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(54) Title: METHODS FOR ANALYZING LTC4 SYNTHASE POLYMORPHISMS AND DIAGNOSTIC USE

(57) Abstract

This invention relates to single nucleotide polymorphisms in the $LTC_4$ synthase gene, EMBL accession no. U50136, particularly at one or more of positions 375, 815, 1003, 2169 and 2742. The invention also relates to methods and materials for analysing allelic variation in the $LTC_4$ synthase gene, and to the use of $LTC_4$ synthase polymorphism in the diagnosis and treatment of leukotriene mediated diseases such as asthma and allergic rhinitis.

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# METHODS FOR ANALYZING LTC4 SYNTHASE POLYMORPHISMS AND DIAGNOSTIC USE

This invention relates to polymorphisms in the $LTC_4$ synthase gene. The invention also relates to methods and materials for analysing allelic variation in the $LTC_4$ synthase gene, and to the use of $LTC_4$ synthase polymorphism in the diagnosis and treatment of leukotriene mediated diseases such as asthma and allergic rhinitis.

The cysteinyl-leukotrienes, $LTC_4$, $LTD_4$ and $LTE_4$, are potent bronchoconstrictors, increase vascular permeability and increase mucus production in airways. They are implicated in the pathophysiology of asthma and allergic rhinitis and are found at elevated levels in bronchoalveolar lavage from asthma patients, particularly after allergen challenge. $LTD_4$ and $LTE_4$ may also enhance the neurogenic inflammatory response in airways. Compounds which inhibit leukotriene synthesis *e.g.* the 5-lipoxygenase inhibitor, zileuton, or the leukotriene receptor antagonist, zafirlukast, have been shown to be effective against asthma and rhinitis (Busse W.W, Clin. Exp. Allergy, **26**, 868-879, 1996; see particularly Figure 1 therein which shows the arachidonic acid cascade, indicating the role of $LTC_4$ synthase in catalysing the formation of $LTC_4$).

Leukotrienes are derived from membrane phospholipids. Arachidonic acid is released from the phospholipid by cytosolic phospholipase A2 and converted to $LTA_4$ by 5-lipoxygenase in the presence of 5-lipoxygenase activating protein, FLAP. Polymorphisms in 5-LO have been reported in international patent application WO 97/42347, Brigham & Women's Hospital. $LTA_4$ is conjugated with reduced glutathione by $LTC_4$ synthase to form $LTC_4$. The biologically active metabolites, $LTD_4$ and $LTE_4$ are formed, following carrier mediated export of $LTC_4$, by the sequential action of gamma-glutamyl transpeptidase and dipeptidases.

The $LTC_4$ synthase gene has been cloned and published as a 4,465 nucleotide genomic sequence comprising 1,446 nucleotides of sequence 5' to the coding sequence, the 5 exons and intervening introns and 3' sequence extending 398 nucleotide beyond the polyA signal (Penrose *et al.*, J. Biol. Chem., **271**, 11356-11361, 1996; EMBL accession no. U50136).

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics") . Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to

elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

Clinical trials have shown that patient response to treatment with leukotriene antagonists is heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy with leukotriene antagonists.

The present invention is based on the discovery of five single nucleotide polymorphisms (SNPs) in the $LTC_4$ synthase gene. Three SNPs have been found in the 5' untranslated region of the gene and two in the first intron, located at positions 375, 815, 1003, 2169 and 2742 respectively, based on the numbering of U50136. Before our first filing date, we believe there has been no disclosure of polymorphism/allelic variation in the $LTC_4$ synthase gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in $LTC_4$ synthase in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the $LTC_4$ synthase gene.

The term human includes both a human having or suspected of having a leukotriene mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to leukotriene mediated disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 375 is presence of G and/or A.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 815 is presence of C and/or A.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1003 is presence of A

and/or C. Testing for the presence of the C allele at this position is especially preferred because, without wishing to be bound by theoretical considerations, of its association with increased levels of $LTC_4$ synthase (as explained herein).

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2169 is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2742 is presence of C and/or T.

The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

In another aspect of the invention we provide a method for the diagnosis of leukotriene mediated disease, which method comprises:

i) obtaining sample nucleic acid from an individual,

ii) detecting the presence or absence of a variant nucleotide at one or more of positions 375, 815, 1003 and 2169 in the $LTC_4$ synthase gene and

iii) determining the status of the individual by reference to polymorphism in the $LTC_4$ synthase gene.

The published sequence of the $LTC_4$ synthase gene, EMBL accession number U50136, is shown in SEQ ID NO: 1 in which the variant sites discovered in the present invention are at positions 375, 815, 1003, 2169 and 2742.

