001
432	12-624-107	T	C	1–989
433	12-624-146	T	C	1–1001
434	12-624-288	T	G	1–1001
435	12-624-293	T	C	1–1001
436	12-421-135	T	—	1–1001
438	12-449-63	AT	—	1–107; 314–656
439	12-454-242	AT	—	1–436; 498–587; 719–740
440	12-463-250	CAT	—	1–30; 102–601; 752–1001
441	12-462-199		deletion	1–1001
442	10-430-287	T	—	1–330; 442–740; 782–1001
443	12-718-432	T	—	1–1001
444	12-269-301	T	—	774–820
450	12-345-410		deletion	1–649
460	12-586-443	C	—	1–42; 120–911
461	12-593-287		deletion	1–17; 455–942
462	12-795-383		insertion	166–500; 540–1001
465	12-659-251	deletion	—	1–929
466	12-912-419	A	—	1–686
467	12-914-28	T	—	1–806
468	12-624-307	T	—	1–1001
469	10-290-326	A	G	1–197; 437–1000
472	12-449-300	T	C	1–542; 908–1000
484	10-290-328	deletion		1–194; 434–1000

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TABLE 13

SEQ ID NO.	BIALLELIC MARKER ID	ORIGINAL ALLELE	ALTERNATIVE ALLELE
10	12-441-343	G	A
18	12-456-380	G	T
32	10-429-84	T	C
45	10-153-329	T	G
46	10-95-342	G	A
47	10-100-277	T	C
57	10-76-217	C	T
59	10-77-316	A	T
60	10-155-78	T	C
61	10-155-104	C	G
62	10-156-52	G	A
63	10-157-39	C	T
64	10-157-131	A	G
65	10-157-166	G	A
66	10-157-246	G	A
67	10-159-161	T	A
68	10-159-162	C	A
69	10-83-169	T	C
70	10-84-152	C	T
71	10-84-243	C	T
74	10-85-43	C	T
76	10-85-320	A	T
81	12-254-180	G	A
83	12-271-118	T	C
87	10-213-292	C	G
90	2-1-216	G	A
91	2-1-397	T	C
92	2-3-232	C	T
93	2-4-51	A	C
94	2-4-126	A	G
95	2-5-202	A	G
96	2-5-275	G	A
97	2-5-346	C	T
98	2-8-171	A	G
99	2-9-188	G	A
100	2-9-223	T	G
103	2-11-284	A	G
106	2-12-223	A	T
107	2-14-239	C	T
108	2-14-370	C	G
109	2-17-104	A	G
110	2-17-396	A	C
111	2-22-43	A	G
112	2-22-138	G	A
113	2-23-82	A	G
114	2-23-166	G	A
115	2-23-244	G	T
116	2-24-115	C	T
117	2-25-36	G	C
118	2-27-185	G	A
119	2-27-378	G	C
120	2-29-142	G	A
124	2-29-314	C	T
125	2-32-68	A	G
126	2-35-357	T	C
127	2-36-256	G	C
128	2-36-354	A	C
129	2-42-236	C	T
130	2-43-139	G	A
131	2-44-215	C	T
132	2-45-38	C	T
133	2-45-183	T	C
134	2-45-335	T	A
135	2-45-394	C	T
136	2-48-39	A	G
137	2-48-72	C	T
138	2-48-156	T	G
139	2-48-285	A	G
140	2-49-167	A	G
151	12-324-219	C	T
171	12-346-204	G	A

TABLE 13-continued

SEQ ID NO.	BIALLELIC MARKER ID	ORIGINAL ALLELE	ALTERNATIVE ALLELE
172	10-364-55	T	G
173	10-364-108	T	C
174	10-364-267	T	A
175	10-367-20	T	G
176	10-351-389	A	G
177	10-353-102	T	A
178	12-474-346	G	A
179	10-354-320	G	A
180	10-354-360	A	G
181	10-355-87	G	A
182	10-358-60	G	A
183	12-468-63	T	C
184	12-468-388	T	C
185	12-468-491	G	A
186	12-469-132	T	C
187	12-469-245	G	A
188	12-472-435	G	A
189	12-473-311	A	C
190	12-473-483	T	C
191	12-475-85	G	T
193	12-477-100	A	G
194	12-477-331	G	A
195	12-477-332	T	C
196	12-477-44	C	G
197	12-478-223	G	A
198	12-478-320	G	A
199	12-479-289	G	T
200	12-482-237	A	G
201	12-482-285	T	A
202	12-482-482	A	T
203	12-483-322	A	T
204	12-484-46	G	A
205	12-490-312	A	T
206	12-491-295	A	G
207	12-493-417	G	C
208	12-494-373	C	T
209	12-495-166	C	T
210	12-495-272	A	T
211	12-495-424	T	C
212	12-500-220	A	G
213	12-501-155	G	T
214	12-503-52	T	G
215	12-503-62	T	A
216	12-504-54	G	A
217	12-504-96	C	T
218	12-504-428	G	C
219	12-507-53	T	C
220	12-507-92	A	G
221	12-507-159	T	G
222	12-507-177	C	G
223	12-508-29	G	A
224	12-509-42	G	A
225	12-509-126	G	A
226	12-510-59	G	A
227	12-511-74	T	C
229	10-327-120	C	T
230	10-331-179	G	A
233	10-321-226	A	G
234	12-183-98	G	A/T
241	12-192-63	C	G/T
243	12-192-268	G	C
245	12-192-352	G	A
250	10-442-133	G	C
252	10-445-281	C	T
253	12-668-362	T	A
261	12-680-331	C	T
262	10-151-154	G	A
264	10-138-352	T	C
266	12-587-379	A	C
267	12-588-103	G	A
273	12-602-350	C	A
278	12-785-393	G	A
283	12-793-383	T	G

TABLE 13-continued

SEQ ID NO.	BIALLELIC MARKER ID	ORIGINAL ALLELE	ALTERNATIVE ALLELE
288	12-809-119	C	G
290	10-265-178	G	A
292	10-403-312	C	T
298	10-410-280	C	T
299	10-410-337	A	G
300	12-121-326	G	A
307	12-126-222	C	T
309	12-128-225	G	T
312	12-130-260	C	T
314	12-132-157	T	C
334	12-146-47	A	G
335	12-148-283	G	A
343	12-154-480	C	T
344	12-155-403	C	A
349	12-161-157	G	A
358	12-639-95	G	A
370	12-646-429	T	C
371	12-646-433	T	G
388	12-906-149	G	A
389	12-906-154	A	C
390	12-906-251	A	T
395	12-909-176	C	T
396	12-909-484	G	T
403	10-448-266	A	C
405	10-455-367	T	C
408	12-10-303	C	T
413	10-460-221	C	T
437	12-442-133	C	—
445	2-13-398	G	—
446	2-28-132	—	T
447	2-39-27		deletion
448	2-45-155		deletion
449	2-4-391	G	—
452	10-360-190	—	T
453	10-365-374	—	A
454	10-367-58	—	insertion
455	12-468-424	—	T
457	12-499-86		deletion
458	12-500-217		insertion
459	12-511-101	A	—
463	10-494-332	insertion	
470	10-290-37	C	T
471	10-523-232	C	T
473	10-186-212	G	C
474	10-286-289	C	G
475	10-286-345	A	T
476	10-286-375	A	G
477	10-289-201	T	C
478	10-321-28	G	A
479	10-98-265	A	G
480	12-157-115	T	C
481	12-472-48	T	G
482	12-477-151	A	G
483	12-479-214	G	T

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TABLE 14

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE
9	12-441-233	G	A
11	12-442-221	T	C
48	10-102-294	C	T
50	10-414-243	A	G
52	10-418-177	A	G
56	10-76-177	A	T
58	10-76-333	G	C
72	10-84-277	A	G

TABLE 14-continued

SEQ ID NO.	BIALLELIC MARKER ID	1ST ALLELE	2ND ALLELE
73	10-84-295	A	G
75	10-85-117	G	T
77	10-86-121	A	C
80	12-254-115	A	T
88	10-214-279	C	T
89	10-214-380	A	G
122	2-29-205	C	T
123	2-29-206	A	G
145	12-631-208	A	G
242	12-192-64	T	C
244	12-192-334	G	A
263	10-138-206	C	T
276	12-783-421	C	T
282	12-792-233	G	A
291	10-266-203	C	T
294	10-408-356	C	T
295	10-409-148	G	C
296	10-409-249	G	C
311	12-130-203	C	T
345	12-156-91	A	G
352	10-471-84	A	T
353	10-471-85	A	C
357	12-637-219	G	A
393	12-907-482	A	T
412	10-457-284	G	T
451	10-358-353		deletion
456	12-481-293	T	—

[0745]

TABLE 15

SEQ ID NO.	POSITION RANGE OF PREFERRED SEQUENCES
9	940–1001
18	1–425; 920–1001
32	1–175; 713–787
50	683–1001
52	1–450; 606–1001
58	732–832
59	612–725
87	717–742; 881–899; 951–1001
88	1–24; 276–305
103	369–387
129	433–449
145	152–241; 540–933
151	1–176; 395–857
171	1–98; 753–798
212	986–1001
229	1–439; 639–1001
230	254–396; 551–908
233	1–161; 283–406; 583–695; 843–1001
261	1–452
267	552–1001
273	1–86; 282–487; 963–1001
276	1–421; 633–1001
278	158–317; 834–1001
283	1–112
288	1–174; 252–399; 583–987
290	1–342; 790–1001
291	86–460
292	654–1001
298	637–799
299	580–742
300	1–297; 727–803
307	1–433; 869–1001
309	1–17
311	928–953
312	871–896; 972–1001

TABLE 15-continued

SEQ ID NO.	POSITION RANGE OF PREFERRED SEQUENCES
314	289–755
334	1–402; 638–678; 969–1001
335	1–223; 656–923
343	629–1000
349	98–251; 960–1001
352	1–52; 399–436
353	1–52; 399–436
357	527–810
358	1–53; 506–521; 596–617
370	1–80; 675–1001
371	1–80; 675–1001
388	1–293; 748–833
389	1–288; 743–828
390	1–191; 646–731
403	745–1001
405	984–1001
408	797–897; 931–1001
412	1–207; 979–1001
455	1–22
463	1–299; 400–463
470	1–485; 725–1000
471	1–432; 514–1000
474	1–353; 656–1000
475	1–298; 601–1000
476	1–268; 571–1000
477	1–452; 538–1000
479	385–391
480	910–954
482	443–531; 730–854
483	347–392

[0746]

TABLE 16

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
1	481–500	502–521
2	481–500	502–521
3	481–500	502–521
4	481–500	502–521
5	441–460	462–481
6	481–500	502–521
7	482–500*	502–521
8	481–500	502–521
9	482–500*	502–521
10	481–500	502–521
11	481–500	502–521
12	482–500*	502–521
13	481–500	502–520*
14	481–500	502–521
15	481–500	502–520*
16	481–500	502–521
17	481–500	502–521
18	481–500	502–521
19	481–500	502–521
20	481–500	502–521
21	481–500	502–521
22	481–500	502–521
23	481–500	502–521
24	481–500	502–521
25	481–500	502–520*
26	481–500	502–521
27	481–500	502–521
28	481–500	502–521
29	481–500	502–521
30	481–500	502–521

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
31	481–500	502–521
32	481–500	502–521
33	481–500	502–521
34	481–500	502–521
35	213–231*	233–252
36	266–285	287–306
37	477–499*	501–520
38	461–479*	481–500
40	406–425	427–446
41	481–500	502–520*
42	484–502*	504–523
43	481–500	502–521
44	482–500*	502–521
45	310–329	331–349*
46	322–341	343–361*
47	258–276*	278–297
48	278–296*	298–317
49	482–500*	502–520*
50	481–500	502–521
51	482–500*	502–520*
52	481–500	502–520*
53	482–500*	502–521
54	482–500*	502–521
56	158–177	179–198
57	198–217	219–238
58	315–333*	335–354
59	481–500	502–521
60	58–77	79–98
61	84–103	105–124
62	32–51	53–71*
63	20–38*	40–59
64	111–130	132–150*
65	146–165	167–186
66	226–245	247–266
67	142–160*	162–181
68	142–161	163–182
69	150–168*	170–189
70	132–151	153–172
71	224–242*	244–263
72	257–276	278–297
73	275–294	296–314*
74	24–42*	44–63
75	97–116	118–136*
76	300–319	321–340
77	102–120*	122–141
78	481–500	502–521
79	431–449*	451–470
80	226–245	247–266
81	292–310*	312–331
82	479–498	500–519
83	481–500	502–521
84	482–500*	502–521
85	483–502	504–522*
86	482–500*	502–521
87	484–502*	504–523
88	481–500	502–521
89	481–500	502–520*
90	196–215	217–236
91	377–396	398–417
92	212–231	233–252
93	31–50	52–71
94	106–125	127–146
95	182–201	203–222
96	255–274	276–295
97	326–345	347–366
98	151–170	172–191
99	168–187	189–208
100	203–222	224–243
101	87–106	108–127
102	357–376	378–397
103	264–283	285–304

