Title: SYSTEM AND METHOD FOR OPTIMIZING DRUG THERAPY

Abstract: Systems and the use of genotyping in the individualization of therapy and/or individualization of drug dosing are provided. More specifically, a pharmacokinetic model is described for the individualization of drug therapy.
System and method for optimizing drug therapy

Field of the invention
The present invention relates to the individualization of therapy. More specifically, the present invention relates to systems and the use of genotyping aiming at the individualization of therapy and/or individualization of drug dosing. More specifically, the present invention relates to the use of genotyping in the individualization of therapy with a therapeutic agent or a class of therapeutic agents in accordance with a novel pharmacokinetic model which is particularly designed for the medication of populations of patients with a high proportion of a particular metabolic phenotype uncommon to Caucasians, such as the metabolic phenotype of ultra-fast metabolizers for the cytochrome P450 CYP2D6.

Background of the invention
It is well recognized that most medications exhibit wide interpatient variability in their efficacy and toxicity. For many medications, these interindividual differences are due in part to polymorphisms in genes encoding drug metabolizing enzymes, drug transporters, and/or drug targets (e.g., receptors, enzymes), summarized in Evans and Johnson, Annual Review of Genomics and Human Genetics 2 (2001), 9-39. Pharmacogenomics is a burgeoning field aimed at elucidating the genetic basis for differences in drug efficacy and toxicity, and it uses genome-wide approaches to identify the network of gene variations that govern an individual's response to drug therapy. For some genetic polymorphisms (e.g., thiopurine S-methyltransferase), monogenic traits have a marked effect on pharmacokinetics (e.g., drug metabolism), such that individuals who inherit an enzyme deficiency must be treated with markedly different doses of the affected medications (e.g., 5%-10% of the standard thiopurine dose). Likewise, polymorphisms in drug targets (e.g., beta adrenergic receptor) can alter the sensitivity of patients to treatment (e.g., beta-agonists), changing the pharmacodynamics of drug response. Recognizing that most drug effects are determined by the interplay of several gene products that govern the pharmacokinetics and pharmacodynamics of medications, pharmacogenomics research aims to elucidate these polygenic determinants of drug effects. The ultimate goal is to provide new strategies for optimizing drug therapy based on each patient's or preferably population of patients' genetic determinants of drug efficacy and toxicity.
A metabolic phenotype is a functional profile of an individual's metabolic capacity. Metabolic phenotypes have been generally characterized for a number of enzymes, as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultra-extensive (UEM) or ultra-fast metabolizers (UM), the latter phenotype being rather rarely found in Caucasians.

Currently, the determination of an individual's phenotype for a given metabolic enzyme can be performed either via direct metabolic phenotyping or indirect extrapolation of an individual's genotype to a given phenotype. Direct phenotyping involves the use of a probe substrate known to be metabolized by a given enzyme. The rate of metabolism of the probe substrate is measured and this rate of metabolism is used to determine a metabolic phenotype. Although labor intensive and costly procedures for direct phenotyping have been known for many years these procedures are not readily adaptable for a clinical environment, nor are they practical for measuring multiple phenotypic determinants. For example, enzymatic phenotypes may be determined by measurements of the molar (or chiral) ratio of metabolites of a drug or a probe substrate in a urine sample from an individual by high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) or stereo-selective capillary gas chromatography. These determination methods are time-consuming, laborious, and employ systems and equipment that are not readily available in a clinical laboratory.

As the direct phenotyping techniques are time consuming and not readily accessible in clinical settings, physicians routinely prescribe treatment regimes without knowledge of an individual's metabolic capability (phenotype) or genotype for drug-specific metabolism. Accordingly, a trial and error treatment regime is initiated, often at the expense of severe side effects and loss of valuable treatment time.

In order to gain approval from a governing regulatory body (e.g. the FDA) a drug must be proven to be safe and effective. This currently involves the testing of the drug in normal healthy volunteers and in individuals with the disease the drug is designed to treat. Huge numbers of individuals are involved and these trials can take upwards of 7 years to complete. The reason for the large number of individuals is to obtain statistical significance to prove the safety and efficiency of the drug. New drug entities go through rigorous clinical trials prior to their approval for use in humans. These clinical trials are extremely lengthy and costly. During the course of clinical-testing, many promising new drug candidates are abandoned due
to unacceptable toxicity profiles. In some cases the unacceptable toxicity occurs only in a minority of the general individual population. Often the occurrence of isolated toxicity is the result of a specific metabolic phenotype. Unfortunately, the ability to select defined individual populations for clinical trials has not been available on a routine basis, and this has resulted in the early termination of trials on otherwise promising new drug candidates.

As a result of the highly variable inter-individual rates of metabolism for some drugs, in which the variability is related to side-effects and/or efficacy, the inclusion of individuals with a phenotype or genotype which makes them prone to decreased drug response or to increased adverse effects can result in an overall decrease in the drug efficiency ratings or safety profiles of the trials. If this decrease in response rate or decreased safety profile is significant the drug may not be able to gain regulatory approval.

Furthermore, the treatment success for many diseases, including cancer and infectious diseases, is correlated with the use of optimal drug dosages, for both single drugs and for drugs in combination. Optimal dosages guarantee that the plasma drug concentration(s) remain well above the minimum effective concentrations (MECs) of all the administered drugs. Often, for example, the higher the MEC of a particular drug is in a particular patient, the lower the disease sensitivity is to that particular drug, resulting in lower likelihood of effective treatment. The probability of treatment success depends on the fact that the MEC is drug-specific, and that for the same drug the MEC also varies across the patient population. Also, different drugs are more effective in some patients than in other patients due to inter-individual differences in pharmacokinetics. Hence, it also may happen that drugs or dosages of drugs that might be effective or only effective for a particular patient population are not revealed in the clinical trials since said patient population forms a too minor part of the commonly selected patient profile.

**Summary of the invention**

One aim of the present invention is to provide a method for selecting an individual treatment regime and to provide a means for individualizing the dose of a desired treatment regime. Accordingly, another aim of the present invention is to provide a method for the individualization of therapy for a subject or a population of subjects, which is substantially homogeneous with respect to their metabolic profile, i.e. with respect to at least one, preferably the predominant metabolic factor for a given drug.
Preferably, the present invention employs genotyping to identify an individual's genotypic metabolic profile for use in determining an individual dosage regime for a given therapy or treatment. In this manner, an individual's genotype may be quantified with respect to one or more metabolic factors for the individualization of therapy. Alternatively, phenotyping may be employed, together with genotyping or alone for the purpose of individualization of therapy according to the present invention.

According to one aspect of the present invention, metabolic determinants are employed in the individualization of therapy. These metabolic determinants may be genotypic and/or phenotypic determinants. Although phenotypic testing is believed to be a more comprehensive and accurate assessment of drug therapy than genotypic testing, phenotypic testing can take longer and may generally be more expensive than genotypic testing. Compared with phenotypic testing, genotypic testing has advantages, including the relative simplicity, low cost, and the speed with which the test can be performed. According to an embodiment of the present invention, a metabolic profile based on at least one metabolic determinant may be provided for use in the individualization of therapy and/or drug dosing.

The present invention may be employed in connection with the individualization of a variety of therapies and/or treatments characterized by a metabolic factor of interest, such as enzymatic and/or metabolic pathway activity.

Thus, the present invention is generally directed to a method of determining a dosage regime for at least one drug or pro-drug, preferably two drugs or pro-drugs for an individual subject, wherein an individualized dosage of a drug selected from a drug or class of drugs known for treating a condition is determined for said subject, said method comprising:

(a) determining a metabolic profile of said individual corresponding to at least one metabolic factor known to influence the metabolism or action of said class of drugs; and

(b) calculating said individualized dosage of said drug according to a pharmacokinetic model.

In accordance with the present invention said pharmacokinetic model takes into account:
the rate of metabolism of at least one drug, preferably at least two drugs, the metabolism or action of which is known to be influenced by a metabolic factor, for example a metabolizing enzyme such as CYP2D6;

(ii) in at least two different groups of subjects, wherein said subjects of each group are substantially homogeneous with respect to the genetic determinant(s) for said metabolic factor for said at least one drug; wherein

(iii) the metabolic phenotype of said group of subjects preferably can be characterized as poor metabolizers (PM), intermediate metabolizers (IM) extensive metabolizers (EM), or ultra-extensive metabolizers (UM); and wherein

(iv) said rate of metabolism in relation to the metabolic phenotype of said group of subjects provides a linear function that can be calculated to a metabolic quotient for each of said PM, IM, EM, and UM.

In accordance with the examples, the present invention provides methods for improved means and methods for the efficient treatment and/or the prevention of depression (Trimipramine) and hypertension (Propaphenone, Metoprolol). The invention provides also precise dose adjustments for poor (PM), intermediate (IM), extensive (EM) and in particular ultra fast (UM) metabolizers. Besides the above, the present invention takes into account the fact that the metabolites of some drugs are also active.

Once the pharmacokinetic model of the present invention is established for a given drug or class of drugs, this pharmacokinetic model can be used to determine the optimal, individualized, dosage or dosage regimen of said drug or for drugs that belong to the same class of drugs, in particular for populations of ultra fast metabolizers (UM).

In combination with the knowledge of the corresponding genotype, i.e. genotypic determinant of a given patient this allows to quickly and easily determine the individualized dosage of the drug for said patient. For example, if a patient has been tested to display the genetic determinant of CYP2D6 for an UM, the dosage of a given drug previously developed for a genetically mixed population of predominantly EMs can immediately be adapted to said UM for which the common dosage would be ineffective. The ability to quickly and accurately screen individuals for their metabolic capacity could potentially allow the approval of promising drug treatments for a selective segment of the population such as PMs and UM, that otherwise would lack satisfactory response rates or safety profiles.
Furthermore, the present invention relates to a method for the preparation of a medication for an individual subject or a subpopulation of subjects comprising reformulation of the drug or pro-drug in accordance with the above described method, wherein preferably said individual subject or a subpopulation of subjects are ultra fast metabolizers (UMs), preferably of CYP2D6. The present invention also concerns medications obtainable by said method, wherein said medications optionally further comprise reformulations of the drug with different dosages for PM, IM, EM and/or UM.

The present invention also concerns a method for selecting a suitable dosage regimen for a subject or formulation of drug or class of drugs known for treating a condition, wherein said method comprises:
(a) determining the presence or absence of at least one genetic determinant in a sample obtained from said subject; and
(b) selecting a suitable therapy for said subject based on the pharmacokinetic model of the present invention.

Furthermore, it is an object of the present invention to use genotyping for a method of selecting a suitable dosage regimen for a subject in accordance with the present invention, wherein an individual is genotyped for a specific metabolic factor and a corresponding genotypic determinant is characterized. Preferably, said genotypic determinant is used to quantify an individualized dosage regime of a drug.

It is another object of the present invention to provide a genomic assay for use in the individualization of therapy and/or treatment, said assay comprising:
(a) a means for identifying a genetic marker corresponding to an individual's capacity for the metabolism of a given drug or class of drugs;
(b) a means for quantifying said genetic marker to provide an indicator of metabolic capacity specific for said drug or class of drugs; and
(c) a means for correlating said indicator with a therapeutically effective dosage of said drug or class of drugs for said individual according to the pharmacokinetic model of the invention.

According to another aspect, the invention relates to a method of selectively treating an individual with a drug or class of drugs; said method comprising:
(a) genotyping an individual to identify at least one allelic polymorphism known to influence the metabolism of said drug or class of drugs;

(b) phenotyping said individual to confirm their phenotypic capacity to metabolize said at drug of class of drugs;

(c) calculating a therapeutically-effective dosage of said drug or class of drugs specific for said individual based on said genotyping and phenotyping in accordance with the pharmacokinetic model of the present invention; and

(d) selectively treating said individual with the same.

In yet another embodiment of the invention, a kit for use in a method of the present invention is provided, said kit comprising oligonucleotides, reference samples, amplification and/or sequencing means, buffer, detergents, biochemical reagents, detection means, or the like and optionally a reformulated drug with a drug dose which is tailored to poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) or ultra fast metabolizers (UM).

For the purpose of the present invention the following terms are defined below.

The term "metabolic determinant" is intended to mean a qualitative or quantitative indicator of a metabolic-specific capacity of an individual.

The term "individualization" as it appears herein with respect to therapy is intended to mean a therapy having specificity to at least an individual's phenotype as calculated according to a predetermined formula on an individual basis.

The term "biological sample" is intended to mean a sample obtained from a biological entity and includes, but is not to be limited to, any one of the following: tissue, cerebrospinal fluid, plasma, serum, saliva, blood, nasal mucosa, urine, synovial fluid, microcapillary microdialysis and breath.

The term "therapeutic agent" or "drug" are used interchangeably herein and are intended to mean an agent(s) and/or medicine(s) used to treat the symptoms of a disease, physical or mental condition, injury or infection.
The term "treatment" is intended to mean any administration of a pharmaceutical compound to an individual to treat, cure, alleviate, improve, diminish or inhibit a disease, physical or mental condition, injury or infection in the individual.

The term "individual treated" is intended to mean any individual being subjected to the administration of (i) a pharmaceutical compound, for treating, curing, alleviating, improving, diminishing or inhibiting a disease, physical or mental condition, injury or infection, or (ii) a probe substrate for determining multi-determinant metabolic phenotype.

The term "clinical data" may include previously recorded patient data, including genotypic variations or patterns with specific therapy sensitivities, data from phenotype/genotype relational databases, 50% inhibitory concentrations and minimum effective concentrations of various therapies, known drug-drug interactions, indications, or contraindications, etc. This clinical data may be generated on-site, off-site, or may be obtained from public databases or journals, or forwarded by researchers in the field.

The term "dosage" includes the size, frequency, formulation, co-medication and number of doses of at least one therapy to be given to a patient. This also includes newly prescribed therapies and/or therapies, both singly and in combination and is irrespective of the way of administration.

The term "patient" or "subject" includes any organism, particularly a human or other mammal, suffering from a disease, in need or desire of treatment for a disease, or in need of testing or screening for a disease. A patient includes any mammal, including farm animals or pets, and includes humans of any age or state of development.

The term "pharmacokinetic model" or "population pharmacokinetic model" predicts an individual plasma concentration of a drug using a set of mathematical equation. An "optimized" population pharmacokinetic model is a model that has been adjusted to minimize the difference between at least one data point in the model and at least one actual measurement from a subject. The pharmacokinetic model which describes the drug's behavior in an organism can be chosen out of variety of models known to the person skilled in the art, including, but not limited to, models based on one compartment, two or more compartments, and using either zero order, first order, second order or higher order kinetics. The model may be a predicted model, wherein the model is chosen based on data known in the art for a
therapy. Alternatively, the model may be measured by analyzing patient samples and determining the pharmacokinetic model thereon (measured model).

For example, based on literature data in combination with drug concentration determinations in defined groups of subjects such as described in the examples indications for a model may be provided. The model may allow one to predict or estimate parameters required, e.g. AUC (Area Under the Curve) s. Patient parameters may also be included in the model, e.g. age, gender, weight, body mass index (Bayes approach). In accordance with the pharmacologic model of the present invention, this combination of data and mathematic equations allows the prediction of parameters including the dosage regimen needed to obtain a certain drug concentration.

The term "pharmacologic exposure" is the extent to which a patient is exposed to a therapy. A measure of exposure is, e.g. steady state trough concentration (Css) and area under the curve (AUC).

The term "therapeutic agent regime" or "dosage regimen" is the course of action or use of a therapeutic agent or drug, or combination of therapeutic agents or drugs in treating a patient including, for example, at least one of dosage, schedule of administration, choice and/or combination of therapeutic agents.

The term “functional CYP2D6 polypeptide” as used herein refers to a polypeptide with wild type CYP2D6 activity corresponding to the polypeptide of GeneBank accession No.: GI: 181304 which forms the *1 (wild type) allele or to a polypeptide encoded by the *2 allele as depicted above. Preferred methods for diagnosing a CYP2D6 duplication are described below in more detail. The phenotype of each subject (enzymatic CYP2D6 activity) can be determined e.g. by measuring the sparteine oxidation, the dextromethorphan and debrisoquine metabolic ratio as described in Bock, Pharmacogenetics 4 (1994), 209-218; Griese, Pharmacogenetics 8 (1998), 15-26; and Sachse et al., Am. J. Hum. Genet. 60 (1997), 284-295.

The term “polynucleotides” or “polypeptides” refers to different variants of a polynucleotide or a polypeptide specified in accordance with the uses of the present invention. Said variants comprise a reference or wild type sequence of the polynucleotides or polypeptides specified herein as well as variants which differ there from in structure or composition. The term “polynucleotide” as used herein preferably encompasses the nucleic acid sequence
specifically referred to by SEQ ID NOs and as well as polynucleotides comprising the reverse complementary nucleic acid sequence thereto.

Reference or wild type sequences for the CYP2D6 polynucleotides are GeneBank accession No.: GI:181303 or GeneBank accession No.: GI:181304 for the CYP2D6 polypeptide. The differences in structure or composition usually occur by way of nucleotide or amino acid substitution(s), addition(s) and/or deletion or (single or multiple) gene duplications; see CYP2D6 Allele Table.

Preferably, said nucleotide substitution(s), addition(s) or deletion(s) referred to in accordance with the use of the present invention result(s) in one or more changes of the corresponding amino acid(s) of the polypeptides. The variant polynucleotides also comprise fragments of said polynucleotides or polypeptides. The polynucleotides or polypeptides as well as the aforementioned fragments thereof are characterized as being associated with a CYP2D6 dysfunction or dysregulation comprising, e.g., insufficient and/or altered drug metabolism. Said dysfunctions or dysregulations referred to in the present invention cause a drug induced disorder or drug resistance. The present invention also encompasses all embodiments described in connection with polynucleotides in WO01/55432, WO01/59152, WO9711175, United state patents 5,981,174; 6,183,963; 5,648,482; 5,912,120; and 5,719,026.