Allelic variation at position 375 consists of a single base substitution from G (the published base), for example to A. Allelic variation at position 815 consists of a single base substitution from C (the published base), for example to A. Allelic variation at position 1003 consists of a single base substitution from A (the published base), for example to C. Allelic variation at position 2169 consists of a single base substitution from C (the published base), for example to T. Allelic variation at position 2742 consists of a single base substitution from C (the published base), for example to T. The status of the individual may be determined by reference to allelic variation at one, two, three, four or all five of the above loci.

Sanak *et al.* (1998), Lancet, **350**, 1599, have reported an increased risk of aspirin induced asthma (AIA) being associated with the polymorphism at position 1003. This work

suggests that the presence of the C allele at position 1003 leads to increased levels of $LTC_4$ synthase (see also Cowburn *et al.* (1998), J. Clin. Invest., **101**, 834). AIA affects about 10 % of adult asthmatics. Aspirin and other cyclo-oxygenase inhibitors cause release of LTs into airways, leading to an asthma attack in susceptible individuals. Clinical approaches to deal with AIA include pretreatment with anti-leukotriene drugs (Szczeklik (1997), Allergy, **52**, 613-9). Commentators have written approvingly of the clinical utility of detection of $LTC_4$ polymorphisms (Holgate (1998), Lancet, **351**, 1300-1301, see last paragraph in particular). Anti-leukotriene drugs have been reviewed in the following publications: Horwitz *et al.* (1998), Am J Respir Crit Care Med, **157**, 1363 (see particularly Table 1 for a list of drugs); and Tan (1998), Current Opinion in Pulmonary Medicine, **4**, 25.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before use in the analysis of $LTC_4$ synthase variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

**Abbreviations:**

| | |
|---|---|
| AIA | Aspirin induced asthma |
| ALEX™ | Amplification refractory mutation system linear extension |
| APEX | Arrayed primer extension |
| ARMS™ | Amplification refractory mutation system |
| b-DNA | Branched DNA |
| CMC | Chemical mismatch cleavage |
| bp | base pair |
| COPS | Competitive oligonucleotide priming system |
| DGGE | Denaturing gradient gel electrophoresis |
| FLAP | 5-lipoxygenase activating protein |
| FRET | Fluorescence resonance energy transfer |
| LCR | Ligase chain reaction |
| 5-LO | 5-Lipoxygenase |
| LT | Leukotriene |
| MASDA | Multiple allele specific diagnostic assay |
| NASBA | Nucleic acid sequence based amplification |
| OLA | Oligonucleotide ligation assay |
| PCR | Polymerase chain reaction |
| PTT | Protein truncation test |
| RFLP | Restriction fragment length polymorphism |
| SDA | Strand displacement amplification |
| SNP | Single nucleotide polymorphism |
| SSCP | Single-strand conformation polymorphism analysis |
| SSR | Self sustained replication |
| TGGE | Temperature gradient gel electrophoresis |

Table 1 - Mutation Detection Techniques

**General**: DNA sequencing, Sequencing by hybridisation

**Scanning**: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

**Hybridisation Based**

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

**Extension Based:** ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

**Incorporation Based:** Mini-sequencing, APEX

**Restriction Enzyme Based:** RFLP, Restriction site generating PCR

**Ligation Based:** OLA

**Other:** Invader assay

Table 2 - Signal Generation or Detection Systems

**Fluorescence:** FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

**Other:** Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the efficacy of therapeutic compounds in the treatment of asthma, rhinitis and other leukotriene mediated diseases. The polymorphisms identified in the present invention occur in the 5' untranslated region and the first intron of the $LTC_4$ synthase gene, regions which are of

importance in the control of gene transcription and gene translation. Furthermore, each of the variant positions is located within a known transcription factor binding site; it is believed that substitution of A at variant position 375 modifies an AP-2 CS4 transcription factor binding site, substitution of A at variant position 815 modifies an AP-2 CS5 transcription factor binding site, substitution of C at variant position 1003 modifies the glucocorticoid receptor binding site GGGACA and substitution of T at variant position 2169 disrupts an MREc-(3) transcription factor binding site.

Example 3 below describes another polymorphism which is substitution of T for C at position 2742. This variant disrupts a RIPE3b site (Shieh and Tsai, J. Biol. Chem. **266**, 16708-16714, 1991).

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the $LTC_4$ synthase gene may therefore exhibit differences in their ability to regulate enzyme biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in enzyme regulation arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. $LTC_4$ synthase polymorphism may therefore have the greatest effect on the efficacy of drugs designed to modulate the activity of $LTC_4$ synthase or other components of the leukotriene pathway. However, the polymorphisms may also affect the response to agents acting on other biochemical pathways regulated by leukotrienes. The diagnostic methods of the invention may therefore be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by leukotrienes. $LTC_4$ synthase polymorphism may be particularly relevant in the development of asthma and other inflammatory diseases such as allergic rhinitis and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the $LTC_4$ synthase gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant

impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of sites recognised by restriction enzymes. In the accompanying Example 1 we provide details of convenient sites that are lost or gained as a result of $LTC_4$ synthase gene polymorphisms. The person of ordinary skill will be able to design and implement diagnostic procedures based on the detection of restriction fragment length polymorphism due to the loss or gain of one or more of the sites listed in Examples 1 or 3.