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
104	136–155	157–176
105	359–378	380–399
106	203–222	224–243
107	219–238	240–259
108	350–369	371–390
109	84–103	105–124
110	375–394	396–415
111	23–42	44–63
112	118–137	139–158
113	62–81	83–102
114	146–165	167–186
115	224–243	245–264
116	95–114	116–135
117	16–35	37–56
118	165–184	186–205
119	358–377	379–398
120	122–141	143–162
121	146–165	167–186
122	184–203	205–224
123	185–204	206–225
124	293–312	314–333
125	48–67	69–88
126	337–356	358–377
127	236–255	257–276
128	333–352	354–373
129	216–235	237–256
130	119–138	140–159
131	193–212	214–233
132	18–37	39–58
133	163–182	184–203
134	315–334	336–355
135	374–393	395–414
136	19–38	40–59
137	52–71	73–92
138	136–155	157–176
139	265–284	286–305
140	147–166	168–187
141	482–500*	502–521
142	481–500	502–520*
143	482–500*	502–521
144	457–476	481–499*
145	414–432*	434–453
146	481–500	502–521
147	483–502	504–523
148	481–500	502–521
149	481–500	502–521
150	481–500	502–520*
151	337–356	358–377
152	454–472*	474–493
153	481–500	502–521
154	481–500	502–521
155	459–478	480–498*
156	480–499	501–520
157	481–500	502–521
158	481–500	502–521
159	483–502	504–523
160	483–502	504–523
161	481–500	502–521
162	481–500	502–521
163	361–380	382–401
164	442–460*	462–481
165	453–472	474–493
166	471–490	492–511
167	481–500	502–520*
168	481–500	502–521
169	481–500	502–520*
170	481–500	502–521
171	481–500	502–521
172	481–500	502–521
173	481–500	502–521
174	481–500	502–521

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
175	477–496	498–517
176	481–500	502–521
177	481–500	502–521
178	482–500*	502–521
179	481–500	502–521
180	481–500	502–521
181	481–500	502–521
182	481–500	502–521
183	481–500	502–520*
184	481–500	502–521
185	481–500	502–521
186	481–500	502–521
187	481–500	502–521
188	481–500	502–521
189	481–500	502–521
190	481–500	502–521
191	481–500	502–521
192	465–484	486–505
193	481–500	502–521
194	481–500	502–521
195	481–500	502–521
196	481–500	502–521
197	481–500	502–521
198	481–500	502–521
199	481–500	502–521
200	481–500	502–521
201	481–500	502–521
202	481–500	502–521
203	479–498	500–519
204	481–500	502–521
205	481–500	502–521
206	481–500	502–521
207	481–500	502–521
208	481–500	502–521
209	481–500	502–521
210	481–500	502–521
211	481–500	502–521
212	481–500	502–521
213	481–500	502–521
214	481–500	502–521
215	481–500	502–521
216	479–498	500–519
217	481–500	502–521
218	482–500*	502–521
219	454–473	475–493*
220	481–500	502–521
221	480–499	501–520
222	481–500	502–521
223	481–500	502–521
224	481–500	502–521
225	481–500	502–521
226	481–500	502–521
227	483–500*	502–521
228	481–500	502–521
229	481–500	502–521
230	481–500	502–521
231	481–500	502–521
232	481–500	502–521
233	481–500	502–521
234	481–500	502–521
235	481–500	502–521
236	481–500	502–520*
237	482–500*	502–521
238	482–500*	502–521
239	481–500	502–521
240	481–500	502–520*
241	481–500	502–521
242	482–501	503–522
243	481–500	502–521
244	481–500	502–521
245	481–500	502–521

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
246	481–500	502–521
247	481–500	502–521
248	481–500	502–521
249	481–500	502–521
250	482–500*	502–521
251	464–483	485–503*
252	482–500*	502–521
253	481–500	502–521
254	481–500	502–521
255	481–500	502–521
256	482–500*	502–521
257	482–500*	502–521
258	233–252	254–273
259	359–378	380–399
260	414–433	435–454
261	483–502	504–523
262	134–153	155–173*
263	186–204*	206–225
264	332–350*	352–371
265	482–500*	502–521
266	479–498	500–519
267	482–500*	502–521
268	481–500	502–521
269	482–500*	502–521
270	481–500	502–520*
271	481–500	502–520*
272	481–500	502–520*
273	481–500	502–521
274	481–500	502–520*
275	481–500	502–521
276	481–500	502–521
277	481–500	502–521
278	481–500	502–521
279	481–500	502–521
280	481–500	502–521
281	481–500	502–521
282	481–500	502–521
283	485–504	506–525
284	481–500	502–521
285	481–500	502–521
286	481–500	502–521
287	481–500	502–521
288	481–500	502–521
289	481–500	502–521
290	481–500	502–521
291	483–502	504–523
292	481–500	502–521
293	481–500	502–521
294	481–500	502–521
295	481–500	502–521
296	481–500	502–521
297	481–500	502–521
298	481–500	502–521
299	481–500	502–521
300	481–500	502–520*
301	481–500	502–521
302	481–500	502–521
303	481–500	502–521
304	482–500*	502–521
305	481–500	502–521
306	481–500	502–521
307	481–500	502–521
308	481–500	502–521
309	481–500	502–520*
310	481–500	502–521
311	481–500	502–521
312	481–500	502–521
313	481–500	502–521
314	235–254	256–275
315	481–500	502–521
316	646–665	667–686

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
317	481–500	502–521
318	229–248	250–269
319	481–500	502–521
320	481–500	502–521
321	481–500	502–521
322	481–500	502–520*
323	481–500	502–520*
324	481–500	502–521
325	481–500	502–521
326	482–500*	502–521
327	481–500	502–521
328	481–500	502–521
329	481–500	502–520*
330	481–500	502–520*
331	481–500	502–521
332	481–500	502–521
333	481–500	502–521
334	481–500	502–521
335	481–500	502–521
336	481–500	502–520*
337	481–500	502–521
338	481–500	502–521
339	481–500	502–521
340	481–500	502–521
341	481–500	502–521
342	482–500*	502–520*
343	481–500	502–521
344	481–500	502–521
345	482–500*	502–520*
346	481–500	502–521
347	481–500	502–521
348	460–479	481–500
349	481–500	502–521
350	478–497	499–518
351	484–502*	504–523
352	484–502*	504–523
353	483–502	505–523*
355	483–502	504–523
356	483–502	504–523
357	480–498*	500–519
358	480–498*	500–519
359	479–498	500–519
360	479–498	500–519
361	479–498	500–519
362	479–498	500–518*
363	479–498	500–519
364	479–498	500–519
365	479–498	500–519
366	412–431	433–452
367	368–387	389–408
368	483–502	504–523
369	484–502*	504–523
370	414–433	435–454
371	418–437	439–458
372	483–502	504–523
373	231–250	252–271
374	408–427	429–447*
375	481–500	502–521
376	278–297	299–318
377	367–385*	387–406
378	437–456	458–477
379	481–500	502–521
380	479–498	500–519
381	479–498	500–519
382	479–498	500–519
383	483–502	504–523
384	483–502	504–523
385	481–500	502–521
386	286–305	307–326
387	477–496	498–517
388	482–500*	502–521

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
389	481–500	502–521
390	481–500	502–521
391	481–500	502–521
392	224–243	245–263*
393	481–500	502–521
394	33–52	54–73
395	173–192	194–213
396	481–500	502–521
397	327–346	348–366*
398	483–502	504–523
399	221–239*	241–260
400	481–500	502–521
401	365–383*	385–404
402	483–502	504–523
403	483–502	504–523
404	483–502	504–523
405	483–502	504–523
406	484–502*	504–523
407	479–498	500–518*
408	482–500*	502–521
409	482–500*	502–521
410	479–498	500–518*
411	481–500	502–520*
412	483–502	504–523
413	483–502	504–523
414	483–502	504–523
415	483–502	504–523
416	483–502	504–523
417	483–502	504–523
418	481–500	502–520*
419	481–500	502–521
420	479–498	500–518*
421	479–498	500–519
422	479–498	500–519
423	481–500	502–520*
424	481–500	502–520*
425	479–498	500–519
426	481–500	502–520*
427	483–502	504–522*
428	483–502	504–523
429	344–363	365–384
430	481–500	502–521
431	481–500	502–521
432	469–488	490–509
433	481–500	502–521
434	481–500	502–521
435	481–500	502–521
436	481–500	—
437	—	502–520*
438	481–500	—
439	481–500	—
440	481–500	—
441	481–500	—
442	481–500	—
443	481–500	—
444	482–500	—
445	481–500	—
446	481–500	—
447	481–500	—
448	481–500	—
449	481–500	—
450	481–500	—
451	481–500	—
452	481–500	—
453	481–500	—
454	481–500	—
455	481–500	—
456	481–500	—
457	481–500	—
458	481–500	—
459	481–500	—

TABLE 16-continued

SEQ ID NO.	POSITION RANGE OF MICROSEQUENCING PRIMERS	COMPLEMENTARY POSITION RANGE OF MICROSEQUENCING PRIMERS
460	481–500	—
461	481–500	—
462	481–500	—
463	481–500	—
465	409–428	—
466	481–500	—
467	286–305	—
468	481–500	—
469	481–500	502–521
470	481–500	502–521
471	481–500	502–521
472	481–500	502–521
473	192–211	213–232
474	481–500	502–521
475	481–500	502–521
476	481–500	502–521
477	481–500	502–521
478	481–500	502–521
479	245–264	266–285
480	481–500	502–521
481	481–500	502–521
482	481–500	502–521
483	481–500	502–521
484	481–500	502–521

[0747]

TABLE 17

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
1	362–380	792–812
2	310–327	751–771
3	304–321	745–765
4	82–99	540–557
5	308–325	830–847
6	304–321	803–823
7	131–150	561–580
8	287–304	805–825
9	284–303	716–734
10	394–413	826–844
11	270–289	704–721
12	444–462	874–893
13	73–91	577–596
14	139–158	634–652
15	372–392	808–826
16	429–449	865–883
17	233–252	693–712
18	122–141	582–601
19	298–317	772–792
20	296–315	770–790
21	200–217	679–696
22	442–459	921–938
23	228–245	760–777
24	378–396	911–928
25	203–221	736–753
26	37–55	570–587
27	222–241	655–675
28	436–455	880–900
29	476–493	945–962
30	266–283	735–752
31	278–295	613–632
32	418–436	823–842
33	216–235	646–665
34	91–109	510–528

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
35	137–153	586–604
36	137–153	586–604
37	206–225	727–746
38	400–419	856–876
40	146–165	588–607
41	62–81	504–523
42	210–230	591–610
43	307–326	797–817
44	277–296	767–787
45	1–20	402–421
46	3–21	404–422
47	1–18	355–372
48	1–18	356–375
49	108–125	539–556
50	259–276	592–609
51	229–246	630–649
52	325–342	659–676
53	357–377	795–815
54	186–205	621–641
56	1–18	416–435
57	1–18	416–435
58	1–18	416–435
59	185–203	593–610
60	1–18	424–442
61	1–18	424–442
62	1–19	401–420
63	1–18	412–431
64	1–18	412–431
65	1–18	412–431
66	1–18	412–431
67	1–19	403–422
68	1–19	403–422
69	1–19	336–353
70	1–18	406–425
71	1–18	406–425
72	1–18	406–425
73	1–18	406–425
74	1–18	405–424
75	1–18	405–424
76	1–18	405–424
77	1–18	334–352
78	228–247	660–678
79	298–318	806–826
80	132–152	586–603
81	132–152	586–603
82	308–328	779–798
83	122–141	598–618
84	390–409	768–788
85	323–339	800–819
86	411–427	761–777
87	212–230	590–608
88	154–174	746–763
89	124–143	647–664
90	1–18	417–437
91	1–18	417–437
92	1–18	406–426
93	3–24	405–429
94	3–24	405–429
95	1–25	400–420
96	1–25	400–420
97	1–25	400–420
98	1–18	405–427
99	1–21	396–420
100	1–21	396–420
101	1–18	423–443
102	1–18	423–443
103	1–18	429–446
104	1–18	429–446
105	1–18	429–446
106	1–25	399–420
107	1–23	398–418