The term “hybridizing” as used herein refers to polynucleotides which are capable of hybridizing to the above polynucleotides or parts thereof which are associated with a CYP2D6 dysfunction or dysregulation. Thus, said hybridizing polynucleotides are also associated with said dysfunctions and dysregulations. Preferably, said polynucleotides capable of hybridizing to the aforementioned polynucleotides or parts thereof which are associated with CYP2D6 dysfunctions or dysregulations are at least 70%, at least 80%, at least 95% or at least 100% identical to the polynucleotides or parts thereof which are associated with CYP2D6 dysfunctions or dysregulations. Therefore, said polynucleotides may be useful as probes in Northern or Southern Blot analysis of RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Also comprised in accordance with the use of the invention are hybridizing polynucleotides which are useful for analyzing DNA-Protein interactions via, e.g., electrophoretic mobility shift analysis (EMSA). Preferably, said hybridizing polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing
polynucleotide to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.

It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions she/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books, such as Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. Preferred in accordance with the use of the present inventions are polynucleotides which are capable of hybridizing to the above polynucleotides or parts thereof which are associated with a CYP2D6 dysfunction or dysregulation under stringent hybridization conditions, i.e. which do not cross hybridize to unrelated polynucleotides such as polynucleotides encoding a polypeptide different from the CYP2D6 polypeptides of the invention.

Moreover, methods for determining whether a subject comprises a polynucleotide referred to herein above are well known in the art. To carry out said methods, it is necessary to take a sample comprising biological material, such as isolated cells or tissue, from said subject. Further, the methods known in the art could comprise for example, PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques. A preferred and convenient method to be used in order to determine the presence or absence of one or more of the above specified polynucleotides is to isolate blood cells from a subject and to perform a PCR based assay on genomic DNA isolated from those blood cells, whereby the PCR is used to determine whether said polynucleotides specified herein above or parts thereof are present or absent. Most preferably, said method for diagnosing a duplication of a functional CYP2D6 polynucleotide is by XbaI RFLP analysis or allele specific polymerase chain reaction (ASPCR) analysis as described in more detail in Bock, Pharmacogenetics 4 (1994), 209-218; Griese, Pharmacogenetics 8 (1998), 15-26; Johannsson, Proc. Natl. Acad. Sci. USA 90 (1993), 11825-11829; Johannsson, Pharmacogenetics 6 (1996), 351-355 and Sachse, Am. J. Hum. Genet. 60 (1997), 284-295.
The term “corresponding” as used herein means that a position is not only determined by the number of the preceding nucleotides and amino acids, respectively. The position of a given nucleotide or amino acid in accordance with the use of the present invention which may be deleted, substituted or comprise one or more additional nucleotide(s) may vary due to deletions or additional nucleotides or amino acids elsewhere in the gene or the polypeptide. Thus, under a “corresponding position” in accordance with the present invention is to be understood that nucleotides or amino acids may differ in the indicated number but may still have similar neighboring nucleotides or amino acids. Said nucleotides or amino acids which may be exchanged, deleted or comprise additional nucleotides or amino acids are also comprised by the term “corresponding position”. Said nucleotides or amino acids may for instance together with their neighbors form sequences which may be involved in the regulation of gene expression, stability of the corresponding RNA or RNA editing, as well as encode functional domains or motifs of the protein of the invention.

The term “pharmaceutical composition” as used herein comprises the substances of the present invention, and optionally one or more pharmaceutically acceptable carriers.

**Brief description of the drawings**

**Fig. 1:** Measured concentration-time data (open circles connected by thin lines) after intravenous infusion of 49.9 mg trimipramine over a period of 2 hours. The three groups represent genotypically identified poor metabolizers (PM) of CYP2D6 substrates, homozygous extensive metabolizers (EM) and ultra rapid metabolizers (UM). The latter group represents carriers of one wild type allele and one duplication allele, i.e. these subjects carry three genes coding for active CYP2D6.

**Fig. 2:** Measured concentration-time data after the 5th dose of multiple oral dosing of 50 mg trimipramine. The three groups represent poor, extensive and ultra fast metabolizers of CYP2D6 substrates.

**Fig. 3:** Measured concentration-time data for plasma after oral application of 100 mg normal release metoprolol.
**Fig. 4:** Dosage recommendations for CYP2D6 genotypes determined in accordance with the present invention. The recommended average dose given in the literature for Imipramin, Doxepin, Trimipramin, Maprotilin, Desipramin, Nortriptylin, Clomipramin, Amitriptylin was 150 mg, Mianserin: 60 mg, Paroxetine: 20 mg and Venlafaxin: 150 mg. The determined optimal dosage in % of the standard dose for PMs, IMs, EMs and UMs is given. The standard dose for the antihypertensive drug metoprolol is 100 mg.

**Detailed description of the invention**

The present invention relates to the individualization of therapy and/or treatment. In particular, the present invention relates to the individualization of therapy and/or treatment with a given drug or drugs. Based on a genotypic and/or phenotypic characterization of an individual's capacity to metabolize a given drug or drugs and a pharmacokinetic model, the present invention provides a system and method for determining an individualized dosage of that drug(s) based thereon.

The present invention is based on a method and pharmacokinetic model, which involves the genotyping of the patients prior to administering a drug so that its dosage is tailored to the patient's genetic profile. As an example for this method, the effective drug dosages for antidepressant drugs, including the antidepressant drug trimipramine, and for the antihypersensitive drug metoprolol (propaphenone) which are all metabolized by CYP2D6, have been determined. The method also relates to pharmaceutical compositions and the preparation of them, which are tailored to the genetic profile of these patients. In accordance with the present invention said pharmacokinetic model takes into account:

(i) the rate of metabolism of at least one drug, preferably at least two drugs the metabolism or action of which is known to be influenced by a metabolic factor, for example a metabolizing enzyme such as CYP2D6;

(ii) in at least two different groups of subjects, wherein said subjects of each group are substantially homogeneous with respect to the genetic determinant(s) for said metabolic factor for said at least one drug; wherein

(iii) the metabolic phenotype of said group of subjects preferably can be characterized as poor metabolizers (PM), intermediate metabolizers (IM) extensive metabolizers (EM), or ultra-extensive metabolizers (UM); and wherein
(iv) said rate of metabolism in relation to the metabolic phenotype of said group of subjects provides a linear function that can be calculated to a metabolic quotient for each of said PM, IM, EM, and UM.

The present invention is based on a pharmacokineti model that will change drug therapy for ever. It will abolish the development of drugs for a statistically average patient. Based on this model the drug concentration of drugs that are metabolized for instance by CYP2D6 is adjusted to the genetic profile of a patient. The result is that the drug is effective and causes less side effects.

In a preferred embodiment, the method of the invention comprises the following steps:

1. determining the presence or absence of at least one variant allele in the genome of a subject in a sample obtained from said subject;
2. determining for instance if the CYP2D6 wild type gene is amplified and how many copies are present;
3. selecting a suitable therapy for said subject based on the results obtained in (1) and (2);
4. selecting the formulation, which contained the drug at a concentration tailored to the genetic profile of the patient;
5. if the drug causes elevated levels of transaminases at their highest concentration then another drug, which is not metabolized by, for instance, CYP2D6 is selected.

Genotyping is performed by analyzing the genetic sequence of a gene coding for a specific enzyme often by a polymerase chain reaction assay (PCR) or a PCR with a restriction fragment length polymorphism assay (PCR-RFLP) or a PCR and dideoxysequencing. The gene is examined for the presence of genetic mutations that can be linked to increased or decreased enzyme levels or activity, which in turn result in a specific phenotype, i.e. a poor metabolizer vs. an extensive metabolizer. The genotype is a theoretical measurement of what an individual's phenotype should be. In principle, the genetic mutation or allele may present in any region of the gene including the coding region, promoter, enhancer sequences, intron, exon, intron/exon junction and vice versa, poly signal and the like, but also including polymorphisms which are not located within the gene encoding the metabolic factor but which nevertheless display a tight linkage to the observed phenotype.

In accordance with the method of present invention two examples have been selected. The first relates to the use of trimipramine for the preparation of a pharmaceutical composition for treating and/or preventing medium to severe depression in patients having in its genome more
than two copies of a functional CYP2D6 gene. Dose recommendations are given in % of the standard dosage. The second example relates to the dose adjustment of the anti-hypertensive drug propafenone (metoprolol) in patients expressing three copies of the wild type CYP2D6 gene.

Surprisingly, the assessment of the genetically determined variability in drug concentration revealed an almost linear relationship within the different metabolic phenotypes, i.e., poor metabolizers (UM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultra fast metabolizers (UM). Furthermore, the metabolic phenotypes correspond to the number of alleles of the corresponding genetic determinant, here CYP2D6 genotype, which means that two 0 alleles correspond to PMs, one wild type and one 0 allele correspond to IM, two wild type alleles correspond to EM and UM contain an additional wild type allele, for example due to gene duplication.

Also encompassed by the use of the present invention are new formulations of the drugs or derivatives of those substances which are obtainable by way of any chemical modification, wherein said derivatives are equally well therapeutically suited for the use of the present invention. The goal of the dose adjustment or chemical modification is to reach in PMs, IMs and UM the therapeutically effective dose in a patient independent of his genotype. The administration of a drug dose which is tailored to the genetic profile of a patient is especially critical for drugs which have a narrow therapeutic index i.e. the toxic concentration of a drug and the minimal effective concentration of it are narrow. To determine whether a derivative of the substances of the invention is therapeutically equally well suited for the use of the invention biological assays and kinetic studies well known in the art can be performed.

Thus, the present invention provides specific methods for improving the efficacy of the currently available drug therapies. As an example dose recommendations are given for drugs which are used to treat intermediate to severe depression and are metabolized by CYP2D6 and an antihypertensive drug, propafenone for which dose recommendation for CYP2D6 ultra fast metabolizers are given. This method can be applied to other drug metabolizing enzymes such as CYP2C9, CYP2C19, TPMT (thiopurine s-methyltransferase), FMO3 (flavin-containing monooxygenase 3), DPD (dihydropyrimidine dehydrogenase), NAT2 etc.. The present invention avoids the aforementioned insufficient activity or non-activity, which are accompanied with the said therapies. So far these therapies are not available yet but are
nevertheless highly desirable in order achieve therapeutic results and to prevent drug induced
drug resistance or unwanted side effects. A correlation of genetic factors and the efficacy of
trimipramin, or the antihypersensitive drug propaphenone therapy have not been made
available yet. If there would be an association between CYP2D6 genotype and efficacy of
drugs metabolized by CYP2D6 existed before the onset of the therapy, this would provide an
improvement over the currently available therapies. The method of the present invention is
applied for polymorphisms which influence the metabolism of specific drugs and/or specific
drugs that occur at a high frequency in a specific population.

The definitions and explanations of the terms made above apply mutatis mutandis to all of the
methods described herein. The term "suitable therapy" as used herein means that a substance
according to the invention is selected and said substance is administered in a certain dosage to
a subject, wherein said substance and said dosage are selected based on the knowledge of the
presence or absence of at least one, preferably at least two variant alleles referred to in
accordance with the use of the invention. Said substance and said dosage of the substance are
selected in a way that they are most effective on one hand and on the other hand they do not
cause toxic or undesirable side effects.

The present invention adds to the art a combination of a bio-analytical method with
population based modeling to determine a patient specific measure of pharmacological
therapy exposure, and a dosage determination. The combination of the genetic determinants,
i.e. genotype and patient specific pharmacokinetic parameters provides a single measure to
manage therapy.

Without intending to be bound by theory it is believed that the here described population
pharmacokinetic model therapy using one or more plasma concentrations of at least two
groups of metabolic phenotypes, measured at any time point after drug intake, can has the
potential to predict the dosage regimen for said drug or a corresponding drug for an individual
subject or population of subjects based on their genetic make up.

Thus, the present invention provides a method of determining a dosage regime for at least one
drug or pro-drug, preferably two drugs or pro-drugs for an individual subject, wherein an
individualized dosage of a drug selected from a drug or class of drugs known for treating a
condition is determined for said subject, said method comprising:
(a) determining a metabolic profile of said individual corresponding to at least one metabolic factor known to influence the metabolism or action of said class of drugs;

(b) calculating said individualized dosage of said drug according to a pharmacokinetic model.

Examples for factors that may be taken into account and corresponding drugs are listed in the annexed tables 1 and 2. Although most drugs are metabolized by a primary enzymatic pathway, for example CYP3A4 or CYP2D6 metabolize many antihistamine agents (drugs), it is often the case that a given drug is metabolized by multiple enzymes and/or metabolic pathways. As a result, it may be preferred to characterize an individual's metabolic profile for a plurality of metabolic enzymes prior to selecting a corresponding drug treatment regime. Accordingly, in one embodiment of the method of the present invention, said pharmacokinetic model comprises different genetic determinants specific for said at least one metabolic factor, wherein said genetic determinants are correlated to a rate of drug metabolism specific of said different genetic determinants, and a metabolic quotient for said at least one drug is determined based on the respective rate of drug metabolism specific of said different genetic determinants.

As already explained before, the pharmacokinetic model of the present invention typically comprises determining a pharmacologic exposure of said drug or class of drugs in at least two different subjects with different genetic determinants. Preferably, said genetic determinants are specific for poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) or ultrarapid metabolizers (UM) of said drug or class of drugs. In a particular preferred embodiment of the pharmacokinetic model of the present invention, at least one pharmacologic exposure of said drug or class of drugs is performed in subjects with a genetic determinant specific for ultrarapid metabolizers (UM). As described above and shown in the examples the genetic determinant of UM usually comprise at least one gene duplication, resulting for example in three copies of a wild type allele of a metabolic enzyme, e.g., CYP2D6. Of course, it may also be that more than three copies of a given allele are present.

Knowledge of an individual's metabolic profile, as determined by genotyping, may be applied clinically in determining a specific drug dosage based on the individual's capacity to metabolize the drug. Alternatively, a metabolic profile as determined by phenotyping, or a
combination of phenotyping and genotyping may be employed in accordance with the present invention for purpose of individualization of therapy or treatment. Other factors representing an individual's capacity to metabolize a drug may also find application in the present invention, together with a metabolic profile for providing the individualization of therapy. Usually, said at least one metabolic factor is specific an activity level of an enzyme activity known to influence the metabolism of said drug or class of drugs.

According to one aspect of the present invention, an individual's metabolic profile is characterized on the basis of metabolic factors specific to a given therapy or treatment by genotyping. For example, genotyping may be employed to identify an allelic variation in an individual corresponding to a metabolic factor of interest, such as enzymatic activity specific to a candidate drug treatment or therapy. Metabolic determinants based on the identified allelic variations are subsequently characterized and used to quantify an individual dosage regimen based on the pharmacokinetic model for that individual patient. In this regard, genotyping may be employed alone or in combination with phenotyping for the purpose of (1) individualizing a dosage regimen for a given treatment or therapy; and (2) identifying a metabolic profile representative of a high risk individual with respect to a given treatment or therapy.

A system of the present invention is exemplified in accordance with a protocol for determining phenotypic determinants for CYP2D6. This protocol can be adapted to provide a system for determining phenotypic determinants for a specific enzyme or metabolic pathway, such as for example CYP2C9 or CYP2C19, identified as a metabolic factor for a specific drug or drug class of interest, in accordance with the present invention. The determination of at least one metabolic determinant for a specific enzyme or metabolic pathway may be performed as a single determination or in combination with methods for determining a metabolic profile including other metabolic factors of interest.

These enzymes are involved in the metabolism of a large number of drugs, and as a result have important implications in the outcome of many individual drug treatment regimes, as well as clinical trial studies.

These enzymes and their corresponding metabolic determinants as described herein are provided as representative examples of determinants for the purposes of exemplifying the
present invention. However, the present invention is not limited thereto. The present invention further provides a corresponding protocol for providing genotypic determinants for drug-specific metabolic factors, including without limitation those metabolic factors and drugs shown in the appended tables and/or metabolic pathways related thereto.

Preferably, said activity level of said enzyme and/or said metabolic pathway is specific for at least one cytochrome P450, N-acetyltransferase, flavin-containing monooxygenase form 3 or thiopurine s-methyltransferase metabolic pathway. An overview on cytochromes P450 and their impact on drug treatment is given in Tredger et al., Hospital Pharmacist 9 (2002), 167-173. Hence, a metabolic profile, according to an embodiment of the present invention may be specific to at least one of the following enzymes, without limitation: NAT1, NAT2, CYP2A6, CYP2D6, CYP3A4, CYP2E1, CYP2C9, CYP1A2 and CYP2C19, FMO3, TPMT, or dihydropyrimidine dehydrogenase (DPD). Examples of the association of alleles of the given enzymes with a metabolic phenotype can be found in the literature.

For example, the influence of CYP2C9 genetic polymorphisms on pharmacokinetics of celecoxib and its metabolites is described in Kirchheiner et al., Pharmacogenetics 13 (2003), 473-480.


Nucleotide polymorphisms in the MDR1 gene encoding the P-glycoprotein, a transmembrane efflux pump that extrudes a wide variety of drugs, thereby reducing their intracellular access,


Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein (MDR-1) in a Russian population are described in Gaikovitch et al., Eur. J. Clin. Pharmacol. 59 (2003), 303-312.

Another metabolic enzyme is flavin-containing monooxygenase 3 (FMO3). The human flavin-containing monooxygenases catalyze the oxygenation of nucleophilic heteroatom-containing drugs, xenobiotics and endogenous materials. Evidence for six forms of the FMO gene exist but it is FMO form 3 (FMO3) that is the prominent form in adult human liver that is likely to be associated with the bulk of FMO-mediated metabolism. An understanding of the substrate specificity of human FMO3 is beginning to emerge and several examples of drugs and chemicals extensively metabolized by FMO3 have been reported. Expression of FMO3 is species- and tissue-specific, but unlike human cytochrome P450 (CYP450), mammalian FMO3 does not appear to be inducible. Interindividual variation in FMO3-dependent metabolism of drugs, chemicals and endogenous materials is therefore more likely to be due to genetic and not environmental effects. Certain mutations of the human FMO3 gene have been associated with abnormal N-oxygenation of trimethylamine. Deficient N-oxygenation of trimethylamine results in a condition called trimethylaminuria. Some treatment strategies for this inborn error of metabolism are discussed. Other common variants of the FMO3 gene including E158K, V257M and E308G have been observed. An overview is given in Cashman, Pharmacogenomics 3 (2002), 325-339. Polymorphisms of the fmo3 gene in caucasian and african-american populations are described in, for example, Lattard et al., Drug Metab. Dispos. 31 (2003), 854-860; Park et al., Pharmacogenetics 12 (2002), 77-80; Hernandez et al., Hum. Mutat. 22 (2003), 209-213; and Zeng et al., Zhonghua Yi Xue Yi Chuan Xue Za Zhi 20 (2003), 318-321.