In yet a further aspect the invention provides a variant of the $LTC_4$ synthase gene comprising one or more of the specific polymorphisms at positions 375, 815, 1003 and 2169.

Further aspects of this invention comprise the 5' untranslated region of the $LTC_4$ synthase gene comprising a polymorphism at one or more of positions 375, 815 and 1003. In particular the polymorphism at position 375 is G to A. In particular the polymorphism at position 815 is C to A. In particular the polymorphism at position 1003 is A to C. Another aspect of this invention comprises the first intron of the $LTC_4$ synthase gene comprising a polymorphism at position 2169; in particular this polymorphism is C to T.

According to another aspect of the present invention there is provided a nucleic acid comprising the 5' untranslated region of $LTC_4$ synthase comprising a polymorphism corresponding with one or more of positions 375, 815 and 1003 as defined by the positions in SEQ ID NO: 1 and in which there is an A at position 375, an A at position 815 and a C at position 1003. The 5' untranslated region of $LTC_4$ synthase is defined as positions 1-1446 of SEQ ID NO: 1. Fragments of the 5' untranslated region comprising at least one of these allelic variants are also within the scope of the invention.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases. Complementary strands are also within the scope of the invention.

According to another aspect of the present invention there is provided a nucleic acid comprising the first intron of the $LTC_4$ synthase gene comprising a polymorphism at one or more of positions 2169 and 2742 as defined by the position in SEQ ID NO: 1 and in which there is a T at position 2169 and there is a T at position 2742. The first intron of the $LTC_4$ synthase gene is defined as positions 1505-2949 of SEQ ID NO: 1. Fragments of the first

intron comprising at least one of these allelic variants are also within the scope of the invention.

The invention further provides nucleotide primers which detect the $LTC_4$ synthase gene polymorphisms of the invention.

According to another aspect of the present invention there is provided a diagnostic nucleic acid primer capable of detecting a $LTC_4$ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

A diagnostic nucleic acid primer is defined as an allele specific primer, used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays, see Example 2 herein. The diagnostic primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

We provide diagnostic primers comprising the sequences set out below as well as derivatives thereof wherein about 6-8 of the nucleotides at the 3' terminus are identical to the sequences given below and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the diagnostic primer. Conveniently, the sequence of the diagnostic primer is as written below, or more preferably as described in Example 2 below. The diagnostic primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

| Primer number* | Allelic variant detected | Diagnostic (Allele Specific) Primer sequence |
|---|---|---|
| 1 | 375 A | GGGGCGGCCGGGGGCGCTCCAGGCGGGGCA |
| 2 | 815 A | CTTGGACAGGTTTCCTCCTGGCAGGGTGGA |
| 3 | 1003 C | GGGTTGCCAGGAACAGCCTGGATGGGGACC |
| 4 | 2169 T | ATGGTCCGACGGGAGGTCTGGGGAGGGAGT |
| 5 | 375 A | CTCCTGCCTGGAGTTCTGGGTGTCTCCCTT |
| 6 | 815 A | TAGTCGTTGTAGGGGTTCCATGCACAAGGT |
| 7 | 1003 C | TAACTCCTCCACCCACCTTATCTGTTCCCG |
| 8 | 2169 T | GACCACACACAGACCAGTGCTGGCTGTGCA |

* Primers 1-8 are represented as SEQ ID NO: 2-9 respectively.

The primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a $LTC_4$ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the $LTC_4$ gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided a diagnostic kit comprising a diagnostic primer of the invention and/or an allele-specific oligonucloetide primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

The $LTC_4$ synthase gene has been mapped to chromosome 5q35 (Penrose *et al*, J. Biol. Chem. **271**, 11356-11361, 1996). In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers for this region in linkage studies. This particularly applies to the polymorphism at 1003 because of its relatively high frequency, (Krugylak, Nature Genetics, **17**, 21-24, 1997).

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with an antileukotriene drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in $LTC_4$ synthase in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the $LTC_4$ synthase gene; and

ii) administering an effective amount of an antileukotriene drug.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antileukotriene drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs.

According to another aspect of the present invention there is provided use of an antileukotriene drug in preparation of a medicament for treating a leukotriene mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an antileukotriene drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

Suitable antileukotriene drugs include leukotriene $D_4$ receptor antagonists, FLAP antagonists and 5-lipoxygenase inhibitors (see particularly Table 1 in the following publication for a list of drugs, Horwitz *et al.* (1998), Am J Respir Crit Care Med, **157**, 1363), preferably leukotriene $D_4$ receptor antagonists, more preferably montelukast and zafirlukast, and of these zafirlukast is most preferred.