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
108	1–23	398–418
109	1–18	427–445
110	1–18	427–445
111	1–18	416–436
112	1–18	416–436
113	1–25	396–420
114	1–25	396–420
115	1–25	396–420
116	1–18	416–434
117	1–21	396–420
118	1–19	405–429
119	1–19	405–429
120	1–21	422–439
121	1–21	422–439
122	1–21	422–439
123	1–21	422–439
124	1–21	422–439
125	1–21	413–432
126	1–18	404–423
127	1–21	411–435
128	1–21	411–435
129	1–18	428–449
130	1–18	395–419
131	3–23	398–420
132	1–21	418–442
133	1–21	418–442
134	1–21	418–442
135	1–21	418–442
136	1–24	404–426
137	1–24	404–426
138	1–24	404–426
139	1–24	404–426
140	1–20	396–420
141	459–476	859–878
142	126–143	526–545
143	451–468	853–872
144	388–407	758–775
145	226–245	705–725
146	220–238	636–655
147	478–496	820–837
148	95–112	504–521
149	297–317	742–759
150	416–435	868–886
151	139–157	579–599
152	139–157	579–599
153	122–140	562–582
154	472–491	926–945
155	449–468	982–999
156	86–105	615–633
157	285–305	751–770
158	290–310	756–775
159	184–202	616–634
160	113–131	545–563
161	85–102	534–552
162	313–331	792–812
163	252–272	681–701
164	252–272	681–701
165	252–272	681–701
166	252–272	681–701
167	403–422	927–947
168	81–101	513–532
169	154–173	685–705
170	53–70	558–578
171	248–267	684–704
172	447–464	849–867
173	394–411	796–814
174	235–252	637–655
175	478–495	889–908
176	113–132	518–537
177	400–417	800–819
178	430–447	830–849

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
179	182–199	582–601
180	142–159	542–561
181	415–434	821–840
182	442–460	870–889
183	439–458	946–966
184	113–132	620–640
185	10–29	517–537
186	370–387	812–832
187	257–274	699–719
188	68–86	533–553
189	192–210	740–758
190	20–38	568–586
191	108–126	566–585
192	453–471	911–930
193	62–82	580–600
194	294–314	812–832
195	295–315	813–833
196	6–26	524–544
197	234–254	704–723
198	331–351	801–820
199	213–230	678–698
200	223–243	720–737
201	271–291	768–785
202	468–488	965–982
203	311–331	802–820
204	86–106	528–546
205	189–209	621–641
206	266–286	777–795
207	90–109	514–534
208	127–144	571–591
209	336–355	784–802
210	230–249	678–696
211	78–97	526–544
212	283–303	711–731
213	168–188	636–655
214	80–100	535–552
215	90–110	545–562
216	446–463	993–1013
217	406–423	953–973
218	75–92	622–642
219	422–441	982–1001
220	410–429	970–990
221	341–360	901–921
222	324–343	884–904
223	473–491	907–925
224	460–479	889–909
225	376–395	805–825
226	107–127	539–559
227	125–145	558–575
228	191–208	596–613
229	382–400	805–824
230	326–345	728–747
231	148–167	550–569
232	240–257	658–675
233	276–293	692–711
234	136–155	581–598
235	424–444	855–875
236	348–368	784–803
237	105–125	541–560
238	437–456	839–859
239	436–455	838–858
240	384–402	832–849
241	75–94	544–563
242	76–95	545–564
243	280–299	749–768
244	346–365	815–834
245	364–383	833–852
246	363–381	878–893
247	171–189	686–707
248	159–177	674–695
249	17–35	532–553

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
250	369–386	777–794
251	237–253	567–586
252	221–238	624–641
253	390–410	844–861
254	454–474	883–901
255	411–431	840–858
256	345–365	774–792
257	354–372	784–804
258	9–29	439–458
259	9–29	439–458
260	9–29	439–458
261	173–192	645–665
262	1–18	367–384
263	1–18	406–425
264	1–18	406–425
265	88–107	552–572
266	478–498	857–877
267	60–77	585–603
268	190–210	636–654
269	384–402	830–849
270	138–158	658–675
271	378–397	805–825
272	307–325	704–724
273	153–171	550–570
274	240–260	668–688
275	429–446	858–878
276	81–98	510–530
277	302–322	791–811
278	109–129	598–618
279	74–94	583–602
280	423–443	876–896
281	291–311	671–690
282	284–304	712–732
283	365–385	866–884
284	169–189	605–625
285	135–155	596–615
286	450–469	894–914
287	427–446	871–891
288	383–402	888–908
289	126–146	558–577
290	324–341	662–681
291	301–320	701–720
292	190–208	593–611
293	448–465	848–867
294	146–165	546–565
295	354–372	779–798
296	253–271	678–697
297	228–245	645–664
298	222–239	639–658
299	165–182	582–601
300	178–196	637–656
301	162–180	595–612
302	122–140	555–572
303	334–352	830–848
304	309–327	805–823
305	203–221	699–717
306	444–462	940–958
307	267–286	703–722
308	342–361	778–797
309	276–295	706–725
310	326–344	779–797
311	301–319	733–753
312	244–262	676–696
313	104–124	594–612
314	99–117	557–577
315	68–86	526–546
316	250–270	800–818
317	250–270	800–818
318	12–32	442–461
319	186–204	623–641
320	87–105	524–542

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
321	112–130	549–567
322	122–139	602–620
323	368–386	868–888
324	173–191	673–693
325	117–135	617–637
326	181–200	640–658
327	414–433	873–891
328	187–205	637–657
329	181–199	631–651
330	50–68	533–552
331	148–167	651–669
332	12–31	515–533
333	271–291	655–674
334	144–164	528–547
335	375–395	765–783
336	403–423	793–811
337	368–387	800–820
338	328–345	827–845
339	306–323	805–823
340	232–249	731–749
341	50–68	553–572
342	386–405	867–887
343	23–43	522–540
344	99–116	628–647
345	412–429	844–862
346	67–87	513–533
347	230–247	691–710
348	446–463	907–926
349	345–363	729–749
350	478–497	909–927
351	479–498	880–899
352	420–439	788–807
353	420–439	788–807
355	171–189	582–600
356	220–238	624–641
357	230–250	698–717
358	144–164	573–593
359	290–310	719–739
360	156–176	629–649
361	296–316	769–789
362	324–344	797–817
363	412–432	885–905
364	127–147	600–618
365	129–149	602–620
366	163–183	636–654
367	163–183	636–654
368	117–135	592–612
369	87–105	562–582
370	6–26	474–494
371	6–26	474–494
372	359–378	788–808
373	129–147	607–627
374	129–147	607–627
375	100–118	578–598
376	184–203	615–635
377	184–203	615–635
378	184–203	615–635
379	131–150	562–582
380	390–410	903–921
381	76–96	595–613
382	168–188	687–705
383	108–128	566–586
384	384–404	863–883
385	120–139	552–572
386	173–193	692–712
387	418–435	979–1000
388	353–372	809–829
389	348–367	804–824
390	251–270	707–727
391	52–71	508–528
392	46–65	533–553

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
393	20–39	507–527
394	18–38	505–525
395	18–38	505–525
396	18–38	505–525
397	272–292	704–724
398	209–229	641–661
399	219–237	653–673
400	437–457	908–928
401	279–298	773–793
402	252–271	746–766
403	238–257	660–679
404	172–189	578–597
405	135–152	545–564
406	346–366	801–821
407	386–406	847–865
408	335–355	787–807
409	237–257	680–700
410	121–140	565–584
411	339–357	781–801
412	220–238	621–639
413	283–301	686–704
414	272–290	675–693
415	269–287	672–690
416	268–286	671–689
417	219–237	622–640
418	444–464	901–921
419	179–199	689–707
420	114–134	597–615
421	175–195	658–676
422	228–248	711–729
423	251–271	776–795
424	461–481	981–1001
425	343–363	781–799
426	424–442	952–971
427	309–326	777–797
428	455–473	907–927
429	40–59	551–569
430	114–134	562–582
431	115–135	563–583
432	127–147	575–595
433	179–199	627–647
434	321–341	769–789
435	326–346	774–794
436	367–385	797–817
437	184–203	616–633
438	86–106	546–563
439	260–279	755–773
440	255–272	773–790
441	303–322	736–756
442	215–233	617–635
443	479–499	913–932
444	204–222	634–654
445	104–121	506–525
446	370–387	769–793
447	475–495	870–892
448	347–367	764–788
449	111–132	513–537
450	96–113	601–621
451	149–167	577–596
452	312–329	713–731
453	128–145	530–548
454	444–461	855–874
455	76–95	583–603
456	333–353	774–793
457	136–156	567–586
458	286–306	714–734
459	152–172	585–602
460	59–78	523–543
461	251–271	771–788
462	119–136	679–696
463	170–188	574–591

TABLE 17-continued

SEQ ID NO.	POSITION RANGE OF AMPLIFICATION PRIMERS	COMPLEMENTARY POSITION RANGE OF AMPLIFICATION PRIMERS
465	179–198	611–631
466	83–103	554–574
467	279–298	773–793
468	340–360	788–808
469	177–196	576–595
470	465–484	864–883
471	270–288	527–545
472	325–345	783–800
473	1–20	413–432
474	213–231	613–631
475	158–176	558–576
476	128–146	528–546
477	307–324	700–719
478	474–491	890–909
479	1–19	404–422
480	387–407	833–853
481	454–472	919–939
482	113–133	631–651
483	288–305	753–773
484	174–193	573–592

[0748]

TABLE 18

SEQ ID NO.	POSITION RANGE OF PROBES
1	489–513
2	489–513
3	489–513
4	489–513
5	449–473
6	489–513
7	489–513
8	489–513
9	489–513
10	489–513
11	489–513
12	489–513
13	489–513
14	489–513
15	489–513
16	489–513
17	489–513
18	489–513
19	489–513
20	489–513
21	489–513
22	489–513
23	489–513
24	489–513
25	489–513
26	489–513
27	489–513
28	489–513
29	489–513
30	489–513
31	489–513
32	489–513
33	489–513
34	489–513
35	220–244
36	274–298
37	488–512
38	468–492
40	414–438
41	489–513

TABLE 18-continued

SEQ ID NO.	POSITION RANGE OF PROBES
42	491–515
43	489–513
44	489–513
45	318–342
46	330–354
47	265–289
48	285–309
49	489–513
50	489–513
51	489–513
52	489–513
53	489–513
54	489–513
56	166–190
57	206–230
58	322–346
59	489–513
60	66–90
61	92–116
62	40–64
63	27–51
64	119–143
65	154–178
66	234–258
67	149–173
68	150–174
69	157–181
70	140–164
71	231–255
72	265–289
73	283–307
74	31–55
75	105–129
76	308–332
77	109–133
78	489–513
79	438–462
80	234–258
81	299–323
82	487–511
83	489–513
84	489–513
85	491–515
86	489–513
87	491–515
88	489–513
89	489–513
90	204–228
91	385–409
92	220–244
93	39–63
94	114–138
95	190–214
96	263–287
97	334–358
98	159–183
99	176–200
100	211–235
101	95–119
102	365–389
103	272–296
104	144–168
105	367–391
106	211–235
107	227–251
108	358–382
109	92–116
110	383–407
111	31–55
112	126–150
113	70–94
114	154–178
115	232–256