A further enzyme under consideration in the method of the present invention is TPMT (Thiopurine S-Methyltransferase), a cytosolic enzyme that inactivates Mercaptopurine, azathioprine, and thioguanine; drugs that are used to treat many diseases, including cancer,
acute leukemias, inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis and autoimmune myasthenia gravis. They are also used as immunosuppressants after organ transplants. Adverse reactions to these drugs include hepatitis, pancreatitis, infections, and severe myelosuppression. There are 8 known mutation alleles. TPMT*3A accounts for 85%, and TMPT*2 and TPMT*3C each accounts for about 5% of all known mutations in Caucasians. The TPMT*2 allele showed a 100-fold decrease, and the TPMT*3A and *3C alleles showed a greater than 200-fold decrease in TPMT enzyme activity. There is a 98% concordance between phenotypic measurement of TPMT activity in Red Blood Cells and genotypic measurement.


There is a rapidly growing body of evidence linking genetic polymorphisms with functional changes in proteins that are responsible for the metabolism and disposition of many medications. Likewise, polymorphisms in genes encoding the targets of medications (e.g. receptors) can alter the pharmacodynamics of the drug response by changing receptor sensitivity; see for review Johnson and Evans, Trends Mol Med 8 (2002), 300-305. Thus, said at least one metabolic factor can also be a drug receptor, translocator, ion-channel, transporter, or ligand binding molecule. Kerwin et al., Expert Rev. Mol. Diagn. 1 (2001), 275-280, describes some targets such as serotonin receptors and transporters, dopamin receptor and histamine 2 for genetic strategies for the personalization of antipsychotic treatment. Drug receptor/effector polymorphisms and pharmacogenetics are described by Johnson and Lima in Pharmacogenetics 13 (2003), 525-534. Another review on published examples of inherited differences in drug metabolizing enzymes, drug transporters, and drug targets (for example, receptors) to illustrate the potential importance of inheritance in determining the efficacy and toxicity of medications in humans is provided by Evans, Gut 52:ii (2003), 10-18; for review see also Weinshilboum, N. Engl. J. Med. 348 (2003), 529-537 and Goldstein, N. Engl. J. Med. 348 (2003), 553-556.
Hence, the influence of polymorphic genes on drug action on the receptor or target molecule is well known. A drug can only exert its action on receptors and other target molecules if it can bind to it. In addition if the receptor is overexpressed in the diseased status of the cell then more drug molecules might be necessary to inactivate sufficient receptor molecules. There exists several examples where polymorphisms in genes encoding receptors or drug targets influence drug action.

**Table: Influence of drugs on receptors and other target molecules**

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Clinical Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Adrenergic Receptor</td>
<td>Albuterol</td>
<td>Bronchodilator response is dependent on specific haplotype combinations. Agonist mediated efficacy is dependent on polymorphisms.</td>
</tr>
<tr>
<td>Dopamine Transporter (9x40bp Variable Number of Tandem Repeats)</td>
<td>L-Dopa</td>
<td>Influence on drug induced psychosis and dyskinesa</td>
</tr>
<tr>
<td>5-Lipoxygenase</td>
<td>Zileuton</td>
<td>No effect in patients who share a specific tandem repeat in the promoter.</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>Tacrine</td>
<td>Only in ApoE negative Alzheimer patients</td>
</tr>
<tr>
<td>MGMT (O⁶-methylguanine-DNA methyltransferase)</td>
<td>Alkylating Agents</td>
<td>Promoter methylation results in good survival prognosis for glioma patients</td>
</tr>
<tr>
<td>KCNE2 (T8A in MiRP1)</td>
<td>Sulfamethoxazol, Procainamid, Oxatomid</td>
<td>Drug induced Long-QT-Syndrome</td>
</tr>
<tr>
<td>Glycoprotein IIIa (PLA1/PLA2)</td>
<td>Aspirin, Abciximab</td>
<td>Reduced response in patients carrying the PLA2 polymorphism.</td>
</tr>
<tr>
<td>CETP (B1/B2)</td>
<td>Pravastatin</td>
<td>Slower development of arteriosclerosis in B1B1 patients.</td>
</tr>
<tr>
<td>α-Adducin</td>
<td>Hydrochlorothiazide</td>
<td>Higher sensitivity in patients who share the 460Gly/Trp polymorphism</td>
</tr>
<tr>
<td>ACE (I/D)</td>
<td>Enalaprilat</td>
<td>Better response of patients bearing the ACE-II-Alele</td>
</tr>
</tbody>
</table>

As shown in the examples, said rate of drug metabolism in the pharmacogentic model of the present invention is calculated based on multiple or single dosing of said drug. Furthermore,
said drug metabolism is usually measured by bioavailability and/or systemic clearance of the
drug. Preferably, as performed in the examples, said drug is administered by infusion or oral
application. The pharmaceutical exposure, i.e. measurement of drug
concentration/metabolization is obtained from at least one sample such as plasma, a blood, a
saliva, a tumor, a tissue, and a bodily fluid. Preferably, plasma/serum samples are used. In
addition, the pharmacologic exposure is commonly determined as a trough steady-state
concentration (Css) or area under curve (AUC).

The area under the plasma (serum, or blood) concentration versus time curve (AUC) has
number of important uses in toxicology, biopharmaceutics and pharmacokinetics. Toxicology
AUC can be used as a measure of drug exposure. It is derived from drug concentration and
time so it indicates how much of a drug stays how long in a body. A long, low concentration
exposure may be as effective as a shorter but higher concentration. Some drugs are dosed
using AUC to quantify the maximum tolerated exposure (AUC Dosing). The AUC measured
after administration of a drug product is an important parameter in the comparison of drug
products. Studies can be performed whereby different drug products may be given to a panel
of subject on separate equations. These bioequivalency or bioavailability studies can be
analyzed by comparing AUC values.

In the present pharmacokinetic model AUC values are used to determine pharmacokinetic
parameters, such as clearance or bioavailability, F; see example 2. The pharmacokinetic
behavior of a drug depends on the animal physiology (i.e., renal and hepatic function, organ
perfusion, etc.) and the drug’s physicochemical features (lipid solubility, ionization, etc.). The
two main pharmacokinetic parameters are clearance and volume of distribution. Changes on
these parameters due to disease, organ dysfunction, age, etc, may require adjusting dosages of
drugs given to animals.

Clearance: The systemic clearance is representative of the elimination of the drug from the
body. Physiologically it represents the rate of elimination of a drug relative to the plasma
concentration. The concept of clearance is extremely useful for dosage adjustment because at
the typical therapeutic doses, the systemic clearance of a drug is usually constant over the
interval of plasma concentrations of clinical importance. For certain drugs the elimination is
dose-dependent due to saturation of the process at high concentrations, and clearance cannot
be considered constant beyond a certain level.
Area Under the Curve (AUC): This parameter is a reflection of the extent of drug bioavailability and graphically consists of the area that is contained by the concentration-time profile. This parameter represents better than most other parameter the exposure of the animal to the drug after each dose. AUC is usually obtained by a numerical integration procedure known as the trapezoidal rule method. The AUC is independent of the administration route and the drug elimination, as long as the processes of elimination are constant. When dose-independent (linear) kinetics apply, the AUC is directly proportional to the dose and is related to this and to the clearance by this useful expression:

\[
AUC_0^\infty = \frac{F \times D}{Cl}
\]

where \( F \) is the fraction of dose absorbed, \( D \) is the dose and \( Cl \) is the systemic clearance. As can be deducted from this expression, when clearance is constant (linear kinetics) an increase in dose (with unchanged \( F \)) will lead to a proportional increase in AUC. This is a measure of drug availability that for certain drugs can be used as a target exposure to modify dosage regimens accordingly. Likewise, clearance is related to average concentration at steady state as follows:

\[
AUC_0^t = CSS_{avg} \times t
\]

where \( CSS_{avg} \) is the average concentration at steady state and \( t \) is the interdose interval. The combination of both expressions allows to easily modify the dose to achieve a new target average concentration at steady state, if this is needed. For example, if a subject is being medicated long-term with phenobarbital and after a few months on therapy the average steady state concentration decreases 25% due to a similar increase in clearance, the dose will need to be increased by 25% in order to revert to the target concentration at steady state.

Peak concentration: This parameter, also known as \( C_{max} \) represents the maximum plasma drug concentration obtained after extravascular administration of the drug. \( C_{max} \) is a measure of dose intensity and may be related to both the therapeutic and toxic effects. When the absorption and elimination processes are linear (not saturated) and the volume of distribution and \( F \) are unchanged, the \( C_{max} \) is proportional to the dose. This relationship can be used to modify drug concentrations to achieve new therapeutic targets. For example, when a
concentration-dependent antibiotic such as an aminoglycoside is administered to an animal the aim is usually to obtain a certain Cmax to MIC ratio (e.g., Cmax/MIC = 10). If a new bacterial isolate has a higher MIC, the dose may need to be modified to account for this new target. Under the conditions expressed before the dose could just be multiplied by the ratio of MIC's, unless toxicity is a limiting factor. In the case of aminoglycosides, this adjustment may be followed by an increase in the interdose interval in order to decrease the likelihood of toxicity (in such case the total daily dose might remain the same).

Volume of distribution: This parameter provides an indication of "how far" the drug travels inside of the patient body. For lipophilic drugs that easily cross membranes, this parameter is usually high (close to 1 L/kg or higher), indicating that the drug reaches concentrations (which may be therapeutic) in many tissues. The rate at which a drug distributes extravascularly can be limited either by perfusion (e.g., lipophilic drugs) or by diffusion (e.g., polar drugs). There are several types of volumes of distribution. For a drug exhibiting a biexponential concentration-time profile, the volume of distribution "area" can be calculated using the following equation:

$$V_a = \frac{Dose}{AUC \times \beta}$$

where $\beta$ is the slope of the elimination phase of the semi logarithmic concentration-time profile after IV bolus administration. The volume of distribution "area" is a proportionality factor between the plasma concentration and the total amount of drug in the body during the terminal phase according to the following equation:

$$Dose = C_t \times V_a$$

here $C_t$ is a plasma concentration within the therapeutic range. This expression can be used to calculate the dose required to achieve a desired plasma concentration. The volume of distribution "at steady state" is useful to calculate the loading dose. This may be necessary in the case of drugs with long half-lives in order to quickly obtain steady-state concentrations.
**Half-life:** This is a hybrid parameter that depends on both volume of distribution and clearance. It is a good indicator of how quickly a drug is excreted from the body. It has also the advantage of being a rather familiar term. It is the time necessary for a plasma concentration to decrease by half after a pseudo-distribution equilibrium is achieved. This is not necessarily the time required for the amount of drug in the body to decrease by half. Half-life is calculated as:

$$T_{1/2} = \frac{0.693 \times V_a}{Cl}$$

Half-life increases when the volume of distribution increases or when the clearance decreases. Although the latter case is more common in practice, an increase in half-life cannot be automatically considered a reflection of a decrease in clearance. Therefore, dose adaptation should be based in clearance and not on half-life. This parameter is useful in the case of multiple administrations to predict drug accumulation, time to steady-state, and dosage interval. It takes 5 half-lives to reach 97% of the concentration at steady-state.

Pharmacokinetics equations can be obtained from the literature; see, e.g., PK Solutions 2.0 User Guide by Farrier, David, Summit Research Services, 68911 Open Field Drive, Montrose, CO 68911 USA, www.SummitPK.com. General purpose modeling and simulation programs such as BOOMER and MULTIFORTE that can be used to calculate the pharmacokinetic model of the present are known to the person skilled in the art and can be obtained, for example, from David Bourne, OUHSC College of Pharmacy, 1110 N. Stonewall Ave, Oklahoma City, OK 73117, U.S.A.; see also http://www.boomer.org.

In a particularly preferred embodiment of the method of the present invention, said pharmacokinetic model is based on a certain subpopulation of subjects. As mentioned before, one of the drawbacks of the current ways to obtain information on drug therapy through clinical trials is that the selected patients represent a genotypic mixed population, which does not reflect different ethnic categories, in particular Arabs, Japanese, Chinese and Indians. Likewise, metabolic phenotypes underrepresented in patients selected for clinical trials are not considered for later prescription of the drug. However, due to the mixed genotypes and thus correspondent phenotypes even the average dosage determined may not be optimal for the majority of patients because of the heterogeneity of the patient population with respect to their
genetic determinants of the metabolic factors influencing the metabolism and/or action of the drug. The pharmacogenetic model of the present invention takes this into account in that the groups of subjects, which are used for the pharmalogical exposure are substantially homogeneous with respect to their genetic background. In this respect, it is also preferred to select the subjects from one and the same ethnic subpopulation. This measure will further contribute to the homogeneity of the group of subjects and thus provide more reliable results. Preferably, said subpopulations are comprised of Arabs, Chinese, Japanese, Koreans, or Indians. In particular for those populations specific medication is required, since their proportion of PMs, IMs, EMs, and UMs differs for at least some metabolic factors.

Therefore, incidences of side effects or non-respondiveness for the average doses of a given drug evaluated for Western Europeans or US people are observed quite often. The method of the present invention overcomes this problem. Therefore, incidences of side-effects and non-response are observed quite often in these populations if they are prescribed the average dose of a given drug that was clinically tested in Caucasians.

A summary on genetic contribution to variable human CYP3A-mediated metabolism in different populations including Chinese, Japanese, etc. is given in Lamba et al., Adv. Drug Deliv. Rev. 54 (2002), 1271-1294. Specific examples for polymorphisms of metabolic factors such as enzymes of the cytochrome P450 system, receptors, etc. can be found in the literature. For example, frequencies of important allelic variants of two drug targets, dipeptidyl carboxypeptidase (DCP1) and cholesteryl ester transfer protein (CETP), and two other drug receptors, beta-2 adrenergic receptor (ADRB2) and 5-hydroxy tryptamine 2A receptor (HTR2A), in the Egyptian population are described in Hamdy et al., Eur. J. Clin. Pharmacol. 58 (2002), 29-36. The frequency of 5,10-methylenetetrahydrofolate reductase C677T polymorphism associated with a common haplotype in whites, Japanese, and Africans is described in Rosenberg et al., Ann. J. Hum. Genet. 70 (2002), 758-762. The frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population is reported in Balram et al., Br. J. Clin. Pharmacol. 56 (2003), 78-83. Genetic polymorphisms of CYP2D6 in Chinese mainland is summarized in Ji et al., Chin. Med. J. 115 (2002), 1780-1784. A high-throughput genotyping method for detecting SNPs in the human drug-metabolizing enzymes and drug targets CYP2A6, CYP2B6, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A5, NAT2, TPMT, DPYD, UGT1A1, ALDH2, ADH2, MDR1, CETP, DCP-1, ADRB2, HTR2A, INPP1, SDF1, and mitochondrial DNA polymorphisms, in the Japanese

The following considerations of active metabolites for genotype-based dose-recommendations may be made. Drugs including antidepressants undergo multiple biotransformations in the liver, producing progressively more polar metabolites which can be eliminated more readily by the liver or the kidneys. If the antidepressant activity of a metabolite is comparable to that of the parent drug (usually demonstrated only in vitro) and if the metabolite exists in considerable concentrations in plasma, it may be considered as a principle metabolite (Sanchez and Hyytel, Cell. Mol. Neurobiol. 19 (1999), 467-489). Such metabolites are preferably taken into account in the dose recommendations by adding their plasma concentrations to those of the parent drug. Active metabolites which are formed in minor quantities, have short half-lives, or are substantially less active than the parent drug may not be considered. Besides their intrinsic potency, the clinical effects of metabolites depend on their volume of distribution and rate of diffusion into the central nervous system (CNS). The lipid solubility of most metabolites is decreased compared to the parent drug which may result in a smaller volume of distribution and a reduced ability to pass the blood–brain barrier.

The dose recommendations are preferably based on pharmacokinetic parameters which are generally linear and thus proportional to dose such as area under the curve (AUC), total clearance (Cl) and steady-state trough levels (Css), because this method of dose adjustment may be sufficiently accurate in most cases. For drugs with non-linear pharmacokinetics, such as clomipramine, desipramine, imipramine, moclobemide, trimipramine, paroxetine, fluvoxamine and possibly fluoxetine, the genotype-based dose adjustments are in theory specific for the doses tested in the studies.

Units of measure usually have to be unified to μmol, L and h, and clearances have been unified to L x h⁻¹ x kg⁻¹. Average dose recommendations (Dₘₑₓ) provided in manufacturers’ information may be considered as the pragmatic results of large-scale studies performed within genetically mixed populations; see the published literature and the summary provided in Kirchheiner et al., Acta Psychiatr. Scand. 104 (2001), 173–192.
When the differences in pharmacokinetic data between UMs and EMs, or IMs and EMs, were statistically significant, dose recommendations were deduced by calculating the ratios of AUC or Css between UMs and EMs and, if available, between IMs and EMs. If several pharmaceutical exposures are available for one drug, the AUCs or Css are preferably summarized by calculating the weighted mean from the various studies.

The metabolic quotient refers to a measure or function of the exposure to a drug in an individual patient (includes, but is not limited to, area under the curve, clearance, and distribution volume) divided by the integer for the metabolic phenotype, i.e. for PM, IM, EM, and UM. Thus, once the MQ is known for at least one drug, the effectiveness of the at least one drug is known and the dosage regimen for said drug can be optimized for the respective metabolic phenotype. Preferably, said metabolic quotient is normalized against other factors that may influence the result. The MQ can be made statistically more significant if said pharmacokinetic model is a measured pharmacokinetic model of a subpopulation of subjects.