Testing for the presence of the C allele at position 1003 is especially preferred because, without wishing to be bound by theoretical considerations, of its association with increased levels of $LTC_4$ synthase (as explained herein).

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

**Identification of Polymorphisms**

**1. Methods**

DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 40 cycles of PCR.

| Fragment | Forward Oligonucleotide | Reverse Oligonucleotide | Annealing Temperature | Time | % DMSO |
|---|---|---|---|---|---|
| 62-1043 | 62-87 | 1021-1043 | 62° | 60 s | 5 |

| | | | | | |
|---|---|---|---|---|---|
| 271-407 | 271-291 | 388-407 | 60° | 45 s | 0 |
| 417-1043 | 417-437 | 1021-1043 | 60° | 45 s | 10 |
| 851-1824 | 851-874 | 1801-1824 | 62° | 60 s | 5 |
| 1503-2400 | 1503-1524 | 2379-2400 | 65° | 60 s | 10 |

For dye-primer sequencing these primers were modified to include T7 and SP6 primer sequences (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the forward and reverse oligonucleotides respectively.

Chemical Mismatch Cleavage (CMC)

CMC was carried out as decribed by Rowley *et al.* (Genomics **30**, 574-582, 1995) using internal labelling of probe and target with fluorescent dyes (RG6 or R110). 6 % Acrylamide gels were run on an automated DNA sequencer (ABI 377, Applied Biosystems) on 12 cm plates (under module GS12-2400A) and analysed with suitable software (ABI GeneScan™ 2.1).

Dye Primer Sequencing

Dye-primer sequencing using T7 and SP6 primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS"™ DNA polymerase, modified in that the annealing temperature was 45° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

**2. Results**

All positions are based on the U50136 numbering.

Variant Position 375

CMC analysis of fragment 1 (62-1043) produced cleavage products of approximately 300 bp and 670 bp in 9/49 subjects. Dye-primer sequence analysis of fragment 1 from 2 subjects showing this pattern revealed a substitution of A for G at position 375. This was

confirmed by sequencing 6 clones of fragment 1 from one of these subjects; 5/6 had A at position 375 and 1/6 had G.

Substituting A for G at position 375 modifies a Mnl I site at position 368. PCR products from $LTC_4$ synthase position 271 to 407 from 49 subjects were digested with Mnl I. This product contains an invariant Mnl I site at position 335 giving an invariant 61 bp fragment and a polymorphic fragment, 72 bp in the absence of site 368 or 33 and 39 base pairs if the Mnl I site at 368 is present. 9/49 subjects gave both the 72 bp and 33/39 bp products indicating that the Mnl I site at position 368 was lost from one allele and these subjects were heterozygous at position 375. The frequency of the A allele at 375 is thus 9/98.

Additional RFLPs generated by this variant are loss of an M.CviA IV and M.Sss I site.

This variant modifies a transcription factor binding site AP-2 CS4.

Variant Position 815

CMC analysis of fragment 1 produced cleavage products of approximately 230 bp and 750 bp in 2/49 subjects. Dye-primer sequence analysis of fragment 1 from 1 subject showing this pattern revealed a substitution of A for C at position 815. This was confirmed by sequencing 8 clones of fragment 1 from this subject. 4/8 had A at position 815 and 4/8 had C.

Substituting A for C at position 815 generates an Ava II site at 813.

PCR products from $LTC_4$ synthase position 417 to 1043 from 53 subjects were digested with Ava II. In 5/53 subjects an Ava II site at position 815 was created. These subjects were heterozygous at position 815. The frequency of the A allele was thus 5/106 alleles.

Additional RFLPs generated by this variant are loss of Aca I, CviK I, M.CviA IV, CviJ I and Hae III sites and gain of Asp697 I, VpaK11A I and Sin I sites.

This variant modifies a transcription factor binding site: AP-2 CS5.

Variant Position 1003

CMC analysis of fragment 1 produced a cleavage product of approximately 940 bp in 22/49 subjects. CMC analysis of fragment 2 (851-1824) produced a band of approximately 800 bp. Dye-primer sequence analysis of fragment 2 from 24 subjects showing this pattern revealed a substitution of C for A at position 1003. This was confirmed by sequencing 14 clones of fragment 1 from 2 subjects with the 940 bp cleavage product. 6/14 had C at position 1003 and 8/14 had A.

Substituting C for A at position 1003 generates an Ava II site at position 999.

PCR products from $LTC_4$ synthase position 417 to 1043 from 53 subjects were digested with Ava II. In 26/53 subjects an Ava II site at position 1003 was created. One of these subjects was homozygous C/C at position 815 and 25 were heterozygous C/A. The frequency of the C allele was thus 27/106 alleles.