	TABLE 18-continued		TABLE 18-continued
SEQ ID NO.	POSITION RANGE OF PROBES	SEQ ID NO.	POSITION RANGE OF PROBES
116	103–127	189	489–513
117	24–48	190	489–513
118	173–197	191	489–513
119	366–390	192	473–497
120	130–154	193	489–513
121	154–178	194	489–513
122	192–216	195	489–513
123	193–217	196	489–513
124	301–325	197	489–513
125	56–80	198	489–513
126	345–369	199	489–513
127	244–268	200	489–513
128	341–365	201	489–513
129	224–248	202	489–513
130	127–151	203	487–511
131	201–225	204	489–513
132	26–50	205	489–513
133	171–195	206	489–513
134	323–347	207	489–513
135	382–406	208	489–513
136	27–51	209	489–513
137	60–84	210	489–513
138	144–168	211	489–513
139	273–297	212	489–513
140	155–179	213	489–513
141	489–513	214	489–513
142	489–513	215	489–513
143	489–513	216	487–511
144	465–489	217	489–513
145	421–445	218	489–513
146	489–513	219	462–486
147	491–515	220	489–513
148	489–513	221	488–512
149	489–513	222	489–513
150	489–513	223	489–513
151	345–369	224	489–513
152	461–485	225	489–513
153	489–513	226	489–513
154	489–513	227	489–513
155	467–491	228	489–513
156	488–512	229	489–513
157	489–513	230	489–513
158	489–513	231	489–513
159	491–515	232	489–513
160	491–515	233	489–513
161	489–513	234	489–513
162	489–513	235	489–513
163	369–393	236	489–513
164	449–473	237	489–513
165	461–485	238	489–513
166	479–503	239	489–513
167	489–513	240	489–513
168	489–513	241	489–513
169	489–513	242	490–514
170	489–513	243	489–513
171	489–513	244	489–513
172	489–513	245	489–513
173	489–513	246	489–513
174	489–513	247	489–513
175	485–509	248	489–513
176	489–513	249	489–513
177	489–513	250	489–513
178	489–513	251	472–496
179	489–513	252	489–513
180	489–513	253	489–513
181	489–513	254	489–513
182	489–513	255	489–513
183	489–513	256	489–513
184	489–513	257	489–513
185	489–513	258	241–265
186	489–513	259	367–391
187	489–513	260	422–446
188	489–513	261	491–515

| TABLE 18-continued | |
SEQ ID NO.	POSITION RANGE OF PROBES
262	142–166
263	193–217
264	339–363
265	489–513
266	487–511
267	489–513
268	489–513
269	489–513
270	489–513
271	489–513
272	489–513
273	489–513
274	489–513
275	489–513
276	489–513
277	489–513
278	489–513
279	489–513
280	489–513
281	489–513
282	489–513
283	493–517
284	489–513
285	489–513
286	489–513
287	489–513
288	489–513
289	489–513
290	489–513
291	491–515
292	489–513
293	489–513
294	489–513
295	489–513
296	489–513
297	489–513
298	489–513
299	489–513
300	489–513
301	489–513
302	489–513
303	489–513
304	489–513
305	489–513
306	489–513
307	489–513
308	489–513
309	489–513
310	489–513
311	489–513
312	489–513
313	489–513
314	243–267
315	489–513
316	654–678
317	489–513
318	237–261
319	489–513
320	489–513
321	489–513
322	489–513
323	489–513
324	489–513
325	489–513
326	489–513
327	489–513
328	489–513
329	489–513
330	489–513
331	489–513
332	489–513
333	489–513
334	489–513

| TABLE 18-continued | |
SEQ ID NO.	POSITION RANGE OF PROBES
335	489–513
336	489–513
337	489–513
338	489–513
339	489–513
340	489–513
341	489–513
342	489–513
343	489–513
344	489–513
345	489–513
346	489–513
347	489–513
348	468–492
349	489–513
350	486–510
351	491–515
352	491–515
353	491–515
355	491–515
356	491–515
357	487–511
358	487–511
359	487–511
360	487–511
361	487–511
362	487–511
363	487–511
364	487–511
365	487–511
366	420–444
367	376–400
368	491–515
369	491–515
370	422–446
371	426–450
372	491–515
373	239–263
374	416–440
375	489–513
376	286–310
377	374–398
378	445–469
379	489–513
380	487–511
381	487–511
382	487–511
383	491–515
384	491–515
385	489–513
386	294–318
387	485–509
388	489–513
389	489–513
390	489–513
391	489–513
392	232–256
393	489–513
394	41–65
395	181–205
396	489–513
397	335–359
398	491–515
399	228–252
400	489–513
401	372–396
402	491–515
403	491–515
404	491–515
405	491–515
406	491–515
407	487–511
408	489–513

TABLE 18-continued

SEQ ID NO.	POSITION RANGE OF PROBES
409	489–513
410	487–511
411	489–513
412	491–515
413	491–515
414	491–515
415	491–515
416	491–515
417	491–515
418	489–513
419	489–513
420	487–511
421	487–511
422	487–511
423	489–513
424	489–513
425	487–511
426	489–513
427	491–515
428	491–515
429	352–376
430	489–513
431	489–513
432	477–501
433	489–513
434	489–513
435	489–513
436	489–513
437	489–513
438	489–513
439	489–513
440	489–513
441	489–513
442	489–513
443	489–513
444	489–513
445	489–513
446	489–513
447	489–513
448	489–513
449	489–513

TABLE 18-continued

SEQ ID NO.	POSITION RANGE OF PROBES
450	489–513
451	489–513
452	489–513
453	489–513
454	489–513
455	489–513
456	489–513
457	489–513
458	489–513
459	489–513
460	489–513
461	489–513
462	489–513
463	489–513
464	489–513
465	417–441
466	489–513
467	294–318
468	489–513
469	489–513
470	489–513
471	489–513
472	489–513
473	200–224
474	489–513
475	489–513
476	489–513
477	489–513
478	489–513
479	253–277
480	489–513
481	489–513
482	489–513
483	489–513
484	489–513

[0749]

TABLE 19

MGST-II ALLELE FREQUENCY DATA (FRENCH and US)

Seq. ID No.	Protein	Biallelic Marker ID	Caucasian French				Caucasian US				
			size	A	C	G	T	size	A	C	G	T
1	MGST-II	12-421/140	Not genotyped for this				189	66.67		33.33	
3	MGST-II	12-424/198	population				184		1.36		98.64
5	MGST-II	12-426/154					190		32.63		67.37
7	MGST-II	12-430/80					189		66.14		33.86
9	MGST-II	12-441/233					190		33.95	66.05	
12	MGST-II	12-447/58					94	1.6		98.4	
13	MGST-II	12-453/429					92		99.46		0.54
14	MGST-II	12-454/363					190		73.16		26.84
15	MGST-II	12-455/326									
21	MGST-II	12-458/196									
25	MGST-II	12-461/299									
436	MGST-II	12-421/135									
437	MGST-II	12-442/133									

[0750]

TABLE 20

ME1 ALLELE FREQUENCY DATA (US)

Seq. ID No.	Protein	Biallelic Marker ID	size	A	C	G	T
31	ME1	10-428-219	189	66.67		33.33	
33	ME1	10-420-284	184		1.36		98.64
35	ME1	12-713-95	190		32.63		67.37
37	ME1	12-716-295	189		66.14		33.86
38	ME1	12-720-80	190		33.95	66.05	
41	ME1	12-721-440	94	1.6			98.4

TABLE 20-continued

ME1 ALLELE FREQUENCY DATA (US)

Seq. ID No.	Protein	Biallelic Marker ID	size	A	C	G	T
42	ME1	12-723-293	92		99.46		0.54
44	ME1	12-724-225	190		73.16		26.84

[0751]

TABLE 21

Allele Frequency Data (French and US)

Seq. ID No.	Protein	Biallelic Marker ID	size	A	C	G	T	size	A	C	G	T
				Caucasian FRENCH					Caucasian US			
336	UGT1A7	12-148-311	88	47.73		52.27		190	44.47		55.53	
345	UGT1A7	12-156-91	90	50.56		49.44		93	50.54		49.46	
300	UGT1A7	12-121-326	93	30.11		69.89		188	28.99		71.01	
309	UGT1A7	12-128-225	93	50.54	49.46			187	55.35	44.65		
304	UGT1A7	12-124-194	85		19.41		80.59					
326	UGT1A7	12-141-159	92	37.5		62.5						
330	UGT1A7	12-143-453	82	50.61		49.39						
322	UGT1A7	12-139-380	85	17.65		82.35		190	20.79		79.21	
342	UGT1A7	12-153-116	90		62.22		37.78					
323	UGT1A7	12-140-134	89			58.99	41.01	183			56.01	43.99
329	UGT1A7	12-142-321	90	54.44		45.56						

[0752]

TABLE 22

Allele Frequency Data (French and US)

Protein	Biallelic Marker ID	size	A	C	G	T	size	A	C	G	T
			Caucasian FRENCH					Caucasian US			
UGT2B4	12-653-423	6	25			75	177	73.45			26.55
UGT2B4	10-470-25	179	56.15			43.85	90	55.56			44.44
UGT2B4	10-494-284	6		16.67		83.33	185		22.97		77.03
UGT2B4	10-471-84	178	24.44			75.56	92	27.17			72.83
UGT2B4	10-471-85	0					188	26.6	73.4		
UGT2B4	12-637-219	0					187		33.42		66.58
UGT2B4	12-639-95	0					187		35.56		64.44
UGT2B4	12-652-203	4	50	50			189	57.67	42.33		
UGT2B4	12-642-417	0					186	73.66		26.34	
UGT2B4	12-648-300	0					91		62.09		37.91

[0753]

TABLE 23

HAPLOTYPE ANALYSIS (MGST-II vs ASTHMA)
297 ALT vs 286 Caucasian US

MARKERS MGST2		12-421/135 inB	12-421/140	12-430/80	2-441/23	12-442/130	12-447/58	2-455/32	12-461/299 markers in bac	2-453/42	12-424/198	2-454/36	2-458/19	2-426/15
size (cases/controls)		282 vs 185	281 vs 183	163 vs 93	85 vs 18	172 vs 94	287 vs 93	167 vs 93	287 vs 18⑦	⑦87 vs 18	260 vs 17⑦	77 vs 17⑦	⑦77 vs 18	⑦72 vs 18
allelic frequency % (case/controls)		85/84(G)	81/79(A)	85(A)	35/35(C)	8/5(C)	45/44(G)	44/41(G)	63/59(T)	60/56(C)	40/38(T)	21/18(A)	81/60(A)	59/58(A)
diff freq all (cases-controls)		10	22	32	03	23	10	24	35	47	17	25	08	13
pvalue		6 6e-01	4 0e-01	1 8e-01	7 5e-01	3 2e-01	7 5e-01	5 8e-01	2 7e-01	1 5e'01	5 8e'01	3 4e-01	7 5e-01	6 5e-01
		1 1	1 1	1 6	1 0	1 4	1 0	1 1	1 2	1 2	1 1	1 2	1 1	1 1
Test Hardy Weinberg Equilibrium cases vs controls		0 00 HW / 0 01 HW	-0 00 HW / 0 00 HW	-0 00 HW / 0 89	0 01 HW / 0 01 HW	-0 00 HW / 0 88	-0 00 HW / -0 04 HW	0 01 HW / 0 02 HW	0 01 HW / -0 03 HW	-0 02 HW / 0 00 HW	0 02 HW / -0 02 HW	0 00 HW / 0 00 HW	0 01 HW / 0 00 HW	0 02 HW / 0 00 HW
PT2														
haplotype 1	286 vs 180			A					T	C				
haplotype 2	157 vs 93					C						G		
haplotype 3	164 vs 94											G		
haplotype 4	276 vs 93						C					A		
haplotype 5	260 vs 170													
haplotype 6	PT3 151 vs 86							C		C	T			
haplotype 7	247 vs 162									C	T			G
haplotype 8	166 vs 92						G	G	C					
haplotype 9	166 vs 93							G	C	T				
haplotype 10	258 vs 165				T					C	T			
haplotype 11	265 vs 166				T				T				A	
haplotype 12	150 vs 86	G												
haplotype 13	281 vs 175							A	T	C	T			
haplotype 14	277 vs 170								T	C			A	
haplotype 15	150 vs 81		A					A						
haplotype 16	284 vs 176				T				T	C	T			
haplotype 17	271 vs 88						G		C	C				G

TABLE 23-continued

HAPLOTYPE ANALYSIS (MGST-II vs ASTHMA)
297 ALT vs 286 Caucasian US

Haplotype		pos vs									
				C	G	T	C	G	T		
haplotype 18	PT4	144 vs 86		C							A
haplotype 19		166 vs 92		T	A	T	T				A
haplotype 20		149 vs 85	G		G	C	G	T	C	T	
haplotype 21		146 vs 79			G	T		G		T	
haplotype 22		271 vs 88	G	C	G		G	T	C	T	
haplotype 23		151 vs 86			G	T	G	T	C	T	
haplotype 24		151 vs 85		C	A		G		C	T	
haplotype 25		151 vs 86		C	G		G		C	T	
haplotype 26		150 vs 81		G	G		G	T	C	T	
haplotype 27		143 vs 85		A	G		G	T	C	T	
haplotype 28		150 vs 86			G		G	T	C	T	
haplotype 29		243 vs 159	G	G			G	T	C	T	G
haplotype 30		240 vs 161	G				G	T	C	T	G

ESTIMATED FREQUENCIES

Test		cases vs controls	haplotype frequencies		p-excess	Odds ratio	Chi-S	Pvalue (1d/f)	
			cases	controls					
Hardy Weinberg Equilibrium									
	PT2								
haplotype 1		286 vs 180	0.390	0.310	11 60	1 42	6 16	1 3e-02	**
haplotype 2		157 vs 93	0.076	0.025	5 18	3 16	5 54	1 8e-02	**
haplotype 3		164 vs 94	0.073	0.025	4 93	3 10	5 28	2 1e-02	**
haplotype 4		276 vs 93	0.058	0.025	4 46	2 89	4 89	2 7e-02	**
haplotype 5		260 vs 170	0.216	0.156	7 14	1 49	4 81	2 7e-02	**
	PT3								
haplotype 6		151 vs 86	0.118	0.011	10 81	12 22	17 23	3.2e-05	****
haplotype 7		247 vs 162	0.097	0.027	7 13	3 60	14 60	1 3e-04	****
haplotype 8		166 vs 92	0.112	0.027	8 69	4 53	11 33	7 3e-04	***
haplotype 9		166 vs 93	0.098	0.022	7 75	4 75	10 38	1 3e-03	***
haplotype 10		258 vs 165	0.150	0.077	7 99	2 13	10 25	1 3e-03	***