In this model, at least one error selected from intra-individual, inter-individual, and residual error is preferably minimized. In a particularly preferred embodiment, said metabolic quotient is calculated as done in the examples by the formula:

\[ D_{Av} = (xD_{PM} + yD_{IM} + zD_{EM} + uD_{UM}) \]  

(1)

To calculate the dose for each genotype, one proceeds as follows:

\[ D_{PM} = D_{EM} \cdot AUC_{EM} \]

(2)

with \[ n = AUC_{EM}/AUC_{PM} \]

\[ D_{IM} = AUC_{EM}/AUC_{IM} \cdot D_{EM} \]

(3)

with \[ m = AUC_{EM}/AUC_{IM} \]

\[ D_{UM} = AUC_{EM}/AUC_{UM} \cdot D_{EM} \]

(4)

with \[ o = AUC_{EM}/AUC_{UM} \]

By substituting equation (2), (3) and (4) into equation (1), these relations can be transformed to percent adjustment compared with the average dose \( D_{Av} (100\%) \) for the EMs:
\[ D_{EM}(\%) = \frac{100}{(x \ast n + y \ast m + z \ast u + u \ast o)} \]  

(5)

And

\[ D_{PM}(\%) = n \cdot D_{EM} \]  

(6)

And

\[ D_{IM}(\%) = m \cdot D_{EM} \]  

(7)

And

\[ D_{UM}(\%) = u \cdot D_{EM} \]  

(8)

wherein

\( D_{AV} \) = Average dose recommendations (\( D_{AV} \)) provided in manufacturers' information may be considered as the pragmatic results of large-scale studies performed within genetically mixed populations;

\( D_{PM}, D_{IM}, D_{EM} \) and \( uD_{UM} \) representing the doses which might have been recommended for the sub-populations poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra-fast metabolizers, respectively;

\( xD_{PM} + yD_{IM} + zD_{EM} + uD_{UM} = % \) values considered as the weighted means from a given population; e.g., with 10% genetically defined PMs, 40% IMs and 50% EMs for CYP2D6 in most Caucasian populations (Sachse et al., Am. J. Hum. Genet. 60 (1997), 284–295) and genotype frequencies for CYP2C19 of 3% PMs, 20% IMs and 77% EMs; Aynacioglu et al., Clin. Pharmacol. Ther. 66 (1999), 185–192.

Dose recommendations are rounded to the nearest number which can be divided by 10.

A similar equation has been used previously by Kirchheiner et al., Acta Psychiatr. Scand. 104 (2001), 173-192, for the CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants based however on published data only and without the notion that data from specific sub-population and/or groups of patients are needed in order to obtain reliable and safe dosage recommendations. Furthermore, it should be clear that instead of AUCs other pharmacokinetic parameters may be used such as total clearance (Cl) and steady-state trough levels (Css).

In one embodiment, a Bayesian model may be used to optimize a population pharmacokinetic model. The concept of Bayesian parameter estimation in the field of therapeutic drug monitoring is known in the art and may be useful in circumstances where drug concentrations are measured during relatively complicated dosage regimens, or where only a few concentration measurements are acceptable. The Bayesian method allows to estimate a
patient's pharmacokinetic parameters, so that therapeutic regimens can be adjusted to achieve specific target concentrations. For this purpose, pre-existing information on population characteristics (means and variances) of pharmacokinetic parameters is used in conjunction with the (limited) concentration-time data of an individual patient. The principle of Bayesian estimation is described, e.g., in Thomson and Whiting, Clin. Pharmacokinet. 22 (1992), 447-467.

In one embodiment, the methods of the present invention further comprise entering said metabolic quotient, and optionally the corresponding genetic determinants in a computer database. The systems and methods of the invention may also, for example, include a database corresponding to the data collected and generated from combined first pharmacokinetic models and/or from combined optimized pharmacokinetic models. This database may include a relational genotype/phenotype database. In a further embodiment, a neural network or computerized platform may also be provided that learns from the patterns in the data collected and generated.

Hence, the systems and methods of the invention may be implemented through any suitable combination of hardware, software and/or firmware. Various system components and analytical tools, such as neural networks or artificial intelligence, can be utilized to further optimize a drug therapy for the treatment of a disease. In addition, consistent with the principles of the invention, a database can be generated through a combination of bioanalytical, population pharmacokinetic, genotypic and phenotypic testing methods to provide individualized therapy regimens that can be administered by physicians and the like.

The invention may be embodied, for example, as a method, a data processing system, a computer program product, a business method, or any combination thereof. Although the invention may be practiced without a computer or software-based platform, using a computer or software-based platform may be desirable, given the complexity of the combination and the volume of data of bioanalytical, population pharmacokinetic, genotypic and phenotypic testing data obtaining methods. Accordingly, the principles of the invention may be implemented as a hardware embodiment, a software embodiment, or any combination thereof, and maybe stored in any computer usable storage medium, i.e., hard disks, CD-ROMs, optical storage devices, magnetic storage devices, etc. In this context, population pharmacokinetic modeling and analysis systems like PDx-POP(TM) described in US patent application
US2003/078760, systems for pharmacogenetics of adverse drug events such as described in US patent application US2003/104453 and computer systems and methods of analyzing an association between patient genotypes and adverse drug phenotypes for providing personalized medical treatment, clinical trial design recommendation, and clinical management as described in international application WO03/039234 can be implemented into the methods of the present invention.

A metabolic profile can be determined by phenotyping (by measuring enzyme activity) or genotyping (by examining the genetic sequence of enzymes). In general, for example, for phenotyping, a probe substrate or drugs, such as those exemplified in the examples are administered to an individual to be phenotyped. A biological samples, such as plasma and/or urine sample is subsequently collected from the individual and analyzed according to a technology, as described herein below, for metabolites corresponding to the probe substrate (s) and the molar ratios of the metabolites calculated to reveal the individual phenotypes.

In general, for example, for genotyping, a blood sample of an individual is obtained, and the genetic sequence of the enzyme(s) is examined for the presence or absence of specific mutations. A specific probe for a known allelic variation may be used to screen for a specific genotype known to effect an individual's specific enzymatic capacity. The combination of mutations on the two alleles is matched to known genotypes. Based on this information, a metabolic profile indicative of an individual's metabolic capacity for a specific metabolic factor is characterized and may be employed in the individualization of therapy as provided by the present invention.

The method of the present invention preferably further includes the use of genotyping to identify individuals having a particular allelic variation known to influence the ability to effectively metabolize a given drug or class of drugs. For example, such an allelic variation may be associated with extremely high risks of toxicity from a given drug treatment regime. According to one embodiment of the present invention, those individuals without the "high risk" genotype may be subsequently phenotyped and an individualized dose determined according at least one phenotypic determinant while the high risk individuals can be readily identified and removed from consideration for the given drug treatment regime. By employing genotyping in combination with phenotyping to screen individuals for treatment
with a given treatment regime, those individuals found to be carriers of a high risk genotype can be eliminated as candidates for such treatment without the necessity of phenotyping.

Alternatively, genotyping may be employed alone, in accordance with an embodiment of the present invention, to characterize a metabolic determinant and to identify an individualized dosage for a given drug treatment regime.

Every individual is genetically predisposed for metabolic function and accordingly, everyone does not metabolize a given drug to the same extent and efficiency. Furthermore, certain enzymes may be inhibited by the metabolism of other agents administered on a coterminous basis, thus effecting an individual's metabolic capacity at any given time.

Due to factors affecting an individual's metabolic capacity with respect to a given enzyme, the phenotypic result will be a decreased clearance or efficiency in the metabolism of certain agents (drugs), and hence the possibility of adverse side effects or non-response. Adverse side effects may be serious, if not life threatening if individuals are treated with an agent they do not respond to because they are not capable of effective metabolism.

For example, certain individuals having a genetic variation resulting in the absence or reduction of CYP2D6 will display an inability to effectively metabolize many drugs such as codeine and tramadol. Clearly, individual variations in CYP2D6 metabolic activity at any given time can have significant impacts on the rates of clearance of CYP2D6-specific drugs and hence the efficacy thereof. For these reasons, the utility of a reliable screening test specific to a metabolic factor such as an enzyme(s) and/or metabolic pathway(s) involved in the metabolism of a given drug or class of drugs, such as CYP2D6 and/or other enzymes, is evident. According to the present invention, metabolic determinants for one or more of the following enzymes may be characterized to provide a metabolic profile on an individual basis:

PCR protocols for determination of CYP2D6 alleles as well corresponding information about polymorphism, substrates, induction and inhibition, interethnic differences, direct phenotyping, genotypic determinants, PCR primers for other metabolic factor such as CYP3A, NAT1, NAT2, CYP1A2, CYP2A6, CYP2C19, CYP2C9, CYP2E1, as well as the
method for the characterization of multiple phenotypic determinants are described in US2003/0091975, the disclosure content of which is incorporated herein by reference. Furthermore, the present invention may also be adapted to provide for the identification of other characteristics or determinants of drug clearance and drug toxicity known to vary on an individual and ethnic basis.

As described before, said step of determining a metabolic profile includes phenotyping and/or genotyping that can be performed by a genormic assay. In accordance with a preferred embodiment of the present invention said genetic determinants comprise at least one allele of CYPD6.

The drug-metabolizing enzyme CYP2D6 metabolizes more than 20% of all prescribed or over the counter drugs. The gene for CYP2D6 maps to chromosome 22q13.1. There exist different gene variants of CYP2D6, which result in defective, qualitatively altered, diminished or enhanced activity (Sachse, Am. J. Hum. Genet., 60 (1997), 284-295). The gene variants comprise different combinations of haplotypes which were found to be associated with differences in the CYP2D6 metabolic activity. A haplotype is composed of various polymorphisms, which are linked on one chromosome. Hence, the CYP2D6 gene is extensively polymorphic with one study identifying 48 mutations and 53 alleles; see also http://www.imm.ki.se/CYPalleles/ for review. An example of a procedure for genotyping CYP2D6 involves the amplification of the entire CYP2D6 coding region (5.1 kb product) by XL-PCR using specific primers. This product is then used for a series of polymerase chain reaction-restriction fragment length polymorphism reactions designed to detect nucleotide point mutations, deletions and insertions compared with the functional CYP2D6*1 allele, e.g. the C188T transition mutation (Garcia-Barcelo et al. 46 (2000) Clinical Chemistry, 18-23).

Examples of alleles with normal (extensive, wild-type function) CYP2D6*1A, CYP2D6*1B, CYP2D6*1C CYP2D6*2A,; CYP2D6*33, CYP2D6*35 alleles resulting in an absence of function CYP2D6*3A, CYP2D6*4A, CYP2D6*4B, CYP2D6*4C, CYP2D6*4D, CYP2D6*4K, CYP2D6*5, CYP2D6*6A, CYP2D6*6B, CYP2D6*6C, CYP2D6*7, CYP2D6*8, CYP2D6*11 & CYP2D6*12; CYP2D6*13, CYP2D6*14A, CYP2D6*15, CYP2D6*16, CYP2D6*19, CYP2D6*20, CYP2D6*21A, CYP2D6*21B, CYP2D6*38 CYP2D6*40, CYP2D6*42, CYP2D6*44 and alleles resulting in a reduced function, CYP2D6*9, CYP2D6*10A, CYP2D6*10B, CYP2D6*17, CYP2D6*36, CYP2D6*41. The
ultra-extensive phenotype appears to arise from the presence of multiple copies of the wild-type CYP2D6 gene (for example, one individual was identified with 13 copies of the gene).

In general, the most frequent mutations are examined and these correspond to the most frequent alleles and genotypes. Those individuals with at least three wild type alleles are identified as ultrafast metabolizers, those with one allele encoding a functional enzyme are identified as extensive metabolizers, while individuals lacking two or more functional CYP2D6 alleles are identified as poor metabolizers. Thus, the gene variants for CYP2D6 result in the poor metabolizer phenotype (PM), the intermediate phenotype (IM), the extensive phenotype (EM) and the ultrafast metabolizer phenotype (UM).

A phenotype means a physical or behavioral trait of an organism. About 5%-10% of Caucasians, who share the so-called poor metabolizers (PMs) phenotype completely lack CYP2D6 activity. This phenotype is inherited as an autosomal-recessive trait. Approximately 2% of Caucasians are so-called ultrarapid metabolizers with more than two active genes due to a duplication or even an amplification of one of the CYP2D6 gene. Johansson et al. found that the CYP2D6 gene was amplified up to 12 fold in a family (Johansson et al., Proc. Natl. Acad. Sci. USA 90 (1993), 11825-11829). In addition, subjects with multiplicated CYP2D6 genes present in 3, 4 or 5 copies on 1 allele have been seen (Dahl et al., J. Pharm. Exp. Therapy 274 (1995), 516-520; Akullu et al., J. Pharm. Exp. Therapy 278 (1996), 441-446).

The percentage of poor, intermediate and ultrarapid metabolizers varies between different populations (Ingelman-Sundberg, Trends Pharmacol. Sci. 20 (1999), 342-349). The poor-hydroxylator phenotype varies among ethnic groups. It can be found in about 9% in the British population, about 1% in Arabs and about 20% in Hong Kong Chinese (Kalow, Canad. J. Physiol. Pharm. 60 (1982), 1-12, Zhou et al., New Eng. J. Med. 320 (1989), 565-570). The occurrence of CYP2D6 gene duplications vary between populations as individuals carrying extra genes as seen in Ethiopia at a frequency of 29% (Akullu et al., J. Pharm. Exp. Therapy 278 (1996), 441-446). In Saudi Arabia 21% of the studied probands showed a gene duplication of the CYP2D6 gene (McLellan et al., Pharmacogenetics 7 (1977), 187-191). In contrast a frequency of 1-2% was observed in Swedish, German, Chinese and black Zimbabwean populations.

It has been shown that there exists an inverse correlation between CYP2D6 activity and the bioavailability of oral spartein, which is metabolized by CYP2D6. PMs had higher spartein Cmax and AUC levels and showed longer half-lives (Eichelbaum et al., Eur. J. Clin.
Pharmacol. 16 (1979), 183-187). The polymorphic oxidation of spartein and debrisoquine
(Mahgoub et al., Lancet (1977), 584-586) is so far the best studied variations of drug
metabolism by CYP2D6. Baumann et al. (Baumann et al., Pharmacopsychiatry 31 (1998), 72)
reported a patient with depression in whom higher doses of conventional antidepressants were
required. Furthermore, Kawanishi et al. (Kawanishi et al., Clin. Genet. 61 (2002), 152-154)
described a patient who was resistant to drug treatment of schizophrenia. All these patients
including those described by Bertilsson et al. (Bertilsson et al., Ther. Drug Monit. 7 (1985),
478-480) had a duplication of the CYP2D6 gene. More than 50 drugs are affected by these
CYP2D6 polymorphisms including antiarrhythmics (e.g. propafenone and flecainide)
antidepressants (e.g. nortriptiline and clomipramine), neuroleptics (perphenazine and
thioridazine), antianginals (perhexilene) and opioids (codeine and dextromethorphan) see
Table 1.
Table: Examples of drugs metabolized by CYP2D6.

<table>
<thead>
<tr>
<th>Beta-Blockers</th>
<th>Antiarrhythmics</th>
<th>Antidepressants</th>
<th>Antipsychotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprenolol</td>
<td>Ajmaline</td>
<td>Amitriptyline</td>
<td>Carvedilol</td>
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<tr>
<td>Bufluralol</td>
<td>Aprindine</td>
<td>Amitriptyline</td>
<td>Haloperidol</td>
</tr>
<tr>
<td>Bunitrolol</td>
<td>Encaïnide</td>
<td>Chlorpromazine</td>
<td>Perphenazine</td>
</tr>
<tr>
<td>Buprananol</td>
<td>Flecaïnide</td>
<td>Clozapine</td>
<td>Risperidone</td>
</tr>
<tr>
<td>Debrisoquine</td>
<td>Mexiletine</td>
<td>Chlorpromazine</td>
<td>Thioridazine</td>
</tr>
<tr>
<td>Indoramin</td>
<td>Propafenone</td>
<td>Desipramine</td>
<td>Zuclopenthixol</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>N-Propylamaline</td>
<td>Doxepin</td>
<td>Venlafaxine</td>
</tr>
<tr>
<td>Propanolol</td>
<td>Sparteine</td>
<td>Fluoxetine</td>
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<tr>
<td>Timolol</td>
<td></td>
<td>Imipramine</td>
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<tr>
<td>Verapamil</td>
<td></td>
<td>Maprotiline</td>
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<td></td>
<td></td>
<td>Methoxyphenamine,</td>
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<td></td>
<td></td>
<td>Minaprine</td>
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<tr>
<td></td>
<td></td>
<td>Nortriptyline</td>
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<td></td>
<td></td>
<td>Paraoxetine</td>
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<td>Perazinê</td>
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<td>Perphenazine,</td>
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<td></td>
<td></td>
<td>Remoxipride,</td>
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<tr>
<td></td>
<td></td>
<td>Trimipramine</td>
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<tr>
<td></td>
<td></td>
<td>Methylenedioxy-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methamphetamine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analgesics</th>
<th>Antitussives</th>
<th>HT3-Antagonists</th>
<th>Addictive Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Dextromethorphan</td>
<td>Tropisetron</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>Codeine</td>
<td>Ethylmorphine</td>
<td>Ondansetron</td>
<td>Methylendioxy-</td>
</tr>
<tr>
<td>Dihydrocodeine</td>
<td>Hydrocodone</td>
<td></td>
<td>Methamphetamine</td>
</tr>
<tr>
<td>Fentanyl</td>
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<tr>
<td>Lidocaine</td>
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<tr>
<td>Norcodeine</td>
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<tr>
<td>Methadone</td>
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<td></td>
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<tr>
<td>Oxycodone</td>
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<tr>
<td>Phenacetin</td>
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<tr>
<td>Tramadol</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cancer Drugs</th>
<th>Antihistamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>Chlorpheniramine,</td>
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<tr>
<td></td>
<td>mequitazine,</td>
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<tr>
<td></td>
<td>promethazine,</td>
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<td></td>
<td>cinnarazine,</td>
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<tr>
<td></td>
<td>flunarizine,</td>
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<tr>
<td></td>
<td>loratadine,</td>
</tr>
<tr>
<td></td>
<td>terfenadine</td>
</tr>
</tbody>
</table>

5 CYP2D6 is inhibited in vitro by quinidine and by viral protease inhibitors as well as by appetite suppressant drugs such as D-and L-fenfluramine. This fact has to be accounted for if a patient is a PM. If the dosage of a drug that is metabolized by CYP2D6 is increased for UMs then the influence of drug-drug interactions has to be accounted for. The dosage has to be reduced if a patient takes at the same time drugs which inhibit the enzyme CYP2D6.