The 1003 C variant is not on the same chromosome as the 815 A variant.

Additional RFLPs generated by this variant are gain of sites for Bcr I, AhaB I, Asp697 I, VpaK11A I, Asu I, Fmu I, Sau96 I, Sin I, Nla IV, Asp I, Asp748 I, BsaC I, Dsa V, Eco1831 I, Hin2 I, Hpa II, Msp I, Bcn I, Nci I, ScrF I and M.Sss I.

This variant modifies the glucocorticoid receptor binding site GGGACA, (Chan *et al.*, J. Biol. Chem. **266,** 22634-22644, 1991).

Sanak *et al.* (1998), Lancet, **350**, 1599, have reported an increased risk of aspirin induced asthma (AIA) being associated with this polymorphism (Sanak's position -444 is equivalent to our position 1003). AIA affects about 10 % of adult asthmatics. Aspirin and other cyclo-oxygenase inhibitors cause release of LTs into airways, leading to an asthma attack. Clinical approaches to deal with AIA include pretreatment with anti-leukotriene drugs (Szczeklik (1997), Allergy, **52**, 613-9). Commentators have written approvingly of the clinical utility of detection of $LTC_4$ polymorphisms (Holgate (1998), Lancet, **351**, 1300-1301, see last paragraph in particular).

Variant Position 2169

Dye-primer sequencing of fragment 6 (1503-2400) from 47 subjects demonstrated a substitution of T for C at position 2169 in 3 subjects.

Substituting T for C at position 2169 generates an Apa LI site at position 647.

Fragment 6 was digested with ApaL I. In 3/54 subjects an Apa LI site was created. These subjects were heterozygous for the RFLP, thus the frequency of the T allele at position 2169 is 3/108 alleles.

Additional RFLPs generated by this variant are loss of M.CviA IV, Bca I, Hinp I, Hinp1 I, Cfo I, Hha I and M.Sss I sites and gain of Aaq I, Bka1125 I, BsaG I, CviR I, BsiHKA I, Bsp 1286 I, Hgi A I and Nsp II sites.

This variant disrupts an MREc-(3) site (Labbe *et al.*, Nuc. Acid Res. **19,** 4225-4231 1991).

Example 2

**Detection of Variants 375, 815, 1003 and 2169 using ARMS™.**

The following primers were used in ARMS™ PCR to distinguish allelic variants at positions 375,815, 1003 and 2169 of the $LTC_4$ synthase gene.

| Allelic Variant Detected | Allele Specific Primer Sequence** | Constant Primer Sequence*** |
|---|---|---|
| 375 G | GGAGTTCTGGGTGTCTCCATC | GGTCAGTCTGGACTTTGCCAC |
| A | GGAGTTCTGGGTGTCTCCATT | |
| 815 C | TAGGGGTTCCATGCACAAGGG | TTGTTACCTTGAGGCAAGAGG |
| C | TAGGGGTTCCATGCACAATGG | |
| A | TAGGGGTTCCATGCACAAGGT | |
| A | TAGGGGTTCCATGCACAATGT | |
| 1003 A | CACCCACCTTATCTGTTCCCT | AGGCTGGCAGGCATGAGGTTT |
| C | CACCCACCTTATCTGTTCCCG | |
| A | GGAACAGCCTGGATGGGGTCA | TTCGTGCCCCTTCCTTGCCTA |
| C | GGAACAGCCTGGATGGGGTCC | |
| 2169 C | CAGACCAGTGCTGGCTGTACG | CTCCAGCTGCTCCTGCACTGA |
| T | CAGACCAGTGCTGGCTGTACA | |

** These primers are represented by SEQ ID NO: 10-21 respectively.

*** These primers are represented by SEQ ID NO: 22-26 respectively.

Genomic DNA (50 ng) was amplified for 35 cycles with the above pairs of primers. The annealing temperatures were 62°, 60°, 64° and 60° for 375, 815, 1003 and 2169 respectively.

Homozygotes for the less common allele were only available for position 1003. The above primers and conditions would distinguish A/A, A/C and C/C genotypes at position 1003.

375 A/A homozygotes were not available so it could not be demonstrated that the G specific primer would not recognise A/A homozygotes but the A specific primer did not

recognise G/G homozygotes. 815 A/A homozygotes were not available so it could not be demonstrated that the C specific primer would not recognise A/A homozygotes but the A specific primer did not recognise C/C homozygotes. 2169 T/T homozygotes were not available so it could not be demonstrated that the C specific primer would not recognise T/T homozygotes but the T specific primer did not recognise C/C homozygotes.