TABLE 23-continued

HAPLOTYPE ANALYSIS (MGST-II vs ASTHMA)
297 ALT vs 286 Caucasian US

haplotype 11	265 vs 166	0 251	0 171	9 58	1 63	7 66	5 5e-03	**
haplotype 12	150 vs 86	0 105	0 034	7 36	3 34	7 59	5 8e-03	**
haplotype 13	281 vs 175	0 340	0 255	11 36	1 50	7 26	6 9e-03	**
haplotype 14	277 vs 170	0 322	0 238	11 02	1 52	7 20	6 9e-03	**
haplotype 15	150 vs 81	0 193	0 098	10 53	2 20	7 07	7 7e-03	**
haplotype 16	284 vs 176	0 262	0 187	9 29	1 55	6 94	8 2e-03	**
haplotype 17	271 vs 88	0 088	0 028	6 12	3 29	6 91	8 2e-03	**
PT4								
haplotype 18	144 vs 86	0 126	0 000	12 60	100 00	23 63	1.2e-06	******
haplotype 19	166 vs 92	0 101	0 000	10 09	100 00	19 85	8.2e-06	******
haplotype 20	149 vs 85	0 131	0 010	12 20	14 29	19 79	8 8e-06	*****
haplotype 21	146 vs 79	0 131	0 009	12 32	15 46	18 98	1 3e-05	*****
haplotype 22	271 vs 88	0 099	0 000	9 89	100 00	18 81	1 4e-05	*****
haplotype 23	151 vs 86	0 123	0 011	11 29	12 37	18 08	2 1e-05	*****
haplotype 24	151 vs 85	0 092	0 000	9 22	100 00	16 66	4 4e-05	*****
haplotype 25	151 vs 86	0 130	0 019	11 28	7 72	16 48	4 9e-05	*****
haplotype 26	150 vs 81	0 119	0 011	10 91	11 94	16 40	5 1e-05	*****
haplotype 27	143 vs 85	0 131	0 019	11 37	7 63	16 31	5 1e-05	*****
haplotype 28	150 vs 86	0 114	0 011	10 33	11 04	16 15	5 7e-05	*****
haplotype 29	243 vs 159	0 105	0 029	7 81	3 96	16 04	6 0e-05	*****
haplotype 30	240 vs 161	0 101	0 028	7 51	3 88	15 38	8 7e-05	*****

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[0754]

TABLE 24

HAPLOTYPE ANALYIS PERMUTATION TEST RESULTS
Asthma

PROTEIN	12-447/58	12-455/326	12-461/299	12-453/429	12-424/198	12-454/363	MARKERS
		markers in bac					
MGST2		G		C	T		HAPLOTYPES (ALT vs US)
		7.52E-01		5.27E-01	7.52E-01		pvalue ALT+ vs ALT-
		0.8		-2.7	-1		(cases vs
		(44 vs 45)		(59 vs 62)	(41 vs 40)		controls)
		5.84E-01		1.47E-01	5.84E-01		pvalue ALT vs
		3.5		4.7	-1.7		(cases vs caucasian US
		(63 vs 60)		(61 vs 56)	(59 vs 61)		controls)

PROTEIN	12-447/58	12-455/326	12-461/299	12-453/429	12-424/198	12-454/363	MARKERS
		markers in bac					
MGST2		G		C	T	G	HAPLOTYPES (ALT vs US)
		7.52E-01		5.27E-01	7.52E-01	4.80E-01	pvalue ALT+ vs ALT-
		0.8		-2.7	-1	-2.5	(cases vs
		(44 vs 45)		(59 vs 62)	(41 vs 40)	(77 vs 80)	controls)
		5.84E-01		1.47E-01	5.84E-01	3.43E-01	pvalue ALT vs
		3.5		4.7	-1.7	-2.5	(cases vs caucasian US
		(63 vs 60)		(61 vs 56)	(59 vs 61)	(79 vs 81)	controls)

PROTEIN	12-447/58	12-455/326	12-461/299	12-453/429	12-424/198	12-454/363	MARKERS
		markers in bac					
MGST2	C	A	T	T			HAPLOTYPES (ALT vs US)
	4.80E-01	7.52E-01	6.55E-01	5.27E-01			pvalue ALT+ vs ALT-
	-3.1	0.8	1.6	-2.7			(cases vs
	(52 vs 55)	(56 vs 55)	(64 vs 63)	(41 vs 38)			controls)
	7.52E-01	5.84E-01	2.73E-01	1.47E-01			pvalue ALT vs
	-1	-2.4	3.5	4.7			(cases vs caucasian US
	(54 vs 55)	(56 vs 58)	(63 vs 60)	(39 vs 44)			controls)

	sample sizes cases vs controls	haplotype frequencies cases	haplotype frequencies controls	p- excess	odds- ratio	chi-S	P value	Av Chi-S	Max Chi-S	> Iter / nb of Iter
HAPLOTYPE GCT										
ALT+ vs ALT-	60 vs 91	0.077	0.141	-7.53	0.50	2.96	8.3e-02 *	1.4	14.6	158/1000
ALT+ vs ALT- (1)	60 vs 91	0.077	0.141	-7.53	0.5	2.96	8.3e-02 *	1.4	14.6	158/1000
ALT vs caucasian US	151 vs 86	0.118	0.011	10.81	12.22	17.23	3.2e-05 ****	2.2	19.5	3/1000
								2.2	22.6	31/10000
ALT vs caucasian US (2)	151 vs 86	0.118	0.011	10.81	12.22	17.23	3.2e-05 ****	2.2	19.5	3/1000
HAPLOTYPE GCTG										
ALT+ vs ALT-	56 vs 88	0.088	0.152	-7.46	0.54	2.47	1.1e-01 *	1.4	16.6	182/1000
ALT+ vs ALT- (1)	56 vs 88	0.088	0.152	-7.46	0.54	2.47	1.1e-01 *	1.4	16.6	182/1000
ALT vs caucasian US	144 vs 86	0.126	0.000	12.6	100	23.52	1.2e-06 ******	2.8	20.1	0/1000
								2.8	28.7	5/10000
ALT vs caucasian US (2)	144 vs 86	0.126	0.000	12.6	100	23.52	1.2e-06 ******	2.8	20.1	0/1000

TABLE 24-continued

HAPLOTYPE ANALYIS PERMUTATION TEST RESULTS
Asthma

HAPLOTYPE
CATT

ALT+ vs ALT−	64 vs 102	0.139	0.075	7	2.01	3.68	5.4e−02	*	1.5	14.4	114/1000
ALT* vs ALT− (1)	64 vs 102	0.139	0.075	7	2.01	3.68	5.4e−02	*	1.5	14.4	114/1000
ALT vs	166 vs 92	0.101	0	10.09	100	19.85	8.2e−06	*****	3.0	29.0	12/1000
caucasian US									3.0	29.0	76/10000
ALT vs	166 vs 92	0.101	0	10.09	100	19.85	8.2e−06	*****	3.0	29.0	12/1000
caucasian US (2)											

[0755]

TABLE 25

SINGLE POINT : ALLELIC ASSOCIATION ANALYSIS
(Zyflo secondary effects)
(89 ALT+ vs 208 ALT−)

SNP CHARACTERISTICS

Seq. ID No.	protein	Biallelic Marker ID	No. in protein	SNP localization	gene or bac size (kb)	fish mapping	Polymorphism
21	MGST2	12-458/196	1	3' gene	bac 145	4q28-3q31	A/T
5	MGST2	12-426/154	3	3' gene	bac 145	4q28-3q31	A/G
9	MGST2	12-441/233	4	3' gene	bac 145	4q28-3q31	C/T
1	MGST2	12-421/140	7	inB	bac 145	4q28-3q31	A/G
436	MGST2	12-421/135	8	inB	bac 145	4q28-3q31	G/T
13	MGST2	12-453/429	9	5' gene	bac 145	4q28-3q31	C/T
12	MGST2	12-447/58	10	5' gene	bac 145	4q28-3q31	C/G
25	MGST2	12-461/299	11	5' gene	bac 145	4q28-3q31	C/T
3	MGST2	12-424/198	12	5' gene	bac 145	4q28-3q31	G/T
14	4.80e−11	4.80e−10	4.80e−09	4.80e−08	4.80e−07	4.80e−06	4.80e−05

| | ALT + | | ALT − | | ASSOCIATION ANALYSIS | |
| | | | | | Diff of | |
Seq. ID No.	sample size	allele frequency (%)	sample size	allele frequency (%)	allele frequency (%)	pvalue (1df)
21	83	77.71	194	83.25	−5.5	1.21e−01
5	85	60.59	188	58.78	1.8	6.55e−01
9	87	60.34	198	65.91	−5.6	1.92e−01
1	86	81.4	195	81.79	−0.4	7.52e−01
436	88	81.82	194	87.37	−5.6	7.83e−02
13	88	59.09	199	61.81	−2.7	5.27e−01
12	89	52.25	198	55.3	−3.1	4.80e−01
25	88	64.2	199	62.56	1.6	6.55e−01
3	80	58.75	180	59.72	−1	7.52e−01
14	4.80e−0	4.80e−03	4.80e−02	4.80e−01	4.80e−00	4.80e−01

[0756]

TABLE 26A

HAPLOTYPE ANALYSIS (MGST2)

89 ALT+ vs 208 ALT-

MARKERS	12-421/ 135	12-421/ 140	12-430/ 80	12-441/ 233	12-442/ 133	12-447/ 58	12-455/ 326	12-461/ 299	12-453/ 429	12-424/ 198	12-454/ 363	12-458/ 196	12-426/ 154
MGST2	in B						markers in bac						
size (cases/controls)	88 vs 194	86 vs 195	66 vs 97	87 vs 198	68 vs 104	89 vs 198	65 vs 102	88 vs 199	80 vs 180	83 vs 194	83 vs 194	83 vs 194	85 vs 188
allelic frequency% (case/controls)	18/12 (T)	18/18 (G)	91/91 (G)	39/34 (C)	92/91 (G)	47/44 (G)	56/55 (A)	64/62 (T)	40/38 (T)	41/40 (T)	22/20 (A)	22/16 (T)	60/58 (A)
diff freq. all. (cases-controls)	56	04	04	56	13	31	08	16	27	10	25	55	18
pvalue cases vs	7 8e-02	7 5e-01	7 5e-01	1 9e-01	6 6e-01	4 8e-01	7 5e-01	6 6e-01	5 3e-01	7 5e-01	4 8e-01	1 2e-01	6 6e-01
	15	10	11	13	12	11	10	11	11	10	12	14	11
Test Hardy Weinberg Equilibrium cases vs	-0 01 HW	-0 00 HW	0.83	0 02 HW	0.85	-0 04 HW	-0 02 HW	0 01 HW	-0 01 HW	-0 04 HW	-0 00 HW	0 03 HW	-0 00 HW
controls	0 00 HW	-0 00 HW	0 00 HW	0 01 HW	0 00 HW	0 01 HW	0 03 HW	0 01 HW	-0 03 HW	0 00 HW	0 00 HW	-0 00 HW	0 03 HW
haplotype 1 77 vs 170													A
haplotype 2 86 vs 198				C									
haplotype 3 81 vs 190		A							T				
haplotype 4 87 vs 198		A		C				T					
haplotype 5 PT2 86 vs 190	T	A											
haplotype 6 86 vs 198				C				T	T				
haplotype 7 86 vs 197						C		T	T				
haplotype 8 79 vs 179				C					T	T			
haplotype 9 81 vs 190		A		C					C				
haplotype 10 77 vs 170								T	T	T			
haplotype 11 77 vs 170								T		T			A
haplotype 12 81 vs 189		A				G							A
haplotype 13 84 vs 188								T					
haplotype 14 75 vs 166		A							T				A
haplotype 15 86 vs 189	T					C			T				A
haplotype 16 83 vs 187							A		T				A
haplotype 17 59 vs 91				C						T		T	
haplotype 18 PT3 73 vs 167				C					T	T	G		A

TABLE 26A-continued

HAPLOTYPE ANALYSIS (MGST2)

haplotype 19	83 vs 187			C				T	T		A
haplotype 20	86 vs 197			C		C		T	T		A
haplotype 21	83 vs 186			C	G	C		T	T		A
haplotype 22	64 vs 98		G			G		T	T		A
haplotype 23	61 vs 95					G	A	T	T		A
haplotype 24	56 vs 85	A					A		T	T	
haplotype 25	79 vs 179	A		C				T	T		A
haplotype 26	85 vs 194	A		C				T	T		A
haplotype 27	59 vs 87	A	G			C			T		A
haplotype 28	81 vs 183									A	A
haplotype 29	77 vs 170	A						T	T		A
haplotype 30	56 vs 83	A	G					T	T		A
haplotype 31 PT4	64 vs 99	G				G	A	T			A