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An example of the need for the individualization of therapy and/or drug dosing is the case of dextromethorphan. According to this example, CYP2D6 is identified as a metabolic factor known to influence the metabolism of dextromethorphan. Dextromethorphan is a nonopioid antitussive with psychotropic effects. However, dextromethorphan doses range from 0 to 6 mg/kg based on individual subject tolerance. Dextromethorphan is activated via the CYP2D6 metabolizing system. Dextromethorphan produces qualitatively and quantitatively different objective and subjective effects in poor vs. extensive metabolizers (mean performance +/-SE, 95+/-0.5% for EMs vs. 86+/-6% for PMs; p < 0.05).

Another important drug that exemplifies the need for the individualization of therapy and/or drug dosing where CYP2D6 is a suitable metabolic factor is in the case of tricyclic antidepressants. Both the PM and UM phenotypes of CYP2D6 are at risk of adverse reactions.

PM individuals given standard doses of these drugs will develop toxic plasma concentrations, potentially leading to unpleasant side effects including dry mouth, hypotension, sedation, tremor, and in some cases life threatening cardiotoxicity. Conversely, administration of these drugs to UM individuals may result in therapeutic failure because plasma concentrations of active drugs at standard doses are far too low. For these reasons, the utility of a reliable methodology for the individualization of therapy and/or drug dosing is evident.

Different probe substrates can be used to determine the CYP2D6 phenotype (dextromethorphan, debrisoquine, bufuralol, antipyrine, theophylline and hexobarbital). In accordance with the present invention, suitable probe substrates include without limitation, dextromethorphan, debrisoquine, bufuralol, spartein, etc. In a preferred embodiment of the present invention, trimipramine or metoprolol and its metabolite is used to determine the CYP2D6 phenotype of the individual.

It has been well established that people who suffer from this deficiency of drug metabolism often experience exaggerated pharmacological or toxic responses when they are treated with usual drug dosages. The problems of ultrafast metabolizers, however, have been neglected since drugs at their standard dosage normally do not cause drug induced adverse reactions in these patients and only 1-3% percent in the Caucasian population are Ultrafast Metabolizers. Since drugs normally work in less than 50% of all patients (Kalow, Meyer and Tyndale
(Eds.), Pharmacogenetics, New York, Marcel Dekker (2001), 17) the low frequency of UMs does not influence the overall efficiency of a drug metabolized by CYP2D6. However, in Arabs, especially in Arabs on the Arabian Peninsula and Ethiopia, the frequency of the ultrafast metabolizer phenotype is very high and therefore these people are treated with drug dosages which are too low to reach the minimum effective concentration in patients. As a result, drug therapies fail in these patients.

In spite of the fact that the percentage of poor metabolizer and ultrafast metabolizer phenotype for drugs metabolized by CYP2D6 greatly vary among different ethnic groups, the Department of Health and Human Services, the FDA, in their draft guidance Docket No. 02D-0018 for industry on the collection of race and ethnicity data in clinical trials for FDA regulated products divided the human race only in five categories for data on race:

1. American Indian or Alaska Native
2. Asian
3. Black or African American
4. Native Hawaiian or Other Pacific Islander
5. White.

There are two categories for data on ethnicity: Hispanic or Latino and Not Hispanic or Latino.

The FDA does not capture ethnic categories that are as important and differ from each other in respect of their drug metabolisms such as Arabs, Japanese, Chinese etc.. The FDA also ignores the fact that both risk for disease and desirable and undesirable drug responses are variable across the human species, and that the variability depends on both genetic and environmental factors, many of which differ between ethnic populations (Pharmacogenetics and Pharmacogenomics in Drug Development and Regulatory Decision Making: Report of the First FDA-PWG-PhRMA-DruSafe Workshop J. Clin. Pharmacol. 43 (2003), 342-358). The FDA also gives no dose recommendations for drugs for ultrafast, intermediate and poor metabolizers, although more than 20% of Arabs are ultrafast metabolizers for CYP2D6, 14 to 20% of Chinese are poor metabolizers for CYP2C19, 9% of Caucasians have a reduced activity in their FMO3 enzyme and more than 80% of Arabs are slow acetylators.

In accordance with the present invention, the mode and population distribution of genetic variations in the CYP2D6 gene - the different alleles of the CYP2D6 gene - have been analyzed by sequence analysis of relevant regions of the human said gene from many
different individuals. It is a well known fact that genomic DNA of individuals, which harbor the individual genetic makeup of all genes, including the CYP2D6 gene, can easily be purified from individual blood samples. These individual DNA samples are then used for the analysis of the sequence composition of the alleles of the CYP2D6 gene that are present in the individual which provided the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of said genes, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (e.g. ABI dye terminator cycle sequencing). Genotyping for CYP2D6 alleles *3, *4, *5 and *6 and gene duplication was performed after the methods described by Sachse (Sachse et al., Am. J. Hum. Genet. 60 (1997), 284-295).

One important parameter that has to be considered in the attempt to determine the individual genotypes and identify novel variants of the CYP2D6 gene by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care has to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations.

Over the past 20 years, genetic heterogeneity has been increasingly recognized as a significant source of variation in drug response. Many scientific communications (Meyer, Ann. Rev. Pharmacol. Toxicol. 37 (1997), 269-296 and West, J. Clin. Pharmacol. 37 (1997), 635-648) have clearly shown that some drugs work better in some patients than in others or may even be highly toxic and that such variations in patients’ responses to drugs can be correlated to a molecular basis. This “pharmacogenomic” concept spots correlations between responses to drugs and genetic profiles of patient’s (Marshall, Nature Biotechnology, 15 (1997), 954-957; Marshall, Nature Biotechnology, 15 (1997), 1249-1252). In this context of population variability with regard to drug therapy, pharmacogenomics has been proposed as a tool useful in the identification and selection of patients which can respond to a particular drug without side effects or non-response. This identification/selection can be based upon molecular diagnosis of gene duplications and genetic polymorphisms by genotyping DNA from leukocytes in the blood of a patient, for example, and characterization of disease (Bertz, Clin. Pharmacokinet. 32 (1997), 210-256; Engel, J. Chromatogra. B. Biomed. Appl. 678 (1996),
93-103). In a second step the patients are given drugs whose dosage is specifically tailored to the specific genetic profile of a patient.

In a preferred embodiment of the method of the present invention said allele is selected from a group consisting of the allele CYP2D6*1, CYP2D6*2, CYP2D6*9 or CYP2D6*10 resulting in the extensive (EM) or ultrafast metabolizer (UM) phenotype if present in more than one copy per chromosome. In another preferred embodiment, said allele is selected from a group consisting of CYP2D6*3, CYP2D6*4, CYP2D6*5, CYP2D6*6, CYP2D6*7, CYP2D6*8, CYP2D6*11, CYP2D6*12 or CYP2D6*15, CYP2D6*16, CYP2D6*19, CYP2D6*20, CYP2D6*36, CYP2D6*38, CYP2D6*40, CYP2D6*42 and CYP2D6*43 alleles resulting in the intermediate (IM) or poor metabolizer (PM) phenotype.

Advantageously, preventive or therapeutically measures which are based on the genotype can be more efficiently applied when taking into consideration said genetic knowledge. Non-effectiveness and undesirable side effects of drugs can be avoided and an effective as well as safe dosage can be determined for the different types of metabolizers due the knowledge of the genetic makeup of the subject. Moreover in accordance with the foregoing, in cases where a given drug causes an unwanted effect, a suitable individual therapy can be designed based on the knowledge of the individual genetic makeup of a subject. The use of the present pharmacokinetic model of the invention, therefore, provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to individually treat the subject with an appropriate dosage and/or an appropriate derivative of said substances. Furthermore, the use of the present invention provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to identify those subjects prior to the onset of the drug therapy and treat only those subjects with an appropriate dosage and/or an appropriate derivative of said substances who are most likely to benefit from therapy with said substances. Thereby, the non-effective treatment due to three active CYP2D6 genes and potentially harmful treatment of those subjects who are poor metabolizers of CYP2D6 can be avoided.

Dependent on the alleles specified above the individuals can be subgrouped into a non-responder group (UMs) and a responder group (EMs) or partial responder group (IMs). According to the present invention, the subjects carrying two deletion polynucleotides
(del/del) are subgrouped as non-responders and subjects carrying at least one insertion polynucleotide as responder or partial responder. Patients carrying gene duplications are subgrouped as ultrafast metabolizers (UMs).

Methods for the determination of an altered expression of a variant allele when compared to its wild type counterpart are well known in the art and comprise inter alia those referred to herein above, e.g. Northern blot analysis, Reverse transcriptase PCR based techniques (RT-PCT), Ribonuclease protection assays, and Real Time PCR (TaqMan) assays. It might be necessary to obtain a sample comprising biological material, such as isolated cells or tissue from the subject prior to perform said methods for determination of the expression levels of the wild type and the variant alleles, respectively. An altered expression in accordance with the use of the present invention means that the expression of the wild type allele differs significantly from the expression of the variant allele.

In a more preferred embodiment of the use of the invention said altered expression is decreased or increased expression. To determine whether the expression of an allele referred to in accordance to the present invention is increased or decreased in comparison to the corresponding wild type allele well known methods such as Northern blot analysis, Reverse transcriptase PCR based techniques (RT-PCT), Ribonuclease protection assays, and Real Time PCR (TaqMan) assays can be applied. As discussed above, it might be necessary to obtain a sample comprising cells or tissue from the subject in order to determine the expression level of the variant allele referred to in the use of the invention. A decrease or increase of the expression is characterized by a significant difference in the expression level of the variant versus the wild type allele in those assays. Also encompassed by decreased expression is the absence of detectable expression of a variant allele.

In a furthermore preferred embodiment of the use of the present invention a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered activity of the polypeptide encoded by the first variant allele compared to the polypeptide encoded by the corresponding wild type allele.

As discussed supra, the variant alleles comprising those polynucleotides specified herein which correspond to coding regions of the CYP2D6 gene effect the amino acid sequences of

Frequently, said altered activity is decreased or increased activity. As discussed for the increase or decrease of expression, a decrease or increase of the activities is characterized by a significant difference in the activity of the variant versus the wild type polypeptide in the assays referred to herein. Also encompassed by decreased activity is the absence of detectable activity of a variant allele.

In particular, the present invention is applicable to humans as is evident from the above. The use of the present invention is to be applied in order to treat patients who suffer from medium to severe depression and hypertension with the drug dosage that is tailored to the genetic makeup of the enzyme(s) that metabolize a specific drug. Therefore the optimal dosages for ultrafast metabolizers (UMs) for trimipramine and propaphenone have been determined by kinetic studies in patients. The pharmacological effects of the above substances or derivatives
thereof are well described in humans. However, the conventional therapies do not take into account the individual genetic makeup of the patient. Here ultrafast metabolizers for CYP2D6 have been selected as examples for this business model since in the Arab population 10-20% of the patient are treated with suboptimal doses due to a gene duplication.

In light of the foregoing, most carefully, said human is selected from the group of Caucasians or preferentially from the Arab population. From the group of Caucasians only 1-3% share the CYP2D6 ultrafast metabolizer phenotype whereas >20% of Arabs are ultrafast metabolizers for CYP2D6 and are therefore more likely to suffer from therapeutic failure. The ultrafast metabolizers for CYP2D6 need to be treated with higher drug doses. On the other hand, patients who share haplotypes which cause a reduced activity of CYP2D6 gene and which affects 7% of Europeans and >20% of Asians are more likely to suffer from drug induced side effects. Therefore, these people need drugs with a lower drug dosage.

In one preferred embodiment said genetic determinants comprise at least one allele of CYP2D6 and/or CYP2C19 and said subpopulation is an Asian population; see Goldstein et al. in Pharmacogenetics 7 (1997), 59-64 for frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations and McLellan et al. in Pharmacogenetics 7 (1997), 187-191 for frequency of CYP2D6 gene duplications in Saudi Arabians.

In another preferred embodiment of the method of the present invention said genetic determinants comprise at least one allele of NAT2, FMO3 or TPMT; see also supra.

In a particular preferred embodiment, the pharmacokinetic model of the invention is established for at least two drugs which are metabolized by CYP2D6 and which preferably have been determined in ultrafast metabolizers (UMs) for CYP2D6 who preferably have three copies of a wild type allele of the CYP2D6 gene. Hence, preferably said individual subjects or subpopulation of subjects are ultrafast metabolizers (UMs) of CYP2D6. Furthermore, in accordance with the present example said drugs used to establish the pharmacokinetic model are trimipramine and metoprolol.

In a further embodiment, the method of the present invention further includes:

c) measuring at least one determinant for drug clearance known to affect the toxicity or efficacy of said drug or class of drugs; wherein said at least one determinant for drug
clearance is factored together with at least said metabolic quotient to determine a therapeutically effective amount of said drug or class of drugs to be prescribed to said individual.

Said at least one determinant for drug clearance can be based on at least one of body surface area and hepatic enzyme levels of said individual.

Said at least one, preferably two drug(s) is/are preferably selected from the group consisting of but not limited to Alprenolol, Bufuralol, Buntritolol, Bupranalol, Metoprolol, Propanolol, Timolol, Aprindine, Encainide, Flecainide, Mexiletine, Propafenone, N-Propylajmaline, Sparteine, Amitriptyline, Clomipramine, Clopromazine, Desipramine, Doxepin, Fluoxetine, Imipramine, Maprotiline, Mianserine, Minaprine, Nortriptyline, Trimipramine, Paroxetine, Haloperidol, Perphenazine, Risperidone, Thoridazine, Zuclopenthizoxol, Carvedilol, Venlafaxine, Codeine, Dihydrocodeine, Oxycodone, Tramadol, Dextromethorphan, Ethylmorphine, Hydrocodone, Tropisetron, Ondansetron, Amphetamine, or Methylendioxy-methamphetamine.

Once the pharmacokinetic model has been established for a given drug or class of drugs said individualized dosage is calculated from the usually applied dose that is recommended for in-patients without taking genetic polymorphisms into consideration, as taken from manufacturers’ recommendations factored against the metabolic quotient.

The methods of the invention may also provide, for example, the optimization of therapy for a disease such as cancer. The invention also provides a method of designing a therapy for a patient, and a method of prescribing a therapy for a patient, including making recommendations for drugs and/or combinations of drugs not yet prescribed for that patient. Accordingly, in a further embodiment the present invention relates to a method for the preparation of an medicament individual subject or subpopulation of subjects comprising reformulation of the drug or pro-drug, wherein said individualized dosage is calculated from the dose usually applied and recommended for in-patients, without taking gene polymorphisms into consideration, as taken from manufacturers’ recommendations factored against the metabolic quotient determined in accordance with the pharmacokinetic model of the present invention.
The appropriate concentration of the therapeutic agent might be dependent on the particular agent. The therapeutically effective dose has to be compared with the toxic concentrations; the clearance rate as well as the metabolic products play a role, as do the solubility and the formulation. Therapeutic efficacy and toxicity of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

The term "pharmaceutical composition" as used herein comprises the substances of the present invention and optionally one or more pharmaceutically acceptable carriers. The substances of the present invention may be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The pharmaceutical compositions can be conveniently administered by any of the routes conventionally used for drug administration, for instance, orally, topically, or by inhalation. The substances may be administered only after the genotype of the patient has been determined. Depending on a patient's genotype three different dosage forms which are suited to treat patients who have three (ultrafast metabolizers), one (intermediate metabolizers) or no wild type gene (poor metabolizers) are given the patient. These dosage forms are prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluents is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluents may include time delay material well known to the art, such as glycercyl mono-stearate or glycercyl distearate alone or with a wax. The substance according to the present invention can be administered in various manners to achieve the desired effect. Said substance can be
administered either alone or in the formulated as pharmaceutical preparations to the subject being treated either orally, topically or by inhalation. Moreover, the substance can be administered in combination with other substances either in a common pharmaceutical composition or as separated pharmaceutical compositions.

The diluents are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to that amount of the substance according to the invention, which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's weight, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

A typical dose can be, for example, in the standard dose (Imipramin 150 mg) range of 37% (for PMs), 91% (for IMs) and 178% (for UM). The standard dose for Imipramine, Doxepin, Trimipramin, Maprotilin, Desipramin, Nortriptylin, Clomipramin, Amitriptylin is 150 mg.; 60 mg for Mianserin, 20 mg for Paroxetine; and 150 mg for Venlafaxin. The determined optimal dosage in % of the standard dose for PMs, IMs, EMs and UM is given in figure 4. The standard dose for the antihypertensive drug metoprolol is 100mg, wherein the optimal dosage for UM has been determined as about 200% of the standard dose; see example 3. Hence, in a particularly preferred embodiment, the individualized dose for UM CYP2D6 genotypes in % of the recommended average dose is 182% for Imipramin, 173% for Doxepin, 173% for Maprotilin, 178% for Trimipramin, 167% for Desipramin, 152% for Nortriptylin, 146% for
Clomipramin, 130% for Amitriptylin, 130% for Venlafaxin, 134% for Mianserin, 138% for Paroxetin, and 190% for Metoprolol.

The pharmaceutical compositions and formulations referred to herein are administered at least once in accordance with the use of the present invention. However, the said pharmaceutical compositions and formulations may be administered more than one time, for example from one to four times daily up to a non-limited number of days.

Specific formulations of the substance according to the invention are prepared in a manner well known in the pharmaceutical art and usually comprise at least one active substance referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent thereof. For making those formulations the active substance(s) will usually be mixed with a carrier or diluted by a diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. A carrier may be solid, semisolid, gel-based or liquid material which serves as a vehicle, recipient or medium for the active ingredients. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington’s Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The formulations can be adopted to the mode of administration comprising the forms of tablets, capsules, suppositories, solutions, suspensions or the like.