Example 3

**Polymorphism at Position 2742**

Dye primer sequencing, as described in Example 1, of fragment 2180-2972 from 5 subjects demonstrated a substitution of T for C at position 2742 in 2 subjects. Template was prepared as described in Example 1 using the conditions set out below.

| Fragment | Forward Oligonucleotide | Reverse Oligonucleotide | Annealing Temperature | Time | % DMSO |
|---|---|---|---|---|---|
| 2180-2973 | 2180-2200 | 2953-2973 | 65° | 60 s | 10 |

This variant disrupts a RIPE3b site (Shieh and Tsai, J. Biol. Chem. **266**, 16708-16714, 1991).

## CLAIMS

1. A method for the diagnosis of a single nucleotide polymorphism in $LTC_4$ synthase in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the $LTC_4$ synthase gene.

2. A method according to claim 1 in which the single nucleotide polymorphism at position 375 is presence of G and/or A.

3. A method according to claim 1 in which the single nucleotide polymorphism at position 815 is presence of C and/or A.

4. A method according to claim 1 in which the single nucleotide polymorphism at position 1003 is presence of A and/or C.

5. A method according to claim 1 in which the single nucleotide polymorphism at position 2169 is presence of C and/or T.

6. A method according to claim 1 in which the single nucleotide polymorphism at position 2742 is presence of C and/or T.

7. A method according to any one of claims 1-6 in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

8. A nucleic acid comprising the 5' untranslated region of $LTC_4$ synthase comprising a polymorphism corresponding with one or more of positions 375, 815 and 1003 as defined by the positions in SEQ ID NO: 1 and in which there is an A at position 375, an A at position 815 and a C at position 1003.

9. A nucleic acid comprising the first intron of the $LTC_4$ synthase gene comprising a polymorphism at one or more of positions 2169 and 2742 as defined by the position in SEQ ID NO: 1 and in which there is a T at position 2169 and there is a T at position 2742.

10. A diagnostic nucleic acid primer capable of detecting a $LTC_4$ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

11. An allele-specific oligonucleotide probe capable of detecting a $LTC_4$ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

12. A diagnostic kit comprising a diagnostic primer as defined in claim 10 and/or an allele-specific oligonucleotide primer as defined in claim 11.

13. A method of treating a human in need of treatment with an antileukotriene drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in $LTC_4$ synthase in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 375, 815, 1003, 2169 and 2742 in the $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the $LTC_4$ synthase gene; and

ii) administering an effective amount of an antileukotriene drug.

14. Use of an antileukotriene drug in preparation of a medicament for treating a leukotriene mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in $LTC_4$ synthase gene as defined by the positions in SEQ ID NO: 1.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Zeneca Limited
(B) STREET: 15 Stanhope Gate
(C) CITY: London
(D) STATE: England
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): W1Y 6LN
(G) TELEPHONE: 0171 304 5000
(H) TELEFAX: 0171 304 5151
(I) TELEX: 0171 304 2042

(ii) TITLE OF INVENTION: METHODS

(iii) NUMBER OF SEQUENCES: 26

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4465 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAGCTCACAG AGCCCCCAGC TGGGGCATAT CTGGTTTCCG GGGGCAGGGG CGATACCCAG 60
AGGAGGAAGA AGGGATTCTG AGAGAGCCCA ACAGGCTCCG AGCCTCAGGC TGGAGCTGAG 120
CTTGGGGCAG CAAGGAAGGA CCAGGTGCGA GGGCAGAACC ATGCGGCCCG ACCCCTGCAG 180
CACGGCCTGT GGCCTCCCCC AGCTCCTGCC CGTGCTTCTG GGTCAGTCTG GACTTTGCCA 240
CTTCTGACCA AAAGCCACCG CAAACCCACT CAAGCCAAAA GAGGAAGTGA CCGTTAGGCC 300
CAACTGGGAA GGCTGGCGGC CAGGGGCACT CCAGGCAGGG CGAGGGGGGC GGCCGGGGGC 360
GCTCCAGGCG GGGCGAGGGA GACACCCAGA ACTCCAGGCA GGAGTCCTCG GGTGCCACCT 420
TTCCTCTCCA CCTGGCCCTG CGTGGGCTCT GTCCTCAGGG TGGCCCGCCG TAGTCCCCCT 480
CCCCACTCTG AGTTTCCTGT CCCAAAGTCC TAAGGAAGTT TCCAGAACTA CATCTCACCA 540