[0757]

TABLE 26B

HAPLOTYPE ANALYSIS (MGST2)

89 ALT+ vs 208 ALT-

MARKERS			ESTIMATED FREQUENCIES						
MGST2									
size (cases/controls)									
allellc frequency% (case/controls)			haplotype frequencies						
diff freq. all. (cases-controls)									
pvalue			cases	controls	p-excess	Odds ratio	Chi-S	Pvalue (1df)	
Test Hardy Weinberg Equilibrium	cases vs controls								
haplotype 1		77 vs 170	0.301	0.180	14.75	1.96	9.11	2.4e-03	***
haplotype 2		86 vs 198	0.175	0.096	8.69	1.99	7.00	7.7e-03	**
haplotype 3		81 vs 190	0.185	0.106	8.78	1.91	6.20	1.2e-02	**
haplotype 4		87 vs 198	0.281	0.196	10.55	1.60	5.03	2.4e-02	**
haplotype 5	PT2	86 vs 190	0.186	0.117	7.82	1.72	4.73	2.8e-02	**
haplotype 6		86 vs 198	0.157	0.049	11.34	3.63	18.68	1.5e-05	*****
haplotype 7		86 vs 197	0.150	0.054	10.15	3.08	14.44	1.4e-04	****
haplotype 8		79 vs 179	0.128	0.046	8.59	3.04	11.15	8.2e-04	***
haplotype 9		81 vs 190	0.134	0.053	8.51	2.74	10.33	1.3e-03	***
haplotype 10		77 vs 170	0.159	0.069	9.69	2.55	9.80	1.7e-03	***
haplotype 11		77 vs 170	0.301	0.180	14.74	1.96	9.08	2.6e-03	***
haplotype 12		81 vs 189	0.124	0.052	7.67	2.61	8.83	2.9e-03	***
haplotype 13		84 vs 188	0.193	0.105	9.87	2.04	7.90	4.7e-03	**
haplotype 14		75 vs 166	0.217	0.122	10.88	2.00	7.34	6.5e-03	**
haplotype 15		86 vs 189	0.114	0.051	6.71	2.41	7.29	6.9e-03	**
haplotype 16		83 vs 187	0.164	0.087	8.40	2.05	6.86	8.6e-03	**
haplotype 17		59 vs 91	0.189	0.087	11.24	2.46	6.81	8.6e-03	**
haplotype 18	PT3	73 vs 167	0.283	0.179	12.74	1.82	6.68	9.6e-03	**
haplotype 19		83 vs 187	0.141	0.028	11.64	5.75	25.13	5.2e-07	******
haplotype 20		86 vs 197	0.154	0.043	11.62	4.04	20.84	4.8e-06	*****
haplotype 21		83 vs 186	0.140	0.047	9.80	3.32	14.32	1.5e-04	****
haplotype 22		64 vs 98	0.147	0.039	11.27	4.26	12.11	4.8e-04	***
haplotype 23		61 vs 95	0.203	0.072	14.08	3.26	11.72	5.9e-04	***
haplotype 24		56 vs 85	0.239	0.092	16.23	3.10	11.45	7.0e-04	***
haplotype 25		79 vs 179	0.130	0.048	8.67	2.99	11.05	8.6e-04	***
haplotype 26		85 vs 194	0.113	0.040	7.65	3.07	10.91	9.1e-04	***
haplotype 27		59 vs 87	0.207	0.080	13.88	3.02	10.06	1.5e-03	***
haplotype 28		81 vs 183	0.121	0.047	7.76	2.77	9.41	2.1e-03	***
haplotype 29		77 vs 170	0.159	0.071	9.53	2.49	9.39	2.2e-03	***
haplotype 30		56 vs 83	0.208	0.082	13.70	2.93	9.17	2.4e-03	***
haplotype 31	PT4	64 vs 99	0.173	0.067	11.40	2.92	9.07	2.6e-03	***

[0758]

TABLE 27

HAPLOTYPE ANALYSIS PERMUTATION TEST RESULTS
Zyflo secondary effects

PROTEIN	12-441/233	12-461/299	12-453/429 markers in bac	12-426/154	MARKERS	
MGST2	C	T	T		HAPLOTYPES (ALT+ vs ALT-)	
	1.92E-01	6.55E-01	5.27E-01		pvalue	ALT+ vs ALT-
	-5.6 (40 vs 34)	1.6 (64 vs 63)	-2.7 (41 vs 38)		diff all. Freq %	
	7.52E-01	2.73E-01	1.47E-01		pvalue	ALT vs caucasian US
	-0.3 (36 vs 35)	3.5 (63 vs 60)	4.7 (39 vs 44)		diff all. Freq %	

PROTEIN	12-441/233	12-461/299	12-453/429 markers in bac	12-426/154	MARKERS	
MGST2	C	T	T	A	HAPLOTYPES (ALT+ vs ALT-)	
	1.92E-01	6.55E-01	5.27E-01	6.55E-01	pvalue	ALT+ vs ALT-
	-5.6 (40 vs 34)	1.6 (64 vs 63)	-2.7 (41 vs 38)	1.8 (61 vs 59)	diff all. Freq %	
	7.52E-01	2.73E-01	1.47E-01	6.55E-01	pvalue	ALT vs caucasian US
	-0.3 (36 vs 35)	3.5 (63 vs 60)	4.7 (39 vs 44)	1.3 (59 vs 58)	diff all. Freq %	

	sample sizes cases vs controls	haplotype frequencies cases	controls	p-excess	odds-ratio	chi-S	P value	PERMUTATIONS Av. Chi-S	Max Chi-S	> Iter / nb of Iter.
HAPLOTYPE CTT										
ALT+ vs ALT-	86 vs 198	0.157	0.049	11.34	3.63	18.68	1.5e-05 *****	1.9	18.9	1/1000
								1.9	25.4	12/10000
ALT+ vs ALT- (1)	86 vs 104	0.157	0.088	7.54	1.93	4.26	3.8e-02 *	1.1	16.5	52/1000
ALT+ vs ALT- (2)	86 vs 94	0.157	0.042	11.91	4.19	13.36	2.5e-04 ****	1.5	15.4	3/1000
ALT vs caucasian US	284 vs 176	0.092	0.071	2.2	1.32	1.18	2.7e-01 *	2.1	20.9	479/1000
ALT vs caucasian US (2)	284 vs 94	0.092	0.047	4.67	2.04	3.77	5.1e-02 *	1.9	17.3	158/1000
ALT vs caucasian US (3)	284 vs 82	0.092	0.116	-2.79	0.77	0.88	3.4e-01	1.8	17.7	498/1000
HAPLOTYPE CTTA										
ALT+ vs ALT-	83 vs 187	0.141	0.028	11.64	5.75	25.13	5.2e-07 ******	2.6	25.2	1/1000
								2.5	35	7/10000
ALT+ vs ALT- (1)	83 vs 98	0.141	0.066	8.03	2.33	5.61	1.7e-02 **	1.7	17.4	59/1000
ALT+ vs ALT- (2)	83 vs 89	0.141	0.034	11.03	4.6	12.42	4.1e-04 ***	1.7	18.3	3/1000
ALT vs caucasian US	270 vs 167	0.082	0.058	2.61	1.46	1.85	1.7e-01 *	2.7	25.9	438/1000
ALT vs caucasian US (2)	270 vs 89	0.082	0.023	6.12	3.89	7.61	5.5e-03 **	2.7	30.3	92/1000
ALT vs caucasian US (3)	270 vs 78	0.082	0.110	-3.06	0.73	1.11	2.7e-01 *	2.3	17.8	499/1000

[0759]

TABLE 28

SINGLE POINT : ALLELIC ASSOCIATION ANALYSIS
(Zyflo secondary effects)
(89 ALT+ vs 208 ALT-)

SNP CHARACTERISTICS

Seq. ID No.	code	protein	Biallelic Marker ID	SNP localization	gene or bac size (kb)	fish mapping	Polymorphism
300	G25.2	UGT1A7	12-121/326	5'gene	bac 130	2q37	A/G
309	G25.2	UGT1A7	12-128/225	5'gene	bac 130	2q37	A/C

TABLE 28-continued

SINGLE POINT : ALLELIC ASSOCIATION ANALYSIS
(Zyflo secondary effects)
(89 ALT+ vs 208 ALT−)

336	G25.2	UGT1A7	12-148/311	5'gene	bac 130	2q37	A/G
345	G25.2	UGT1A7	12-156/91	5'gene	bac 130	2q37	A/G
322	G25.2	UGT1A7	12-139/380	in bac	bac 130	2q37	A/G
323	G25.2	UGT1A7	12-140/134	in bac	bac 130	2q37	G/T
329	G25.2	UGT1A7	12-142/321	in bac	bac 130	2q37	A/G

| | ALT+ | | ALT− | | ASSOCIATION ANALYSIS | |
| | | | | | Diff of | |
Seq. ID No.	sample size	allele frequency (%)&	sample size	allele frequency (%)&	allele frequency (%)&	pvalue (1df)#
300	85	73.53	199	69.1	4.4	2.73E−01
309	89	58.43	199	51.01	7.4	9.43E−02
336	89	57.3	199	50.25	7.1	1.14E−01
345	78	52.56	192	49.22	3.3	4.80E−01
322	88	78.41	198	79.55	−1.1	7.52E−01
323	81	51.85	196	56.89	−5	2.73E−01
329	85	55.88	192	50.78	5.1	2.54E−01

[0760]

TABLE 29A

HAPLOTYPE ANALYSIS G25.2 UGT1A7 (2q37)
CASES (89 ALT +) vs CONTROLS (208 ALT−)

MARKERS PROTEIN UGT1A7 G25.2	12-121/ 326	12-128/ 325	12-148/ 311	12-156/ 91	12-139/ 380	12-140/ 134	12-142/ 321
			5' gene			in Bac	
cases / controls	85 vs 199	89 vs 199	89 vs 199	78 vs 192	88 vs 198	81 vs 196	85 vs 192
frequency % (case/controls)	73/69 (G)	58/51 (A)	57/50 (G)	52/49 (A)	21/20 (A)	48/43 (T)	55/50 (A)
diff freq. all. (cases − controls)	4.4	7.4	7.1	3.3	1.1	5.0	5.1
Pvalue	2.7e−01	9.4e−02	1.1e−01	4.8e−01	7.5e−01	2.7e−01	2.5e−01
Odd ratio	1.2	1.4	1.3	1.1	1.1	1.2	1.2
Test Hardy Weinberg cases vs	0.04 HW	−0.00 HW	0.01 HW	−0.07 HWE	0.01 HW	0.03 HW	0.05 HWE
controls	−0.01 HW	0.00 HW	−0.00 HW	−0.01 HW	0.01 HW	−0.01 HW	−0.00 HW
haplotype 1 PT2 81 vs 196		A				T	
haplotype 2 78 vs 196	G					T	
haplotype 3 85 vs 199	G	A					
haplotype 4 85 vs 199	G		G				
haplotype 5 88 vs 198		A			A		
haplotype 6 81 vs 196			G			T	
haplotype 7 81 vs 192	G						A
haplotype 8 PT3 72 vs 185	G			G			A
haplotype 9 80 vs 191	G				A		A
haplotype 10 71 vs 188				A	G	T	
haplotype 11 77 vs 190		A			G	T	A
haplotype 12 PT4 71 vs 188		A		A	G	T	
haplotype 13 71 vs 188			G	A	G	T	
haplotype 14 74 vs 190	G	A				T	A
haplotype 15 76 vs 189		A			G	T	A
haplotype 16 77 vs 195	G	A			G	T	

[0761]

TABLE 29B

HAPLOTYPE ANALYSIS
G25.2 UGT1A7 (2q37)
CASES (89 ALT+) vs CONTROLS (208 ALT-)