The dosing recommendations will depend on the genotype of the patient and will be indicated in the product labeling. This will allow the doctor to select the drug formulation that contains the drug concentration which is suited to the specific genotype of a patient. It is a prerequisite that the gene for the drug metabolizing enzyme is first analyzed before the specific drug formulation is prescribed. This will avoid that the wrong drug is prescribing the wrong patients at the wrong dose.

The present invention also encompasses all embodiments described in connection with pharmaceutical compositions in US patents: 4,695,578; 4,753,789; 5,578,628; 5,955,488; 6,063,802; 4,886,808; 6,294,548; 4,906,755.

The present invention also relates to the medicament obtainable by the method of the invention, optionally further comprising prescription of the drug for PM, IM, EM and/or UM. In this respect, the present invention also relates to a method for selecting a suitable dosage
regimen or formulation of drug or class of drugs known for treating a condition wherein said method comprises:

(a) determining the presence or absence of at least one genetic determinant as defined in above in a sample obtained from said subject; and

(b) selecting a suitable therapy for said subject based on the pharmacokinetic model of the present invention.

In accordance with the results obtained for the CYP2D6 ultra-fast metabolizers, the medicament manufactured and/or repackaged according to the method of the present invention preferably comprises a multiple (M) (single (S)) dose selected from the group consisting of 250 to 275 mg (80 to 90 mg) Imipramin, 250 to 275 mg Doxepin, 250 to 275 mg Maprotilin, 250 to 275 mg Trimipramin, 240 to 260 mg (75 to 85 mg) Desipramin, 220 to 240 mg (70 to 80 mg) Nortriptylin, 200 to 240 mg (70 to 80 mg) Clomipramin, 185 to 210 mg (60 to 70 mg) Amitriptylin, 185 to 210 mg Venlafaxin, 75 to 85 mg (35 to 45 mg) Mianser in, 25 to 27.5 mg (25 to 27.5 mg) Paroxetin, and 270 to 300 mg Metoprolol. For safety reasons, the dosage indicated for the single doses (S) are preferably lowered by 10%, preferably 20% or even 25% for example if given for the first time. In some embodiments, the therapy regimen may be started with the average dose or even a lower dose. The optimal M and S doses can be calculated according to the percentages given above.

Furthermore, the present invention relates to the use of genotyping for any one of the methods described above, wherein an individual is genotyped for a specific metabolic factor and a corresponding genotypic determinant is characterized. Preferably, said genotypic determinant is used to quantify an individualized dosage regime of a drug. Furthermore, it is preferred that said genotypic determinant is used to identify an individual having a metabolic incompatibility with a selected therapy and/or treatment. In accordance with afore-mentioned methods it is preferred that said metabolic factor is at least one metabolism-specific allelic polymorphism such as an allelic polymorphism in a gene for an enzyme as defined herein.

According to an embodiment of the present invention, genotypic determinants may be employed to individualize an effective dosage regime for an individual. In doing so, an individual having a specific allelic variation corresponding to an enzyme specific inefficiency in metabolism can be identified by genotyping, and a corresponding dosage regime can be determined that will be safe for that individual. According to this example, an allelic variation may be identified as a metabolic factor of interest and metabolic determinants for that
metabolic factor may be characterized to provide a metabolic profile. Based on an individual's metabolic profile, a corresponding individualized drug dosage may be determined.

According to an alternate embodiment of the present invention, a genomic assay is provided to characterize genotypic determinants for use in the individualization of therapy and/or treatment, said assay comprising:

(a) a means for identifying a genetic marker corresponding to an individual's capacity for the metabolism of a given drug or class of drugs;

(b) a means for quantifying said genetic marker to provide an indicator of metabolic capacity specific for said drug or class of drugs; and

(c) a means for correlating said indicator with a therapeutically-effective dosage of said drug or class of drugs for said individual according to the pharmacokinetic model of the present invention.

Preferably, said means for identifying said genetic marker include a genetic probe specific to an allelic polymorphism of interest. In addition, it is advantageous if said allelic polymorphism is specific to a gene associated with drug metabolism. As already described for the methods of the present invention, said allelic polymorphism is preferably specific to a gene known to influence the activity of at least one of the following enzymes: NAT1, NAT2, CYP2A6, CYP2D6, CYP3A4, CYP2E1, CYP2C9, CYP1A2, CYP2C19, or TPMT.

A genomic assay of the present invention may include a means for identifying a genetic marker such as a metabolic factor corresponding to an individual's capacity for the metabolism of a given drug or class of drugs, for example. This genetic marker may be quantified in accordance with the genomic assay to provide an indicator of metabolic capacity. This indicator of metabolic capacity may be a genotypic determinant. This genotypic determinant may be employed to subsequently characterize a metabolic profile specific to an individual's metabolic capacity for the drug or class of drugs of interest. The genomic assay of the present invention may further include means for correlating said indicator with a therapeutically effective dosage of the drug or class of drugs of interest for the individual.

The genomic assay of the present invention may be specific to one or more genotypic determinants for the individualization of therapy and/or treatment with a drug or class of drugs. Typically, without limitation, the genomic assay will provide for the genotyping a
biological sample obtained from an individual to identify one or more genotypic determinants corresponding to metabolic factor(s) of interest. Based on the one or more genotypic determinants a metabolic profile may be characterized. A metabolic profile may correspond to a rate of drug metabolism of an individual. The metabolic profile may be employed in the determination of an individual dosage of a drug of interest.

The pharmacological exposure of an individual to a drug is preferably described by the concept of area-under-the curve (commonly referred to as AUC). AUC is related to clearance by the following equation:

\[ \text{AUC} = \text{dose/clearance} \]

Thus, if the rate of drug clearance for different metabolic phenotypes has been determined in accordance with the model of the present invention, the dose can be individualized to achieve a desired AUC by the equation:

\[ \text{Dose} = \text{desired AUC} \times \text{clearance} \]

An individual's rate of drug clearance is important as it determines the circulating drug concentrations. Both efficacy and toxicity are determined, in part, by the circulating concentrations of the drug.

Other factors can alter drug clearance, such as body surface area, hepatic enzyme levels (e.g. serum alanine aminotransferases (ALT), albumin, alkaline phosphatases and serum a-l-acidicglycoprotein (ARG)), and drug transport proteins (e.g. P-glycoprotein (pgp)). Other individual specific characteristics may play a role in determining individual dose-limiting toxicity. According to another aspect of the present invention, other influencing factors may be accounted for, in addition to the rate of metabolism, in the model for the individualization of therapy. For example, in the case of many chemotherapeutic drugs, myelosuppression is the dose-limiting toxicity, and hence an individual's pretreatment white blood cell (WBC) count could be an important factor in predicting toxicity. Using multivariate analysis such individual factors are examined for correlation to efficacy and toxicity. In accordance with one embodiment of the present invention, factors identified as having a significant correlation to either efficacy or toxicity are included in the model along with drug metabolism.

In some cases multiple drug metabolizing enzymes play key roles in determining the rate of drug metabolism. Therefore, the monitoring of only one metabolizing enzyme in such cases may not provide complete information for individualizing therapy. The use of a multi-
determinant assay allows for the examination of multiple metabolic factors to provide additional metabolism-related information and thereby provides a more accurate model for individualizing therapy. As one drug or drug metabolite can be acted on by several enzymes the use of a multi-determinant assay, which accounts for multiple enzymes associated metabolism, may, in some cases, provide a more accurate model. A multideterminant assay of the present invention may employ genotyping and/or phenotyping to characterize the metabolic profile of an individual.

The knowledge of an individual's metabolic profile for one or more enzymes provides for the identification of drug(s) that could cause significant side effects or be ineffective in treating that individual. In addition, a metabolic profile as provided by the present invention provides for the development of an individualized dosing scheme where a dosage corresponds to a level of enzymatic activity or rate of metabolism expressed by an individual. The implementation of a metabolic phenotyping and/or genotyping in the individualization of treatment and/or individualization of dosing determination in accordance with the present invention will lead to a marked decrease in side effects and an increase in therapeutic efficiency.

The genomic assay may be employed to detect genotypic determinants specific for the metabolism of a given drug or class of drugs. These genotypic determinants may be subsequently employed in order to determine an individual's capacity to metabolize a given drug. The levels of activity of at least one enzyme and/or metabolic pathway as determined by genotyping are incorporated into an individualization of therapy model in accordance with one aspect of the present invention to determine an individualized treatment dosage of a drug that correlates with an individual's capacity to metabolize the same.

The present invention also provides for an individualization model based upon at least an individual's specific phenotype and/or genotype for at least one metabolic factor for use in the individualization of therapy and/or drug dosing. The individualization model of the present invention may further include other enzyme-specific determinants as well as other factors, which have a significant contribution to the clearance of a drug in the body or a significant contribution to toxicity (e.g. pretreatment renal function).

As a result, physicians will be provided with a tool for the individualization of therapy providing an alternative to the trial and error selection of medications based on prognosis and
categorical dosing. Hence, the present invention relates a method of selectively treating an individual with a drug or class of drugs; said method comprising:

(a) genotyping an individual to identify at least one allelic polymorphism known to influence the metabolism of said drug or class of drugs;

(b) phenotyping said individual to confirm their phenotypic capacity to metabolize said a drug or class of drugs;

(c) calculating a therapeutically-effective amount of said drug or class of drugs specific for said individual based on said genotyping and phenotyping in accordance with the pharmacokinetic model of the present invention; and

(d) selectively treating said individual with the same; i.e.

(e) treating the patient with a very low (for PMs), low (for IMs) or very high (for UM) drug dosage.

Preferably, an allelic polymorphism known to adversely influence the metabolism of a drug or class of drugs is identified by genotyping, an individual is identified as high risk and treatment with said drug or class of drugs is avoided.

The present invention also relates to a kit for use in a method of the present invention comprising oligonucleotides, reference samples, amplification and/or sequencing means, buffer, chips or arrays, detergents, biochemical reagents, detection means, or the like and optionally a reformulated drug with a drug dose which is tailored to poor metabolizers (PM), intermediate metabolizers (IM) or ultrafast metabolizers (UM). For use of the CYP450 GeneChip microarray assay see, e.g., Chou et al., Clin. Chem. 49 (2003), 542-551. Such a kit would typically comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents for detection such as primers for genotyping, enzyme substrates or the like. Hence, the present invention provides a convenient and effective tool for use in both a clinical and laboratory environment. The present invention is particularly suited for use by a physician in a clinic, whereby metabolic determinants corresponding to drug specific metabolic factors, can be quickly and easily analyzed. According to an embodiment of the present invention, a ready-to-use kit is provided for fast and accurate determination of at least one metabolic determinant for a metabolic factor specific for the metabolism of a given drug such as described in WO03/008637 which particularly discloses different probe substrates that can be used to determine the NAT2 phenotype. The kit of the present invention may be tailored for phenotypic and/or genotypic screening. According to one embodiment, the assay system and kit preferably employ
antibodies specific to a plurality of metabolites on a suitable substrate allowing for detection of the preferred metabolites in a biological sample of an individual after consumption of a corresponding probe substrate. The assay systems of the present invention may be provided in a plurality of forms including but not limited to a high-throughput assay system or a dipstick based assay.

These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the materials, methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example the public database "Medline" may be utilized, which is hosted by the National Center for Biotechnology Information and/or the National Library of Medicine at the National Institutes of Health. Further databases and web addresses, such as those of the European Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL) are known to the person skilled in the art and can also be obtained using internet search engines. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

The above disclosure generally describes the present invention. Several documents are cited throughout the text of this specification. Full bibliographic citations may be found at the end of the specification immediately preceding the claims. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application and manufacturer's specifications, instructions, etc) are hereby expressly incorporated by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.
EXAMPLES

The examples which follow further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed herein can be found in the cited literature; see also “The Merck Manual of Diagnosis and Therapy” Seventeenth Ed. ed by Beers and Berkow (Merck & Co., Inc. 2003).

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Methods in molecular genetics and genetic engineering are described generally in the current editions of Molecular Cloning: A Laboratory Manual, (Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press); DNA Cloning, Volumes I and II (Glover ed., 1985); Oligonucleotide Synthesis (Gait ed., 1984); Nucleic Acid Hybridization (Hames and Higgins eds. 1984); Transcription And Translation (Hames and Higgins eds. 1984); Culture Of Animal Cells (Freshney and Alan, Liss, Inc., 1987); Gene Transfer Vectors for Mammalian Cells (Miller and Calos, eds.); Current Protocols in Molecular Biology and Short Protocols in Molecular Biology, 3rd Edition (Ausubel et al., eds.); and Recombinant DNA Methodology (Wu, ed., Academic Press). Gene Transfer Vectors For Mammalian Cells (Miller and Calos, eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al., eds.); Immobilized Cells And Enzymes (IRL Press, 1986); Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (Weir and Blackwell, eds., 1986). Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, and Clontech. General techniques in cell culture and media collection are outlined in Large Scale Mammalian Cell Culture (Hu et al., Curr. Opin. Biotechnol. 8 (1997), 148); Serum-free Media (Kitano, Biotechnology 17 (1991), 73); Large Scale Mammalian Cell Culture (Curr. Opin. Biotechnol. 2 (1991), 375); and Suspension Culture of Mammalian Cells (Birch et al., Bioprocess Technol. 19 (1990), 251); Extracting information from cDNA arrays, Herzl et al., CHAOS 11, (2001), 98-107.
Example 1: Development of a population based pharmacokinetic model

Any population pharmacokinetic model known in the art is applicable in the methods of the invention. In one embodiment of the present invention, the concentration data obtained by bioanalysis of human blood samples drawn from patients is used to develop a population pharmacokinetic model. Other information which may be used in such a model includes, but is not limited to information regarding dosage regimen (dose, dosing frequency, therapy formulation, time of administration etc.), the associated sampling time, co-medication, and patient-specific information.

In one embodiment, a structural pharmacokinetic model may be used in the methods of the invention, which describes the concentration-time course of a therapy. The data will determine which structural pharmacokinetic model may be used to mathematically describe the observed concentration-time courses. A population pharmacokinetic model may describe both the pharmacokinetics of a therapy in an 'average' patient and the variability of certain parameter values in the patient population.

In population pharmacokinetic modeling, the observed drug concentrations in the blood may be subject to three types of variability. These are the inter-individual and inter-occasion variability in the pharmacokinetic parameters, and a residual intrapatient variability. The residual variability originates from error in the bio-analysis, misspecification of the time after the last drug intake, model misspecifications etcetera.

The inter-occasion variability of model parameters can originate from several causes, such as variability in hepatic metabolism, increased heart rate, increased water retention etcetera. Inter-individual variability of pharmacokinetic parameters can also originate from several sources, like the individual's composition of metabolizing enzymes, protein composition of the blood, and many others.

A population pharmacokinetic model may comprise co-variates that explain variability of the parameter values. For example, the bodyweight of the patient may be predictive for a certain pharmacokinetic parameter value for that patient. In one embodiment, the developed model may be used to predict pharmacokinetic parameter values of an individual patient using
Bayesian methods. The obtained parameter values may, for example, be used to predict the concentration-time course of the drug in that particular patient. Particularly, it is preferred to use the following equations described and explained above. However, it should be clear that instead of AUCs other pharmacokinetic parameters may be used such as total clearance (Cl) and steady-state trough levels (Css).

The data should consist of a sufficient number of patients to characterize the pharmacokinetic variability which exists in the population. This may include deciding which patients to include to cover the natural variability. For example, one may include patients in a broad range of weight, age, renal function, preferably selected from the same subpopulation, for example the same ethnic group. In addition or alternatively, fractional data from individual patients e.g. a drug level, may be used to derive population pharmacokinetic parameters which may then be used to derive individual patient parameters (via Bayesian approach) again using fractional data (e.g. age, weight etc.) from different individual patients. The patient specific parameters may then be used to calculate, for an individual patient, the through concentration or to recalculate the drug dosage to be administered to a patient. In one embodiment, this approach may be used to optimize the therapy regimen of an individual patient. For example, one may apply a Bayesian single compartment model.

In one embodiment, the optimized pharmacokinetic model may be used in which at least one of three different types of variation and their associated errors are checked and minimized: (1) intra-individual variation, where a single patient's parameters may change over time (this includes measurement and sampling errors); (2) inter-individual variation, where an individual patient's parameters differ from the calculation based on previous research and experience; and (3) residual errors, where the theoretically predicted drug concentration differs from the actual measured blood drug concentration errors. The invention may, for example, address all three sources of error by iterative use of the pharmacokinetic model. The methods of the invention may also be encompassed in a database, a neural network relating to the database, and/or by the combined pharmacokinetic model generated from previously collected and iterated patient data (including data from previously conducted clinical studies). In one method of the invention, a neural network is used to obtain resistance data from genotypic data. In another embodiment, a neural network is used to refine the final pharmacokinetic model in order to minimize the difference between the theoretical drug concentration and the actual concentration.
Example 2: Pharmacokinetic model exemplified for Trimipramine

The tricyclic antidepressant trimipramine was shown to be one of the CYP2D6 substrates, with the most tremendous effects on oral total clearance caused by genetic polymorphisms of CYP2D6. We studied intravenous and multiple dose oral application of 50 mg trimipramine in 5, 7, and 3 healthy subjects with CYP2D6 genotypes predicting deficient, highly active and ultrarapid metabolism. The latter group included carriers of one wild type and one duplication allele. After an overnight fast, all volunteers received an intravenous dose of 50 mg trimipramine dissolved in 30.6 ml of aqueous 5% glucose solution which was infused at a constant rate within an interval of exactly 2 hours. Blood sampling was started 5 min after end of the infusion with scheduled sampling times at 0, 0.5, 1, 4, 6, 22, 30, 46, and 70 h; One pre-dose sample was taken prior infusion start. Urine was collected over 8 h from start of intravenous infusion.