TCTTGAGTCA GCCTTGGCTC AGTGTCCATC TCACAGGCCT GGAAGGGGCA GGAGTCAGCA 600
CTGTCCAGAC CACAGGGCCT GAGTGTGGGG AGGGCAGCCG TCTAGGAAGG TGGTGGAGGG 660
TTGTTACCTT GAGGCAAGAG GGCTGCGGGG CAGAAAGACA CAGCAGGTGA CTGTTGTGGG 720
AGGCCCAAGA GAGGCCTGGG AGAGGGATGG CCCACAAGGG CTGACCCTCC CGCCACCCAG 780
GGGGCCTTGG ACAGGTTTCC TCCTGGCAGG GTGGCCCTTG TGCATGGAAC CCCTACAACG 840
ACTAAGGCTG GCAGGCATGA GGTTTCCTGA AGGAGAAAGA GCTTGTGGGG CCCAGTGTGG 900
CTGGGGGGGC GCTGGGACTC CATTCTGAAG CCAAAGGCAC TGGGAAGGGC TTCCGCAGAG 960
GAGGGTTTGG CAGGGGTTGC CAGGAACAGC CTGGATGGGG ACAGGGAACA GATAAGGTGG 1020
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CCTGGAGAAA GTGGGGTGGT GCAGCAGGGG GCACACCCAC AGCTGGAGCT GACCCAGATG 1140
GACAGCTTGG GCTCTGCCAC GCGGGACTAG GCAAGGAAGG GGCACGAACA AGCAGGAAGT 1200
GGTGAGGCGG TCTCCAGCTA GCTGCTCTCC CCTGCCCAGA CTTTGGTTTC CTCCCTGCTG 1260
GCTTGGCCTG GCTCCCTGGC TCTGTGTGGT ATGGTCACAC CCCCGTGCAC CCCCTCCACT 1320
GAGATGGGGC GGGGAGAGCA CCGAGGCTGC TCTTCCTCTC CTGGGCCGTC CTCTGAGCAG 1380
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CAAGGTGGGC TGGTTCCTAT CTAGGAAGAG GGTGGGCCTT AGATCCCTAC AGCTTGCCCT 1560
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TGCTTCAAAG GCACCTCCAT ACTGGGACCA CGTGGAGCAG GGTAGAGGTG GGACTCCTTC 1860
CTCCAGCCCC CTAAAAAGAG CCTGCTTAAT GCCTTTCTCA GACTGGCCCT AAAGGACACA 1920
TTCCTTGGCC AGATATCCTT GCCACCTAAG AGACACCACT ACTCCACAGT GTGTGGGCTA 1980
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TGGAGGCACA GGGACGCAGG CTTTAGTGAT CCCGGCCTGA GGCAGGGTCA GAGGGCCCTG 2460
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CGCCGCTCAC CACCGGCCCA CCCGAGTTCG AGCGCGTCTA CCGAGCCCAG TGAGGCGCGG 3060
CGGGAGGGCG CGGGGCGGGG AGCGAGCCCC AGGCGGGTCC GGGTCGCAGG ACCATCCCGG 3120
CCGGCGCGCT CATCCCACCC GCCCACCGCA GGGTGAACTG CAGCGAGTAC TTCCCGCTGT 3180
TCCTCGCCAC GCTCTGGGTC GCCGGCATCT TCTTTCATGA AGGTCGGGGT GTGGGGCAGG 3240
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AGGGCTACGC GCGCTCCGCG CAGCTCAGGT GAGGGCCGGG CGGGGAGCGG GGCGGGGCCG 3420
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CGGGCCGGAG CCCAGCGCCT TTGGGGATTC GGTGGGCGAG CCCTGGCGGC GGCCAGAGGA 3540
AGTCCCCGTG GGGCCAGGGT TGCGGCGGGG AAGAAGCGGG CCTCCTCGCG CCACCTCCCC 3600
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GCTCCTCGGA CGGCTCCGGA CGCTGCTGCC GTGGGCCTGA GACCAAGGCC CCCGGGCCGA 3780
CGGAGCCGGG AAAGAAGAGC CGGAGCCTCC AGCTGCCCCG GGGAGGGGCG CTCGCTTCCG 3840
CATCCTAGTC TCTATCATTA AAGTTCTAGT GACCGAGACC CGGGCTGCGT TCTCTGGGTC 3900
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ACAAGGCCGC CCAGTCCTGC CGGGGTTCAC CCTCCTAGCG CTCAGCGGTC TCCTCACCGG 4140
TCCCCCTCCT CAGGGGCCTT CCCTCGACTC TCAGCCGCCG CAGTCCCTCG TCCCCTGGCC 4200
TTCACAGCTG ACACTAGATA GAGCCTGTGG CTCTCTCCCC AGGTGAGGGC AGGGGTTTTT 4260
CTTTTGGTCA GCACTGGATC CCCCTCGTTA ACTGTAGGTG TTCAGGGCAG CCCTCCGAGG 4320
TCCGCAGAGC TGCGGGCACC ATGGGAACGA AGTGAGTCAG TGACAGGCGG TCTCAAGGAA 4380
ATGTCCAGAA GCCTTGGGGA TCCAGGGGAG GCCCACAGAA ACAAAGAAGT GACTTTTAGC 4440
CAAGTATGCA GGAGAAACGG AGGAG 4465