MARKERS
PROTEIN UGT1A7 G25.2
cases/controls
frequency % (case/controls)

diff freq. all. (cases – controls)
Pvalue

Odd ratio
Test
Hard Weinber

Haplotype		cases vs controls	HAPLOTYPE FREQUENCY TEST										OMNIBUS LR TEST			
			Estimation frequency of haplotype				Odds ratio	Statistical test					Likelihood Ratio			omnibus test
			frequency cases (%)	frequency controls (%)	frequency difference	p-excess		Chi-S	pvalue (1df)		Pvalue (100 permutations)	No. of permutations	Likelihood Ratio Test	Pvalue	(# df)	Pvalue (100 permutations)
haplotype 1		81 vs 196	30.1	22.8	7.3	9.42	1.46	3.24	(6.9e-02)	*	(7.00e-02)	[7/100]	4.05	(2.5e-01)	3df (2.1e-01)	NS
haplotype 2		78 vs 196	38.5	30.6	7.9	11.30	1.42	3.11	(7.4e-02)	*	(5.00e-02)	[5/100]	2.63	(4.4e-01)	3df (4.7e-01)	NS
haplotype 3		85 vs 199	58.8	51	7.8	15.96	1.37	2.93	(8.3e-02)	*	(4.00e-02)	[4/100]	2.97	(3.9e-01)	3df (2.1e-01)	NS
haplotype 4		85 vs 199	57.6	50.3	7.3	14.87	1.35	2.61	(1.0e-01)	*	(3.00e-02)	[3/100]	2.62	(4.4e-01)	3df (2.6e-01)	NS
haplotype 5		88 vs 198	12.3	8.3	4	4.45	1.56	2.36	(1.2e-01)	*	(2.10e-01)	[21/100]	3.34	(3.3e-01)	3df (4.1e-01)	NS
haplotype 6		81 vs 196	29	22.8	6.2	8.01	1.38	2.36	(1.2e-01)	*	(1.20e-01)	[12/100]	3.23	(3.5e-01)	3df (2.8e-01)	NS
haplotype 7	PT2	81 vs 192	42	35.4	6.6	10.27	1.32	2.14	(1.4e-01)	*	(8.00e-02)	[8/100]	2.49	(4.8e-01)	3df (4.9e-01)	NS
haplotype 8		72 vs 185	14.1	5.2	8.9	9.35	2.98	11.45	(7.0e-04)	***	(<1.0e-02)	[0/100]	8.15	(3.2e-01)	7df (1.5e-01)	NS
haplotype 9		80 vs 191	11.6	5.4	6.2	6.49	2.28	6.33	(1.1e-02)	**	(7.00e-02)	[7/100]	6.82	(4.4e-01)	7df (5.7e-01)	NS
haplotype 10		71 vs 188	26.2	17.2	9	10.87	1.71	5.27	(2.1e-02)	**	(1.00e-02)	[1/100]	7.69	(3.6e-01)	7df (4.3e-01)	NS
haplotype 11	PT3	77 vs 190	19.5	11.9	7.6	8.61	1.79	5.19	(2.3e-02)	**	(2.00e-02)	[2/100]	6.31	(4.9e-01)	7df (6.1e-01)	NS
haplotype 12		71 vs 188	27.4	17	10.4	12.60	1.85	7.08	(7.7e-03)	**	(2.00e-02)	[2/100]	28.05	(2.1e-02)	15df (2.0e-02)	[S]
haplotype 13		71 vs 188	27.2	17.4	9.8	11.84	1.77	6.15	(1.3e-02)	**	(2.00e-02)	[2/100]	27.71	(2.3e-02)	15df (1.0e-02)	[S]
haplotype 14	PT4	74 vs 190	19.9	12.3	7.6	8.66	1.77	4.97	(2.5e-02)	**	(4.00e-02)	[4/100]	8.66	(8.9e-01)	15df (7.7e-01)	NS
haplotype 15		76 vs 189	15.2	8.7	6.5	7.10	1.88	4.83	(2.7e-02)	**	(1.00e-01)	[10/100]	13.83	(5.3e-01)	15df (7.8e-01)	NS
haplotype 16		77 vs 195	26	17.7	8.3	10.10	1.63	4.76	(2.8e-02)	**	(4.00e-02)	[4/100]	10.64	(7.7e-01)	15df (6.7e-01)	NS

[0762]

TABLE 30

Multipoint analysis: HAPLOTYPE FREQUENCY TEST - OMNIBUS LR TEST G25.2 UGT1A7(2q37)

		12-128/ 225	12-156/ 91	12-139/ 380	12-140/ 134
MARKERS		5' gene		Bac	
HAPLOTYPE 1		A	A	G	T

		12-128/225	12-156/91	12-139/380	12-140/134
pvalue (1df)		9.4e−02	4.8e−01	7.5e−01	2.7e−01
% frequency difference ALT+ vs ALT−		74	33	11	5
(sample sizes) (2 screening)		(89 vs 199)	(78 VS 192)	(88 vs 198)	(81 vs 196)

HAPLOTYPE FREQUENCY TEST

		Estimation frequency of haplotype			Statistical test					
Zyflo secondary effects	sample sizes cases vs controls	frequency cases (%)	frequency controls (%)	frequency difference (%)	p-excess	Odds ratio	Chi-S	pvalue (1df)	Pvalue (100 permuta-tions)	No. of permuta-tions
ALT+ vs ALT− (2 screening)	71 vs 188	27.4	17	10.4	1.85	12.60	7.09	7.7e−03 **	1.90e−02	19/1000
ALT+(1) vs ALT−(1)	52 vs 97	24.9	12.9	12	2.23	13.75	6.83	8.6e−03 **	3.30e−02	33/1000
ALT+(1) vs ALT−(2)	52ncv91	24.9	22.2	2.7	1.16	3.48	0.27	5.8e−01	6.15e−01	615/1000
ALT vs US (2)	259ncv89	20.3	20.8	0.5	0.97	−0	62 0.02	7.5e−01	9.00e−01	900/1000

OMNIBUS LR TEST

Zyflo secondary effects	Likelihood Ratio		omnibus test
	Likelihood Ratio Test	Pvalue (# df)	Pvalue (100 permutations)
ALT+ vs ALT− (2 screening)	28.01	(2.1e−02) 15df	(1.2e−02) S
ALT+(1) vs ALT−(1)	24.05	(6.3e−02) 15df	(5.7e−02) NS
ALT+(1) vs ALT−(2)	25.69	(4.1e−02) 15df	(1.5e−02) S
ALT vs US (2)	15.14	(4.4e−01) 15df	(3.9e−01) NS

[0763]

TABLE 31

SINGLE POINT ALLELIC ASSOCIATION ANALYSIS (Zyflo secondary effects)
(89 ALT+ vs 208 ALT−)

SNP CHARACTERISTICS

Seq. ID No.	code	protein	Biallelic Marker ID	No. in Protein	SNP localization	SNP change	gene or bac size (kb)	fish mapping	Polymorphism
380	G40.3	UGT2B4	12-653/423	inB		bac 120	4q13.3-q21	A/T	
351	G40.3	UGT2B4	10-470/25	2	in2		bac 120	4q13.3-q21	A/T
352	G40.3	UGT2B4	10-471/84	3	ex5	G->A	bac 120	4q13.3-q21	A/T
353	G40.3	UGT2B4	10-471/85	3	ex5		bac 120	4q13.3-q21	A/C
357	G40.3	UGT2B4	12-637/219	6	3'UTR		bac 120	4q13.3-q21	C/T
358	G40.3	UGT2B4	12-639/95	7	in bac		bac 120	4q13.3-q21	C/T

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TABLE 31-continued

SINGLE POINT ALLELIC ASSOCIATION ANALYSIS (Zyflo secondary effects)
(89 ALT+ vs 208 ALT−)

| 377 | G40.3 | UGT2B4 | 12-652/203 | 8 | in bac | | bac 120 | 4q13.3-q21 | A/C |
| 369 | G40.3 | UGT2B4 | 12-642/417 | 9 | in bac | | bac 120 | 4q13.3-q21 | A/G |

	ALT +		ALT −		ASSOCIATION ANALYSIS	
Seq. ID No.	sample size	allele frequency (%) &	sample size	allele frequency (%) &	Diff of allele frequency (%) $	pvalue (1df) #
380	82	81.1	193	72.3	8.8	2.85E-02
351	87	51.15	191	41.88	9.3	4.04E-02
352	84	81.55	194	72.68	8.9	2.53E-02
353	89	80.3	199	72.6	7.7	4.55E-02
357	82	72.56	189	62.96	9.6	3.02E-02
358	82	64.02	189	59.79	4.2	343E−01
377	88	50	199	59.55	-9, 5	3.20E-02
369	86	71.51	194	78.61	−7.1	6.52E−02

[0764]

TABLE 32A

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT−)

MARKERS PROTEIN UGT2B4 (G40.3)	12-653/ 423 inB	10-470/ 25 in2	10-471/ 84	10-471/ 85 ex5	12-637/ 219	12-639/ 95 3'UTR	12-652/ 203	12-642/ 417 in Bac
cases / controls	82 vs 193	87 vs 191	84 vs 194	89 vs 199	85 vs 190	86 vs 190	88 vs 199	85 vs 198
frequency %	81/72	51/41	81/72	80/72	72/62	64/60	50/40	28/21
(case/controls)	(A)	(T)	(T)	(C)	(T)	(T)	(C)	(G)
diff freq all. (cases - controls)	8.8	9.3	8.9	7.7	10.3	4.5	9.5	6.8
Pvalue	2.9e−02	4.0e−02	2.5e−02	4.5e−02	1.8e−02	2.9e−01	3.2e−02	7.8e−02
Odd ratio	1.6	1.4	1.7	1.5	1.6	1.2	1.5	1.4

Test Hardy Weinberg	cases vs controls	−0.01 HW −0.01 HW	0.01 HW 0.01 HW	−0.02 HW −0.01 HW	−0.02 HW −0.01 HW	−0.00 HW −0.02 HW	−0.01 HW −0.01 HW	−0.05 HW 0.01 HW	−0.02 HW 0.00 HW

		12-653/423	10-470/25	10-471/84	10-471/85	12-637/219	12-639/95	12-652/203	12-642/417
haplotype 1 PT2	86 vs 191		T					C	
haplotype 2	83 vs 182		T			T			
haplotype 3	81 vs 185			T		T			
haplotype 4	84 vs 190		A						G
haplotype 5	85 vs 190				C	T			
haplotype 7	88 vs 199				C			C	
haplotype 8	79 vs 184	A				T			
haplotype 9	80 vs 185	A	T						
haplotype 11	82 vs 193	A						C	
haplotype 12	84 vs 194			T				C	
haplotype 13	84 vs 194			T	C				
haplotype 14 PT3	84 vs 183		T				T	C	
haplotype 15	83 vs 182		T			T		C	
haplotype 16	84 vs 190		T					C	A
haplotype 17	80 vs 185	A	T					C	

TABLE 32A-continued

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT-)

haplotype	PT	comparison								
haplotype 18		86 vs 191		T		C			C	
haplotype 20		82 vs 186		T	T				C	
haplotype 21		77 vs 176	A	T			T			
haplotype 22		79 vs 177		T	T		T			
haplotype 23		83 vs 182		T		C	T			
haplotype 25		82 vs 181		T			T			A
haplotype 27		85 vs 190				C	T		C	
haplotype 28		81 vs 185			T	C	T			
haplotype 30		80 vs 184	A					T	C	
haplotype 31		81 vs 185			T		T		C	
haplotype 32		79 vs 184	A				T		C	
haplotype 33		75 vs 179	A		T		T			
haplotype 34		86 vs 190				C		T	C	
haplotype 35	PT4	78 vs 177	A	T				T	C	
haplotype 36		77 vs 176	A	T			T		C	
haplotype 37		83 vs 182		T		C	T		C	
haplotype 38		84 vs 183		T		C		T	C	
haplotype 39		79 vs 177		T	T		T		C	
haplotype 40		80 vs 179		T	T			T	C	
haplotype 41		80 vs 185	A	T		C			C	
haplotype 42		76 vs 180	A	T	T				C	

[0765]

TABLE 32B

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT-)

MARKERS
PROTEIN UGT2B4 (G40.3)
cases / controls
frequency %
(case/controls)
diff freq. all.