After a minimum of 4 days washout, the volunteers took daily oral dosages of 50 mg trimipramine for 4 days at 8 pm, and the fifth dose at 8 am on the second study day, prior to an oral concentration profile with sampling times at 0 h (prior to dosage, trough level) and 1, 2, 3, 5, 8, 24, 32, 48, and 72 h after oral intake. Urine was collected over 8 h after oral dosing. All volunteers had given written informed consent. Trimipramine and desmethyltrimipramine concentrations were measured by HPLC over a time interval of 72 h after each application. Serum concentrations of racemic trimipramine and desmethyltrimipramine were analyzed by reversed phase HPLC with UV detection at 250 nm. Both reference substances in racemic form were kindly provided by Rhone-Poulenc, Rhorer, France. Concentrations between 2.5 and 100 µg/L of trimipramine maleate and desmethyltrimipramine maleate were used for calibration. 1-ml serum samples were mixed with 25 µl of the internal standard (500 ng imipramine in methanolic solution) and 500 µl of 1 M aqueous sodium carbonate. Extraction was performed with 5 ml n-hexane/acetonitrile (98/2 v/v) and 4.5 ml of the organic supernatant was evaporated under nitrogen and dissolved in 100 µl of the mobile phase. 50-µl samples were injected for chromatography and separation was performed on a LiChrospher 100 CN™ column (Merck, Darmstadt, Germany) with a mobile phase consisting of 5 mM sodium phosphate buffer, pH 6.0/methanol/acetonitrile in a ratio of 22/32/46, v/v/v at a flow rate of 1.5 ml/min at room temperature. Limit of quantification was 1 µg/L, both, for the parent drug and for the metabolite.
Both, bioavailability and systemic clearance significantly depended on CYP2D6 genotype with a linear gene dose relationship. Mean systemic availability was 54%, 20% and 9% in carriers of zero, two and three active genes of CYP2D6, respectively, and the corresponding data for systemic clearance were 12.0, 24.2, and 30.3 L/h. Correspondingly, the median total oral clearances were 16, 117, and 183 L/h.

**Table:** Trimipramine pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population estimate</th>
<th>Unit</th>
<th>Precision of estimate</th>
<th>Population variability, 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Cl}_{\text{basal}}$ (CYP2D6 independent clearance) *</td>
<td>12.03</td>
<td>L/h</td>
<td>3.53</td>
<td></td>
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<tr>
<td>$\text{Cl}_{\text{CYP2D6}}$ (Clearance conferred by one CYP2D6 allele) **</td>
<td>6.09</td>
<td>L/h</td>
<td>1.34</td>
<td></td>
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<tr>
<td>Volume of distribution</td>
<td>123.9</td>
<td>L</td>
<td>24.6</td>
<td>109.2 – 140.6</td>
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<tr>
<td>Absorption rate constant</td>
<td>0.11</td>
<td>h⁻¹</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Intercompartmental clearance</td>
<td>9.41</td>
<td>L/h</td>
<td>2.27</td>
<td></td>
</tr>
<tr>
<td>Volume of distribution at steady state</td>
<td>701</td>
<td>L</td>
<td>351</td>
<td></td>
</tr>
</tbody>
</table>

**Clearances in carriers of**

- Zero active CYP2D6 genes (PM) 12.0 L/h 10.6 – 13.6
- Two active CYP2D6 genes (EM) 24.2 L/h 21.3 – 27.5
- Three active CYP2D6 genes (UM) 30.3 L/h 26.7 – 34.4

**Bioavailability in carriers of**

- Zero active CYP2D6 genes 0.44 0.32 - 0.60
- Two active CYP2D6 genes 0.16 0.12 - 0.22
- Three active CYP2D6 genes 0.12 0.09 - 0.16
To have a quantitative estimation of the dose adaptations according to CYP2D6, CYP2D6 metabolizer subgroup-specific doses were calculated based on median oral clearances after multiple dosing using the method described earlier (Kirchheiner et al., 2001). Then, CYP2D6 poor metabolizer should get 20%, intermediate individuals being heterozygous for inactive CYP2D6 alleles should get 80%, extensive metabolizer 140%, and ultrafast metabolizers should get even doses up to 200% of the average standard dose.

**Example 3: Pharmacokinetic model exemplified for Metoprolol**

Metoprolol and hydroxymetoprolol plasma concentration-time courses after oral application of 100 mg normal release metoprolol (Metoprolol-Ratiopharm, Ulm, Germany) were compared between 9 healthy volunteers carrying one CYP2D6 duplication allele combined with one wild-type allele (UMs) and 8 individuals with two CYP2D6 wild-type alleles (extensive metabolizer).

Genotyping for CYP2D6 alleles *3, *4, *5 and *6 and gene duplication was performed after the methods described by Sachse (Sachse et al., 1997). HPLC analyses were performed to determine metoprolol and hydroxy-metoprolol plasma concentrations at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after metoprolol intake after the method given by Mistry et al., J. Pharm. Biomed. Anal. 16 (1998), 1041-1049; see figure 4.

An about twofold difference in the mean metoprolol AUC was found between the ultrarapid and the extensive CYP2D6 metabolizers (p=0.03). Mean AUC in extensive metabolizers was 627 μg · h/L (standard deviation 347 μg · h/L) and 326 μg · h/L (143 μg · h/L) in ultra-rapid metabolizers. AUCs of the hydroxy-metabolite were slightly (11%) higher in the ultra-rapid metabolizers but group differences not statistically significant.

Based on these pharmacogenetic data, ultra-rapid metabolizers carrying CYP2D6 gene duplications should receive about twofold higher doses of metoprolol than carriers of CYP2D6-wildtype.

**Example 4: Dosage for other antidepressant drugs dependent on the CYP2D6 genotype**

As is evident from the above, a prerequisite for selecting a suitable therapy is the knowledge of the presence or absence of a first and/or second variant allele referred to in accordance with
the use of the invention. Therefore, the method of the present invention encompasses the determination of the presence or absence of said variant alleles in a sample which has been obtained from said subject. The sample which is obtained by the subject comprises biological material which is suitable for the determination of the presence or absence of said variant alleles, such as isolated cells or tissue. Methods for the determination of the presence or absence of the variant alleles of the method of the invention comprise those methods referred to herein above. Further examples and a summary of dose recommendations are given in figure 4.

Thanks to the method of the present invention, it is possible to give patients suffering from medium to severe depression or hypertension the right drug at the right dose thereby preventing drug treatment failure and drug induced side effects. Thereby, mistreatment of patients based on wrong medications and drug dosages and the results thereof, such as increased costs in health care, can be efficiently avoided. Furthermore, patients that are at high risk can be excluded from therapy prior to the first dose and/or dosage can be adjusted according to the individual’s genetic makeup prior to the onset of drug therapy. Thus, therapy failure and adverse effects can be avoided and the optimal drug concentration can be reached faster without time-consuming and expensive drug monitoring-based dose finding. This can reduce costs of medical treatment and indirect costs of disease (e.g. shorter time and less frequent hospitalization of patients).
Example 5: Examples of possible metabolic factors and genetic determinants to be analyzed in accordance with the method of the present invention

The following table is taken from the supplementary material of Evans and Relling, Pharmacogenomics: Translating Functional Genomics into Rational Therapeutics, Science 286 (1999), 487-491.

**Table 1:** Genetic polymorphisms of human drug-metabolizing enzymes and transporter.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>Consequences of polymorphism for drug effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I enzymes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Benzo(a)pyrene, phenacetin</td>
<td>Not yet elucidated</td>
<td>(1)</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Acetaminophen, amonafide, caffeine, paraxanthine, ethoxyresorufin, propranolol, fluvoxamine</td>
<td>Not yet elucidated</td>
<td>(2)</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Estrogen metabolites</td>
<td>Not yet elucidated</td>
<td>(3)</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin, nicotine, halothane</td>
<td>Cigarette addiction</td>
<td>(4)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Cyclophosphamide, aflatoxin, mephenytoin</td>
<td>Not yet elucidated</td>
<td>(5)</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Retinoic acid, paclitaxel</td>
<td>Not yet elucidated</td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Tolbutamide, warfarin, phenytoin, nonsteroidal anti-inflammatories</td>
<td>Anticoagulant effect of warfarin</td>
<td>(6)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Mephenytoin, omeprazole, hexobarbital, mephobarbital, propranolol, proguanil, phenytoin</td>
<td>Peptic ulcer cure rates with omeprazole</td>
<td>(7)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Beta blockers, antidepressants, antipsychotics, codeine, debrisoquin, dextromethorphan, encaignide, flecainide, guanoxan, methoxyamphetamine, N-propyljalmine, perhexiline, phenacetin, phenformin, propafenone, sparteine</td>
<td>Tardive dyskinesia from antipsychotics, narcotic side effects, efficacy, and dependence, imipramine dose requirement, beta blocker effect</td>
<td>(8, 9)</td>
</tr>
</tbody>
</table>

**Supplemental Table 1. Cont.**
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>Consequences of polymorphism for drug effects in vivo</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2E1</td>
<td>N-Nitrosodimethylamine, acetaminophen, ethanol</td>
<td>Possible effect on alcohol consumption</td>
<td>(10)</td>
</tr>
<tr>
<td>CYP3A4/3A5/3A7</td>
<td>Macrolides, cyclosporin, tacrolimus, calcium channel blockers, midazolam, terfenadine, lidocaine, dapsone, quinidine, triazolam, etoposide, teniposide, lovastatin, alfentanil, tamoxifen, steroids, benzo(a)pyrene</td>
<td>Not yet elucidated</td>
<td>(11)</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase (ALDH2)</td>
<td>Cyclophosphamide, vinyl chloride</td>
<td>SCE frequency in lymphocytes</td>
<td>(12)</td>
</tr>
<tr>
<td>Alcohol dehydrogenase (ADH3)</td>
<td>Ethanol</td>
<td>Increased alcohol consumption and dependence</td>
<td>(13)</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase</td>
<td>Fluorouracil</td>
<td>5-fluorouracil neurotoxicity</td>
<td>(14)</td>
</tr>
<tr>
<td>NQO1 (DT-diaphorase)</td>
<td>Ubiquinones, menadione, mitomycin C</td>
<td>Menadione-associated urolithiasis</td>
<td>(15)</td>
</tr>
</tbody>
</table>

**Phase II Enzymes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>Consequences of polymorphism for drug effects in vivo</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyltransferase (NAT1)</td>
<td>p-Aminosalicylic acid, p-aminobenzoic acid, sulfamethoxazole</td>
<td>Not yet elucidated</td>
<td>(16)</td>
</tr>
<tr>
<td>N-Acetyltransferase (NAT2)</td>
<td>Isoniazid, hydralazine, sulfonamides, amonafide, procainamide, dapsone, caffeine</td>
<td>Hypersensitivity to sulfonamides, amonafide toxicity, hydralazine-induced lupus, isoniazid neurotoxicity</td>
<td>(16-18)</td>
</tr>
<tr>
<td>Glutathione S-transferase (GSTM1)</td>
<td>Aminochrome, dopachrome, adrenochrome and noradrenochrome</td>
<td>Not yet elucidated</td>
<td>(19)</td>
</tr>
<tr>
<td>Glutathione S-transferase (GSTM3)</td>
<td>Not yet elucidated</td>
<td>(19)</td>
<td></td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>Not yet elucidated</td>
<td>(19)</td>
<td></td>
</tr>
<tr>
<td>(GSTT1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Glutathione S-transferase (GSTP1)</td>
<td>13-cis retinoic acid, ethacrynic acid, acrolein, epirubicin</td>
<td>Not yet elucidated</td>
<td>(19, 20)</td>
</tr>
<tr>
<td>Sulfotransferases</td>
<td>Steroids, acetaminophen, estrogens, dopamine, epinephrine, naringenin</td>
<td>Not yet elucidated</td>
<td>(21)</td>
</tr>
<tr>
<td>Catechol-O-methyltransferase</td>
<td>Estrogens, levodopa, ascorbic acid</td>
<td>Substance abuse, levodopa response</td>
<td>(22)</td>
</tr>
<tr>
<td>Histamine methyltransferase</td>
<td>Histamine</td>
<td>Not yet elucidated</td>
<td>(23)</td>
</tr>
<tr>
<td>Thiopurine methyltransferase</td>
<td>Mercaptopurine, thioguanine, azathioprine</td>
<td>Thiopurine toxicity and efficacy, risk of second cancers</td>
<td>(24, 25)</td>
</tr>
<tr>
<td>UDP-glucuronosyltransferase (UGT1A1)</td>
<td>Irinotecan, bilirubin</td>
<td>Irinotecan glucuronidation</td>
<td>(26)</td>
</tr>
<tr>
<td>UDP-glucuronosyltransferase (UGT2B1)</td>
<td>Opioids, androgens, morphine, naproxen, ibuprofen</td>
<td>Not yet elucidated</td>
<td>(27)</td>
</tr>
</tbody>
</table>

**Transporters**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP</td>
<td>Conjugates</td>
</tr>
<tr>
<td>MDR-1</td>
<td>Natural product anticancer drugs, CYP3A4 substrates, digoxin</td>
</tr>
<tr>
<td>MRP</td>
<td>Glutathione, glucuronide, and sulfate conjugates, nucleoside antivirals</td>
</tr>
</tbody>
</table>
Table 2: Genetic polymorphisms of drug receptors or targets linked to altered drug effects in humans

<table>
<thead>
<tr>
<th>Receptor/target</th>
<th>Medication</th>
<th>Drug effects linked to receptor/target polymorphism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2-Adrenergic receptor</td>
<td>Albuterol</td>
<td>Response in asthmatics</td>
<td>(32)</td>
</tr>
<tr>
<td>5-Lipoxygenase promoter</td>
<td>ABT-761 (zileuton)</td>
<td>Response in asthmatics</td>
<td>(33)</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (ACE)</td>
<td>Enalapril, isoprin, captopril</td>
<td>Renoprotective effects, cardiac indices, blood pressure, IgA nephropathy</td>
<td>(34, 35)</td>
</tr>
<tr>
<td>Cholesteryl ester transfer protein</td>
<td>Pravastatin</td>
<td>Progression of atherosclerosis</td>
<td>(36)</td>
</tr>
<tr>
<td>Stromelysin</td>
<td>Pravastatin</td>
<td>Efficacy in coronary atherosclerosis and restenosis</td>
<td>(37)</td>
</tr>
<tr>
<td>Angiotensin-II T receptor</td>
<td>Perindopril nitrendipine</td>
<td>Change in arterial stiffness</td>
<td>(38)</td>
</tr>
<tr>
<td>Sulfonylurea receptor</td>
<td>Tolbutamide</td>
<td>Serum C-peptide and insulin response</td>
<td>(39)</td>
</tr>
<tr>
<td>5-Hydroxytryptamine 2C receptor</td>
<td>Clozapine</td>
<td>Response in schizophrenia</td>
<td>(40)</td>
</tr>
<tr>
<td>5-Hydroxytryptamine 2A receptor</td>
<td>Clozapine and other neuroleptics</td>
<td>Response in schizophrenia</td>
<td>(41)</td>
</tr>
<tr>
<td>Serotonin transporter promoter</td>
<td>Fluvoxamine</td>
<td>Response in delusional depression</td>
<td>(42)</td>
</tr>
<tr>
<td>Dopamine D2 and D3 receptors</td>
<td>Antipsychotics</td>
<td>Drug-induced tardive dyskinesia</td>
<td>(43)</td>
</tr>
<tr>
<td>Vitamin D receptor</td>
<td>1,25-Dihydroxy vitamin D3</td>
<td>Vitamin D response in rickets</td>
<td>(44)</td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>Dexamethasone</td>
<td>Cortisol and insulin response</td>
<td>(45)</td>
</tr>
<tr>
<td>Nicotinic receptor</td>
<td>Acetylcholine (-) nicotine</td>
<td>Increased sensitivity to agonist effects</td>
<td>(46)</td>
</tr>
<tr>
<td>Delta opioid receptor</td>
<td>Heroin</td>
<td>Addiction</td>
<td>(47)</td>
</tr>
<tr>
<td><strong>Potassium channels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>HERG</td>
<td>Quinidine</td>
<td>Drug-induced long QT syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cisapride</td>
<td>Drug-induced torsade de pointes</td>
<td></td>
</tr>
<tr>
<td>KvLQT1</td>
<td>Terfenadine, disopyramide mefloquine</td>
<td>Drug-induced long QT syndrome</td>
<td></td>
</tr>
<tr>
<td>hHCNE2</td>
<td>Clarithromycin</td>
<td>Drug-induced arrhythmia</td>
<td></td>
</tr>
</tbody>
</table>

**Sodium channel**

<table>
<thead>
<tr>
<th>SCN5A</th>
<th>Mexiletine</th>
<th>Efficacy for long-QT syndrome secondary to SCN5A mutations but not HERG mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inositol-p1p</td>
<td>Lithium</td>
<td>Response of manic depressive illness</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>Cyclosporin A</td>
<td>Response of aplastic anemia</td>
</tr>
<tr>
<td>Apolipoprotein E4</td>
<td>Tacrine</td>
<td>Response of Alzheimer's disease</td>
</tr>
<tr>
<td>Ryanodine receptor</td>
<td>Halothane or succinylcholine</td>
<td>Drug-induced malignant hyperthermia</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>Oral contraceptives</td>
<td>Risk of cerebral-vein thrombosis</td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor</td>
<td>Insulin</td>
<td>Insulin sensitivity</td>
</tr>
</tbody>
</table>