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGGGCGGCCG GGGGCGCTCC AGGCGGGGCA 30

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTTGGACAGG TTTCCTCCTG GCAGGGTGGA 30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGTTGCCAG GAACAGCCTG GATGGGGACC 30

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATGGTCCGAC GGGAGGTCTG GGGAGGGAGT 30

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTCCTGCCTG GAGTTCTGGG TGTCTCCCTT 30

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAGTCGTTGT AGGGGTTCCA TGCACAAGGT 30

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAACTCCTCC ACCCACCTTA TCTGTTCCCG 30

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACCACACAC AGACCAGTGC TGGCTGTGCA 30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGAGTTCTGG GTGTCTCCAT C 21

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGAGTTCTGG GTGTCTCCAT T 21

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TAGGGGTTCC ATGCACAAGG G 21

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TAGGGGTTCC ATGCACAATG G 21

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAGGGGTTCC ATGCACAAGG T 21

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAGGGGTTCC ATGCACAATG T 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CACCCACCTT ATCTGTTCCC T 21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CACCCACCTT ATCTGTTCCC G 21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGAACAGCCT GGATGGGGTC A 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGAACAGCCT GGATGGGGTC C 21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CAGACCAGTG CTGGCTGTAC G 21

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAGACCAGTG CTGGCTGTAC A 21

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGTCAGTCTG GACTTTGCCA C 21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTGTTACCTT GAGGCAAGAG G 21

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AGGCTGGCAG GCATGAGGTT T 21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TTCGTGCCCC TTCCTTGCCT A 21

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTCCAGCTGC TCCTGCACTG A 21

# INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/GB 98/02468

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 95 33839 A (SEARLE & CO ;CREELY DAVID PAUL (US); HAUSER SCOTT DAVID (US); WELS) 14 December 1995 see abstract see page 11, line 3 - page 12, line 7 see page 20, line 3 - line 33 see page 22, line 8 - page 24, line 3; claims 1,12,18; example 4 --- | 1,7,8, 10-14 |
| Y | WO 95 32280 A (BRIGHAM & WOMENS HOSPITAL) 30 November 1995 see abstract see page 5, line 10 - line 16 see page 15, line 25 - page 16, line 1 see page 29, line 7 - page 33, line 10; claims 4,6,11,12,14,20-23,27; examples 1,2,5 --- -/-- | 1,8,13, 14 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 November 1998 | 11/12/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Knehr, M |

2

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 98/02468

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 93 18178 A (PHILADELPHIA CHILDREN HOSPITAL) 16 September 1993<br>cited in the application<br>see the whole document | 1,7,<br>10-12 |
| Y | BUSSE W W: "The role of leukotrienes in asthma and allergic rhinitis"<br>CLINICAL AND EXPERIMENTAL ALLERGY,<br>vol. 26, 1996, pages 868-879, XP002086061<br>cited in the application<br>see abstract<br>see page 870, column 1, paragraph 2 - page 871, column 1, paragraph 1<br>see page 875, column 1, paragraph 1 - page 876, column 2, paragraph 2<br>see page 877, column 1, paragraph 2 - paragraph 3 | 13,14 |
| A | PENROSE J F ET AL.: "Molecular cloning of the gene for human leukotriene C4 synthase"<br>JOURNAL OF BIOLOGICAL CHEMISTRY,<br>vol. 271, no. 19, 1996, pages 11356-11361, XP002086062<br>cited in the application<br>* see especially Fig. 1 *<br>see the whole document | |
| A | WELSCH D J ET AL: "MOLECULAR CLONING AND EXPRESSION OF HUMAN LEUKOTRIENE-C4 SYNTHASE"<br>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,<br>vol. 91, October 1994, pages 9745-9749, XP002044917<br>* see especially Fig. 2 *<br>see the whole document | |
| A | NEWTON C R ET AL: "ANALYSIS OF ANY POINT MUTATION IN DNA. THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)"<br>NUCLEIC ACIDS RESEARCH,<br>vol. 17, no. 7, 11 April 1989, pages 2503-2516, XP000141596<br>cited in the application<br>see the whole document | |
| P,X | SANAK M ET AL.: "leukotriene C4 synthase promotor polymophism and risk of aspirin-induced asthma"<br>THE LANCET,<br>vol. 350, 1997, pages 1599-1600, XP002086063<br>cited in the application<br>see the whole document | 1,4,8,<br>10,11,<br>13,14 |

2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/02468

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 13
is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/GB 98/02468

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9533839 A | 14-12-1995 | AU 2638695 A | 04-01-1996 |
| WO 9532280 A | 30-11-1995 | NONE | |
| WO 9318178 A | 16-09-1993 | NONE | |