HAPLOTYPE FREQUENCY TEST

	(cases - controls) Pvalue Odd ratio		Estimation frequency of haplotype						Statistical test		Pvalue	
	Test Hardy Weinberg	cases vs controls	frequency cases (%)	frequency controls (%)	frequency difference	p-excess	Odds ratio	Chi-S	pvalue (1df)		(100 permutations)	No. of permutations
haplotype 1	PT2	86 vs 191	30.3	19.2	11.1	13.71	1.83	8.32	(3.8e−03)	**	(2.00e−02)	[2/100]
haplotype 2		83 vs 182	50.6	38.7	11.9	19.43	1.62	6.62	(9.6e−03)	**	(<1.0e−02)	[0/100]
haplotype 3		81 vs 185	73.5	62.7	10.8	28.83	1.65	5.80	(1.5e−02)	**	(<1.0e−02)	[0/100]
haplotype 4		84 vs 190	15.6	8.7	6.9	7.50	1.93	5.65	(1.7e−02)	**	(5.00e−02)	[5/100]
haplotype 5		85 vs 190	72.3	62.6	9.7	25.87	1.56	4.87	(2.7e−02)	**	(3.00e−02)	[3/100]
haplotype 7		88 vs 199	50	40.5	9.5	16.03	1.47	4.53	(3.2e−02)	*	(2.00e−02)	[2/100]
haplotype 8		79 vs 184	72	62.5	9.5	25.40	1.54	4.43	(3.4e−02)	*	(1.00e−02)	[1/100]
haplotype 9		80 vs 185	51.2	41.4	9.8	16.88	1.49	4.43	(3.4e−02)	*	(4.00e−02)	[4/100]
haplotype 11		82 vs 193	50.6	40.9	9.7	16.38	1.48	4.38	(3.6e−02)	*	(4.00e−02)	[4/100]
haplotype 12		84 vs 194	50	40.5	9.5	16.02	1.47	4.34	(3.6e−02)	*	(1.00e−02)	[1/100]
haplotype 13		84 vs 194	81	72.7	8.3	30.28	1.60	4.30	(3.8e−02)	*	(<1.0e−02)	[0/100]
haplotype 14	PT3	84 vs 183	22	11.3	10.7	12.00	2.20	10.41	(1.2e−03)	***	(<1.0e−02)	[0/100]
haplotype 15		83 vs 182	29.9	18.1	11.8	14.44	1.93	9.38	(2.2e−03)	***	(<1.0e−02)	[0/100]
haplotype 16		84 vs 190	27.9	16.6	11.3	13.54	1.94	9.27	(2.3e−03)	***	(1.00e−02)	[1/100]
haplotype 17		80 vs 185	33	20.8	12.2	15.42	1.87	8.99	(2.7e−03)	***	(<1.0e−02)	[0/100]
haplotype 18		86 vs 191	31.8	20.2	11.6	14.51	1.84	8.75	(3.0e−03)	***	(<1.0e−02)	[0/100]
haplotype 20		82 vs 186	31.4	20.2	11.2	14.11	1.81	8.00	(4.7e−03)	**	(<1.0e−02)	[0/100]
haplotype 21		77 vs 176	51.3	38.6	12.7	20.72	1.68	7.10	(7.3e−03)	**	(<1.0e−02)	[0/100]
haplotype 22		79 vs 177	51.3	39.2	12.1	19.90	1.63	6.54	(1.0e−02)	**	(2.00e−02)	[2/100]
haplotype 23		86 vs 182	50.6	38.9	11.7	19.13	1.61	6.37	(1.1e−02)	**	(1.00e−02)	[1/100]
haplotype 25		82 vs 181	38.1	27.6	10.5	14.45	1.61	5.79	(1.6e−02)	**	(1.00e−02)	[1/100]
haplotype 27		85 vs 190	42.5	32.2	10.3	15.14	1.55	5.41	(1.9e−02)	**	(1.00e−02)	[1/100]
haplotype 28		81 vs 185	72.8	62.7	10.1	27.18	1.60	5.14	(2 3e−02)	**	(2 00e−02)	[2/100]
haplotype 30		80 vs 184	39.5	29.6	9.9	14.09	1.55	5.00	(2.5e−02)	**	(1.00e−02)	[1/100]
haplotype 31		81 vs 185	42.1	32.2	9.9	14.65	1.53	4.87	(2.7e−02)	**	(1.00e−02)	[1/100]
haplotype 32		79 vs 184	42.6	32.6	10	14.75	1.53	4.76	(2.8e−02)	**	(<1.0e−02)	[0/100]

TABLE 32B-continued

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT−)

MARKERS
PROTEIN UGT2B4 (G40.3)
cases / controls
frequency %
(case/controls)
diff freq. all.

		(cases − controls)		Estimation frequency			HAPLOTYPE FREQUENCY TEST						
											Statistical test		
		Pvalue		of haplotype								Pvalue	
		Odd ratio	frequency	frequency								(100	No. of
Test Hardy		cases vs	cases	controls	frequency	p-	Odds			pvalue		permuta-	permuta-
Weinberg		controls	(%)	(%)	difference	excess	ratio	Chi-S		(1df)		tions)	tions
haplotype 33		75 vs 179	72.7	62.6	10.1	26.95	1.59	4.76	(2.8e−02)	**		(1.00e−02)	[1/100]
haplotype 34		86 vs 192	38.3	29.1	9.2	13.02	1.51	4.64	(3.0e−02)	**		(2.00e−02)	[2/100]
haplotype 35	PT4	78 vs 77	25.5	12.3	13.2	15.12	2.45	13.96	(1.8e−04)	****		(<1.0e−02)	[0/100]
haplotype 36		77 vs 176	33.3	19.5	13.8	17.16	2.06	11.31	(7.3e−04)	***		(<1.0e−02)	[0/100]
haplotype 37		83 vs 182	32.1	19	13.1	16.15	2.01	10.96	(9.1e−04)	***		(<1.0e−02)	[0/100]
haplotype 38		84 vs 183	23.4	12.2	11.2	12.74	2.20	10.89	(9.6e−04)	***		(1 00e−02)	[1/100]
haplotype 39		79 vs 177	31.8	18.9	12.9	15.89	2.00	10.26	(1.3e−03)	***		(<1.0e−02)	[0/100]
haplotype 40		80 vs 179	23	12.1	10.9	12.45	2.18	10.14	(1.4e−03)	***		(<1.0e−02)	[0/100]
haplotype 41		80 vs 185	33.6	20.8	12.8	16.19	1.93	9.86	(1.7e−03)	***		(<1.0e−02)	[0/100]
haplotype 42		76 vs 180	33.7	20.8	12.9	16.28	1.93	9.57	(1.9e−03)	***		(<1.0e−02)	[0/100]

[0766]

TABLE 32C

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT−)

MARKERS
PROTEIN UGT2B4 (G40.3)
cases / controls
frequency %
(case/controls)
diff freq. all.
(cases − controls)
Pvalue

Odd ratio

				OMNIBUS LR TEST			
				Likelihood Ratio		omnibus test	
Test Hardy		cases vs	Likelihood	Pvalue		Pvalue (100	
Weinberg		controls	Ratio Test	(# df)		permutations)	
haplotype 1	PT2	86 vs 191	7.60	(5.3e−02)	3df	(5.0e−02)	S
haplotype 2		83 vs 182	11.10	(1.1e−02)	3df	(1.0e−02)	S
haplotype 3		81 vs 185	6.62	(8.2e−02)	3df	(1.0e−02)	S
haplotype 4		84 vs 190	9.82	(1.9e−02)	3df	(2.0e−02)	S
haplotype 5		85 vs 190	8.14	(4.2e−02)	3df	(6.0e−02)	NS
haplotype 7		88 vs 199	5.52	(1.3e−01)	3df	(6.0e−02)	NS
haplotype 8		79 vs 184	11.39	(9.7e−03)	3df	(1.0e−02)	S
haplotype 9		80 vs 185	5.95	(1.1e−01)	3df	(9.0e−02)	NS
haplotype 11		82 vs 193	6.19	(1.0e−01)	3df	(4.0e−02)	S
haplotype 12		84 vs 194	6.36	(9.4e−02)	3df	(1.0e−02)	S
haplotype 13		84 vs 194	7.40	(6.0e−02)	3df	(2.0e−02)	S
haplotype 14	PT3	84 vs 183	10.02	(1.8e−01)	7df	(2.2e−01)	NS
haplotype 15		83 vs 182	15.78	(2.7e−02)	7df	(4.0e−02)	S
haplotype 16		84 vs 190	15.28	(3.2e−02)	7df	(5.0e−02)	S
haplotype 17		80 vs 185	9.36	(2.3e−01)	7df	(3.0e−02)	S
haplotype 18		86 vs 191	8.60	(2.7e−01)	7df	(7.0e−02)	NS
haplotype 20		82 vs 186	8.98	(2.5e−01)	7df	(7.0e−02)	NS
haplotype 21		77 vs 176	17.48	(1.4e−02)	7df	(1.0e−02)	S
haplotype 22		79 vs 177	12.56	(8.2e−02)	7df	(1.0e−02)	S
haplotype 23		83 vs 182	14.30	(4.5e−02)	7df	(2.0e−02)	S
haplotype 25		82 vs 181	16.77	(1.9e−02)	7df	(4.0e−02)	S
haplotype 27		85 vs 190	10.23	(1.7e−01)	7df	(4.0e−02)	S

TABLE 32C-continued

HAPLOTYPE ANALYSIS
CASES (89 ALT+) vs CONTROLS (208 ALT−)

| | | | OMNIBUS LR TEST | | |
| | | | Likelihood Ratio | | omnibus test |
Test Hardy Weinberg	cases vs controls	Likelihood Ratio Test	Pvalue (# df)		Pvalue (100 permutations)
haplotype 28		81 vs 185	8.79	(2.7e−01) 7df	(5.0e−02) S
haplotype 30		80 vs 184	8.52	(2.8e−01) 7df	(1.9e−01) NS
haplotype 31		81 vs 185	8.04	(3.2e−01) 7df	(6.0e−02) NS
haplotype 32		79 vs 184	12.96	(7.2e−02) 7df	(3.0e−02) S
haplotype 33		75 vs 179	14.87	(3.7e−02) 7df	(1.0e−02) S
haplotype 34		86 vs 190	6.62	(4.6e−01) 7df	(2.6e−01) NS
haplotype 35	PT4	78 vs 177	14.25	(5.0e−01) 15df	(1.4e−01) NS
haplotype 36		77 vs 176	21.26	(1.3e−01) 15df	(1.0e−02) S
haplotype 37		83 vs 182	18.23	(2.5e−01) 15df	(3.0e−02) S
haplotype 38		84 vs 183	11.38	(7.2e−01) 15df	(2.5e−01) NS
haplotype 39		79 vs 177	16.02	(3.8e−01) 15df	(6.0e−02) NS
haplotype 40		80 vs 179	12.39	(6.5e−01) 15df	(3.1e−01) NS
haplotype 41		80 vs 185	18.11	(2.5e−01) 15df	(1.0e−02) S
haplotype 42		76 vs 180	21.92	(1.1e−01) 15df	(1.0e−02) S

MARKERS
PROTEIN UGT2B4 (G40.3)
cases / controls
frequency %
(case/controls)
diff freq. all.
(cases − controls)
Pvalue

Odd ratio

[0767]

TABLE 33

Multipoint analysis: HAPLOTYPE FREQUENCY TEST-OMNIBUS LR TEST
G40.3 UGT2B4 (4q13.3-q21)

MARKERS HAPLOTYPE 1		10-470/ 25 in2 T	12-652/ 203 Bac C
pvalue (1df)	ALT+ vs ALT−	40e−02	32e−02
% frequency difference	(2 screening)	93	95
(sample sizes)		(87 vs 191)	(88 vs 199)

S = Significant
NS = Not Significant

HAPLOTYPE FREQUENCY TEST

Zyflo	Estimation frequency of haplotype				Statistical test						
secondary effects HAPLOTYPE 1(ATTC)	sample sizes cases vs controls	frequency cases (%)	frequency controls (%)	frequency difference (%)	p-excess	Odds ratio	Chi-S	pvalue (1df)		Pvalue (100 permutations)	No. of permutations
ALT+ vs ALT− (2 screening)	86 vs 191	30.3	19.2	11.1	1.83	13.71	8.32	3.8e−03	**	4.00e−03	4/1000
ALT+ (1) vs ALT− (1)	69 vs 103	34.5	16.9	17.6	2.59	21.18	14.03	1.7e-04	****	<1.0e−03	0/1000
ALT+ (1) vs ALT− (2)	69 vs 88	34.5	21.8	12.7	1.89	16.23	6.26	12e−02	**	1.90e−02	19/1000
ALT vs US (2)	277 vs 90	22.5	22.5	0	1.00	0.06	0.00	7.5e−01		9.87e−01	987/1000

TABLE 33-continued

Multipoint analysis: HAPLOTYPE FREQUENCY TEST-OMNIBUS LR TEST
G40.3 UGT2B4 (4q13.3-q21)

Zyflo secondary effects HAPLOTYPE 1(ATTC)	OMNIBUS LR TEST			
	Likelihood Ratio		omnibus test	
	Likelihood Ratio Test	Pvalue (#df)		Pvalue (100 permutations)	
ALT+ vs ALT– (2 screening)	7.60	(5.3e-02)	3df	(5.0e-02)	S
ALT+ (1) vs ALT– (1)	12.22	(6.4e-03)	3df	(8.0e-03)	S
ALT+ (1) vs ALT– (2)	7.65	(5.3e-02)	3df	(8.4e-02)	NS
ALT vs US (2)	1.04	(7.8e-01)	3df	(7.9e-01)	NS

[0768]

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/sequence.html?DocID=20040038231). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A method of determining the genotype of an individual comprising the steps of:

  a) obtaining a biological sample containing a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from said individual; and

  b) determining the identity of a nucleotide at a biallelic marker of SEQ ID NO:15, wherein said nucleotide determines said genotype of said individual.

2. The method of claim 1, further comprising amplifying a portion of said polynucleotide comprising said biallelic marker prior to said determining step.

3. The method of claim 2, wherein said amplifying is performed by PCR.

4. The method of claim 1, wherein said determining is performed by an assay selected from the group consisting of hybridization assay, sequencing assay, microsequencing assay, and enzyme-based mismatch detection assay.

* * * * *