**Table 3**: Further substrates for CYP2C9 and CYP2C19

<table>
<thead>
<tr>
<th><strong>CYP2C9</strong></th>
<th><strong>CYP2C19</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSAIDs:</strong></td>
<td><strong>Proton Pump Inhibitors:</strong></td>
</tr>
<tr>
<td>diclofenac</td>
<td>lansoprazole</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>omeprazole</td>
</tr>
<tr>
<td>meloxicam</td>
<td>pantoprazole</td>
</tr>
<tr>
<td>S-naproxen=&gt;Nor</td>
<td>E-3810</td>
</tr>
<tr>
<td>piroxicam</td>
<td></td>
</tr>
<tr>
<td>suprofen</td>
<td></td>
</tr>
<tr>
<td><strong>Oral Hypoglycemic Agents:</strong></td>
<td><strong>Anti-epileptics:</strong></td>
</tr>
<tr>
<td>tolbutamide</td>
<td>diazepam=&gt;Nor</td>
</tr>
<tr>
<td>glipizide</td>
<td>phenytoin(O)</td>
</tr>
<tr>
<td></td>
<td>S-mephenytoin</td>
</tr>
<tr>
<td></td>
<td>phenobarbitone</td>
</tr>
<tr>
<td>Angiotensin II Blockers:</td>
<td>amitriptyline</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>losartan</td>
<td>carisoprodol</td>
</tr>
<tr>
<td>irbesartan</td>
<td>citalopram</td>
</tr>
<tr>
<td></td>
<td>clomipramine</td>
</tr>
<tr>
<td></td>
<td>cyclophosphamide</td>
</tr>
<tr>
<td></td>
<td>hexobarbital</td>
</tr>
<tr>
<td></td>
<td>imipramine N-DeME</td>
</tr>
<tr>
<td></td>
<td>indomethacin</td>
</tr>
<tr>
<td></td>
<td>R-mephobarbital</td>
</tr>
<tr>
<td></td>
<td>moclobemide</td>
</tr>
<tr>
<td></td>
<td>nelfinavir</td>
</tr>
<tr>
<td></td>
<td>nilutamide</td>
</tr>
<tr>
<td></td>
<td>primidone</td>
</tr>
<tr>
<td></td>
<td>progesterone</td>
</tr>
<tr>
<td></td>
<td>proguanil</td>
</tr>
<tr>
<td></td>
<td>propranolol</td>
</tr>
<tr>
<td></td>
<td>teniposide</td>
</tr>
<tr>
<td></td>
<td>R-warfarin=&gt;8-OH</td>
</tr>
<tr>
<td>amitriptyline</td>
<td>celecoxib</td>
</tr>
<tr>
<td>fluoxetine</td>
<td>fluvastatin glyburide</td>
</tr>
<tr>
<td>phenytoin=&gt;4-OH</td>
<td>rosiglitazone</td>
</tr>
<tr>
<td>rosiglitazone</td>
<td>tamoxifen</td>
</tr>
<tr>
<td>torsemide</td>
<td>S-warfarin</td>
</tr>
<tr>
<td>Drugs metabolized by Arylamide Acetylase 2 (N-acetytransferase 2 (NAT2)) (More than 80% of Arabs, 55% of Europeans and 17% of Asians are Slow Acetylators):</td>
<td></td>
</tr>
<tr>
<td>Isoniazid (Antibuberculosis),</td>
<td></td>
</tr>
<tr>
<td>Hydralazine-HCL (Hypertension),</td>
<td></td>
</tr>
<tr>
<td>Sulfonamamides (Antibiotics),</td>
<td></td>
</tr>
<tr>
<td>Procainamide (Cardiac Drug)</td>
<td></td>
</tr>
<tr>
<td>Dapsone (Leprosy)</td>
<td></td>
</tr>
<tr>
<td>Drugs metabolized by the human flavin-containing monooxygenase form 3 (FMO3) (9% of Caucasians have a reduced activity):</td>
<td></td>
</tr>
<tr>
<td>Perazine</td>
<td></td>
</tr>
<tr>
<td>Sulindac</td>
<td></td>
</tr>
<tr>
<td>Albendazole</td>
<td></td>
</tr>
<tr>
<td>Benzyldamine</td>
<td></td>
</tr>
</tbody>
</table>
Drugs metabolized by the thiopurine S-methyltransferase (TPMT) enzyme (Only 0.3% of Caucasians have enzyme defect, but it can be fatal):

Azathioprine
6-Mercaptopurine
References for Tables 1 and 2


38. A. Benetos et al., Hypertension 28, 1081 (1996).


48. S. G. Priori et al., Circulation99, 674 (1999); C. Donger et al., ibid 96, 2778 (1997).

49. G. W. Abbott et al., Cell 97, 175 (1999).

Claims

1. A method of determining a dosage regime for at least one drug or pro-drug, preferably two drugs or pro-drugs for an individual subject, wherein an individualized dosage of a drug selected from a drug or class of drugs known for treating a condition is determined for said subject, said method comprising:
   (a) determining a metabolic profile of said individual corresponding to at least one metabolic factor known to influence the metabolism or action of said class of drugs; and
   (b) calculating said individualized dosage of said drug according to a pharmacokinetic model.

2. The method of claim 1, wherein said pharmacokinetic model comprises different genetic determinants specific for said at least one metabolic factor; wherein said genetic determinants are correlated to a rate of drug metabolism specific of said different genetic determinants, and a metabolic quotient for said at least one drug is determined based on the respective rate of drug metabolism specific of said different genetic determinants.

3. The method of claim 2, wherein said pharmacokinetic model comprises determining a pharmacologic exposure of said drug or class of drugs in at least two different subjects with different genetic determinants.

4. The method of claim 2 or 3, wherein said genetic determinants are specific for poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) or ultra-rapid metabolizers (UM) of said drug or class of drugs.

5. The method of any one of claims 2 to 4, wherein at least one pharmacologic exposure of said drug or class of drugs in subjects with a genetic determinant specific for ultrarapid metabolizers (UM) has been performed.

6. The method of any one of claims 2 to 5, wherein the genetic determinant comprises at least one gene duplication.
7. The method of any one of claims 2 to 6, wherein at least one of said genetic determinants is analyzed by genotyping and/or phenotyping.

8. The method of any one of claims 1 to 7, wherein said at least one metabolic factor is specific for an activity level of an enzyme activity known to influence the metabolism of said drug or class of drugs.

9. The method of claim 8, wherein said activity level of said enzyme is specific for at least one cytochrome P450 (CYP), N-acetyltransferase (NAT), flavin-containing monoxygenase 3 (FMO3), P-glycoprotein (MDR-1) or thiopurine S-methyltransferase (TPMT) enzyme.

10. The method of any one of claims 1 to 7, wherein said at least one metabolic factor is an activity level of a metabolic pathway known to influence the metabolism of said drug or class of drugs.

11. The method of claim 10, wherein said activity level of said metabolic pathway is specific for at least one cytochrome P450, N-acetyltransferase or thiopurine S-methyltransferase metabolic pathway.

12. The method of any one of claims 8 to 11, wherein said at least one enzyme or metabolic pathway is at least one of the following enzymes: NAT1, NAT2, CYP2A6, CYP2D6, CYP3A4, CYP2E1, CYP2C9, CYP1A2 and CYP2C19, TPMT, FMO3, or dihydropyrimidine dehydrogenase (DPD).

13. The method of any one of claims 1 to 12, wherein at least one metabolic factor is a drug receptor, translocator, ion-channel, transporter, or ligand binding molecule.

14. The method of any one of claims 2 to 13, wherein said a rate of drug metabolism is calculated based on multiple or single dosing of said drug.

15. The method of any one of claims 2 to 14, wherein said drug metabolism is measured by bioavailability and/or systemic clearance of the drug.
16. The method of any one of claims 2 to 15, wherein said drug is administered by infusion or oral application.

17. The method of any one of claims 3 to 16, wherein said pharmaceutical exposure is obtained from a sample chosen from at least one of a plasma sample, a blood sample, a saliva sample, a tumor sample, a tissue sample, and a bodily fluid sample.

18. The method of any one of claims 3 to 17, wherein the pharmacologic exposure is a trough steady-state concentration (CSS) or area under curve (AUC).

19. The method of any one of claims 1 to 18, wherein said pharmacokinetic model is based on a certain subpopulation of subjects.

20. The method claim 19, wherein said subpopulation is comprised of Arabs, Chinese, Japanese, Korean, or Indians.

21. The method of any one of claims 1 to 20, wherein the pharmacokinetic model is a measured pharmacokinetic model of a subpopulation of subjects.

22. The method of any one of claims 2 to 21, wherein said metabolic quotient is calculated by the formula

\[ D_{AV} = (xD_{PM} + yD_{IM} + zD_{EM} + uD_{UM}) \]  \hspace{1cm} (1)

To calculate the dose for each genotype, one proceeds as follows:

\[ D_{PM} = D_{EM} \cdot AUC_{EM} \]  \hspace{1cm} (2)

with

\[ n = AUC_{EM}/AUC_{PM} \]

and

\[ D_{IM} = AUC_{EM}/AUC_{IM} \cdot D_{EM} \]  \hspace{1cm} (3)

with

\[ m = AUC_{EM}/AUC_{IM} \]

and

\[ D_{UM} = AUC_{EM}/AUC_{UM} \cdot D_{EM} \]  \hspace{1cm} (4)

\[ o = AUC_{EM}/AUC_{UM} \]
By substituting equation (2), (3) and (4) into equation (1), these relations can be transformed to percent adjustment compared with the average dose \( D_{AV} \) (100%) for the EMs:

\[
D_{EM}(\%) = \frac{100}{(x \cdot n + y \cdot m + z + u \cdot o)}
\]  
(5)

And

\[
D_{PM}(\%) = n \cdot D_{EM}
\]  
(6)

And

\[
D_{IM}(\%) = m \cdot D_{EM}
\]  
(7)

And

\[
D_{UM}(\%) = o \cdot D_{EM}
\]  
(8)

wherein

\( D_{AV} \) = Average dose recommendations \( D_{AV} \) provided in manufacturers’ information may be considered as the pragmatic results of large-scale studies performed within genetically mixed populations;

\( D_{PM}, D_{IM}, D_{EM} \) and \( uD_{UM} \) representing the doses which might have been recommended for the sub-populations poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra-fast metabolizers, respectively;

\( xD_{PM} + yD_{IM} + zD_{EM} + uD_{UM} = \% \) values considered as the weighted means from a given population.

23. The method of any one of claims 2 to 24, wherein the population pharmacokinetic model is optimized using a Bayesian model.

24. The method of any one of claims 2 to 23 further comprising entering said metabolic quotient, and optionally the corresponding genetic determinants in a computer database.

25. The method of any one of claims 1 to 24, wherein said step of determining a metabolic profile includes phenotyping and/or genotyping.

26. The method of claim 27, wherein genotyping is performed by a genomic assay.
27. The method of any one of claims 2 to 26, wherein said genetic determinants comprise at least one allele of CYP2D6.

28. The method of claim 27, wherein said allele is selected from a group consisting of the allele CYP2D6*1, CYP2D6*2, CYP2D6*9 or CYP2D6*10 resulting in the extensive (EM) or ultra-fast metabolizer (UM) phenotype if present in more than one copy per chromosome.

29. The method of claim 27 or 28, wherein said allele is selected from a group consisting of CYP2D6*3, CYP2D6*4, CYP2D6*5, CYP2D6*6, CYP2D6*7, CYP2D6*8, CYP2D6*11, CYP2D6*12 or CYP2D6*15, CYP2D6*16, CYP2D6*19, CYP2D6*20, CYP2D6*36, CYP2D6*38, CYP2D6*40, CYP2D6*42 and CYP2D6*43 alleles resulting in the intermediate (IM) or poor metabolizer (PM) phenotype.

30. The method of any claims 19 to 29, wherein said genetic determinants comprise at least one allele of CYP2C19 and said subpopulation is an Asian population.

31. The method of any claims 2 to 30, wherein said genetic determinants comprise at least one allele of NAT2, FMO3 or TPMT.

32. The method of any claims 1 to 31, wherein said pharmacokinetic model is established for at least two drugs which are metabolized by CYP2D6 and which have been determined in ultrafast metabolizers (UMs) for CYP2D6 who have three copies of a wild type allele of the CYP2D6 gene.

33. The method of any one of claims 1 to 32, further including:
   (c) measuring at least one determinant for drug clearance known to affect the toxicity or efficacy of said drug or class of drugs; wherein said at least one determinant for drug clearance is factored together with at least said metabolic quotient to determine a therapeutically effective amount of said drug or class of drugs to be prescribed to said individual.
34. The method of claim 33, wherein said at least one determinant for drug clearance is based on at least one of body surface area and hepatic enzyme levels of said individual.

35. The method of any one of claims 1 to 34, wherein the at least one drug is selected from the group consisting of Alprenolol, Bufuralol, Bunitrolol, Bupranolol, Metoprolol, Propanolol, Timolol, Aprindine, Encaidine, Flecainide, Mexiletine, Propafenone, N-Propylajmaline, Sparteine, Amitriptyline, Clomipramine, Clopromazine, Desipramine, Doxepin, Fluoxetine, Imipramine, Maprotiline, Mianserine, Minaprine, Nortriptyline, Trimipramine, Paroxetine, Haloperidol, Perphenazine, Risperidone, Thiordazine, Zuclopenthizoxol, Carvedilol, Venlafaxine, Codeine, Dihydrocodeine, Oxycodone, Tramadol, Dextromethorphan, Ethylmorphine, Hydrocodone, Tropisetron, Ondansetron, Amphetamine, or Methylendioxy-methamphetamine.

36. The method of any one of claims 1 to 36, wherein said individualized dosage is calculated from the dose usually applied dose recommended for in-patients, without taking genetic polymorphisms into consideration, as taken from the average dose according to the manufacturers' recommendations factored against the metabolic quotient.

37. The method of claim 36, wherein the individualized dose for PM, IM, EM and UM CYP2D6 genotypes is given in % of the recommended average doses for Imipramine, Doxepin, Trimipramine, Maprotiline, Desipramine, Nortriptylin, Clomipramine, Amitriptylin, Venlafaxin: all 150 mg; Metoprolol 100 mg, Mianserin: 60 mg; and Paroxetin: 20 mg; as shown in figure 4.

38. The method of claim 36 or 37, wherein the individualized dose for UM CYP2D6 genotypes in % of the recommended average dose is 182% for Imipramin, 173% for Doxepin, 173% for Maprotiline, 178% for Trimipramin, 167% for Desipramin, 152% for Nortriptylin, 146% for Clomipramin, 130% for Amitriptylin, 130% for Venlafaxin, 134% for Mianserin, 138% for Paroxetin, and 190% for Metoprolol.
39. A method for the preparation of an medicament individual subject or subpopulation of subjects comprising reformulation of the drug or pro-drug in accordance with the method of any one of claims 36 to 38.

40. The method of claim 39, wherein said individual subject or subpopulation of subjects are ultrafast metabolizers (UMs) of CYP2D6.

41. The method of claim 39 or 40, wherein said drug is trimipramin or metoprolol.

42. A medicament obtainable by the method of any one of claims 39 to 41; optionally further comprising prescription of the drug for PM, IM, EM and/or UM.

43. The medicament of claim 42 comprising a multiple (M) (single (S)) dose selected from the group consisting of 250 to 275 mg (65 to 90 mg) Imipramin, 250 to 275 mg Doxepin, 250 to 275 mg Maprotilin, 250 to 275 mg Trimipramin, 240 to 260 mg (60 to 85 mg) Desipramin, 220 to 240 mg (60 to 80 mg) Nortriptylin, 200 to 240 mg (60 to 80 mg) Clomipramin, 185 to 210 mg (60 to 75 mg) Amitriptylin, 185 to 210 mg Venlafaxin, 75 to 85 mg (35 to 45 mg) Mianserin, 25 to 27,5 mg (25 to 27,5 mg) Paroxetin, and 270 to 300 mg Metoprolol.

44. A method for selecting a suitable dosage regimen or formulation of drug or class of drugs known for treating a condition wherein said method comprises:
   (a) determining the presence or absence of at least one genetic determinant as defined in any one of claims 2 to 38 in a sample obtained from said subject; and
   (b) selecting a suitable therapy for said subject based on the pharmacokinetic model defined in any one of claims 2 to 38.

45. Use of genotyping for a method of any one claims 39, 40 or 44, wherein an individual is genotyped for a specific metabolic factor and a corresponding genotypic determinant is characterized.

46. The use of claim 45, wherein said genotypic determinant is used to quantify an individualized dosage regime of a drug.
47. The use of claim 45 or 46, wherein said genotypic determinant is used to identify an individual having a metabolic incompatibility with a selected therapy and/or treatment.

48. The use of any one of claims 45 to 47, wherein said metabolic factor is at least one metabolism-specific allelic polymorphism.

49. The use of claim 48, wherein said allelic polymorphism in a gene for an enzyme as defined in any one of claims 9 to 12.

50. A genomic assay for use in the individualization of therapy and/or treatment, said assay comprising:
   (a) a means for identifying a genetic marker corresponding to an individual's capacity for the metabolism of a given drug or class of drugs;
   (b) a means for quantifying said genetic marker to provide an indicator of metabolic capacity specific for said drug or class of drugs; and
   (c) a means for correlating said indicator with a therapeutically-effective dosage of said drug or class of drugs for said individual according to the pharmacokinetic model of any one of claims 2 to 38.

51. The genomic assay of claim 50, wherein said means for identifying said genetic marker includes a genetic probe specific to an allelic polymorphism of interest.

52. The genomic assay of claim 51, wherein said allelic polymorphism is specific to a gene associated with metabolism.

53. The genomic assay of claim 52, wherein said allelic polymorphism is specific to a gene known to influence the activity of at least one of the following enzymes: NAT1, NAT2, CYP2A6, CYP2D6, CYP3A4, CYP2E1, CYP2C9, CYP1A2, CYP2C19, FMO3, or TPMT.

54. A method of selectively treating an individual with a drug or class of drugs; said method comprising:
   (a) genotyping an individual to identify at least one allelic polymorphism known to influence the metabolism of said drug or class of drugs;
(b) phenotyping said individual to confirm their phenotypic capacity to metabolize said at drug of class of drugs;
(c) calculating a therapeutically-effective amount of said drug or class of drugs specific for said individual based on said genotyping and phenotyping in accordance with the pharmacokinetic model of any one of claims 2 to 38; and
(d) selectively treating said individual with the same.

55. The method of claim 54, wherein an allelic polymorphism known to adversely influence the metabolism of a drug or class of drugs is identified by genotyping, an individual is identified as high risk and treatment with said drug or class of drugs is avoided.

56. A kit for use in a method of any one of claims 1 to 40, 44, or 50 to 55 comprising oligonucleotides, a chip or array, reference samples, amplification and/or sequencing means, buffer, detergents, biochemical reagents, detection means, or the like and optionally a reformulated drug with a drug dose which is tailored to poor metabolizers (PM), intermediate metabolizers (IM) or ultrafast metabolizers (UM).
Fig. 1
Fig. 2
Metoprolol mean concentrations and CYP2D6 gene duplication

Fig. 3
Fig. 4