

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
24 January 2002 (24.01.2002)

PCT

(10) International Publication Number  
**WO 02/06523 A2**

- (51) **International Patent Classification<sup>7</sup>:** C12Q 1/68, A61K 31/00
- (21) **International Application Number:** PCT/EP01/07524
- (22) **International Filing Date:** 2 July 2001 (02.07.2001)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
00115353.5 14 July 2000 (14.07.2000) EP
- (71) **Applicant (for all designated States except US):** F. HOFFMANN-LA ROCHE AG [CH/CH]; 124, Grenzacherstrasse, CH-4070 Basle (CH).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** ACUNA, Gonzalo [CH/CH]; 5, Guggenbuehlstrasse, CH-8953 Dietikon (CH). FOERNZLER, Dorothee [DE/CH]; 4, Rathausgasse, CH-5600 Lenzburg (CH). LEONG, Diane, Uratsu [US/US]; 1914 El Dorado Avenue, Berkeley, CA 94707 (US).
- (74) **Agent:** WAECHTER, Dieter; 124, Grenzacherstrasse, CH-4070 Basle (CH).
- (81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- without international search report and to be republished upon receipt of that report
  - with sequence listing part of description published separately in electronic form and available upon request from the International Bureau
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 02/06523 A2

(54) **Title:** METHOD FOR DETECTING PRE-DISPOSITION TO HEPATOTOXICITY

(57) **Abstract:** This invention relates to a method for diagnosing a pre-disposition to drug induced liver toxicity which method comprises determining at least one single nucleotide polymorphism in the UDP-glucuronosyl transferase (UGT1) gene. Said method is based on determining specific single nucleotide polymorphisms in the UGT1 gene in a human being and determining the status of said human being by reference to polymorphism in UGT1. The invention further relates to diagnostic nucleic acids comprising within their sequence the polymorphisms as defined herein, to allele-specific primers and allele-specific oligonucleotide probes capable of hybridizing to such diagnostic nucleic acids and to diagnostic kits comprising one or more of such primers and probes for detecting a polymorphism in the UGT1 gene.

Case 20707Method for detecting pre-disposition to hepatotoxicity

The present invention relates to a method for diagnosing a pre-disposition to drug induced liver toxicity which method comprises determining the polymorphisms in the UDP-glucuronosyl transferase (UGT1) gene.

UGT1 is a member of the UDP glucuronosyltransferase (UGT) gene superfamily. UGT enzymes catalyze the addition of the glucuronosyl group from a nucleotide sugar to a small hydrophobic molecule (aglycone) in order to enhance the water solubility of endo- and xenobiotics. UGT enzymes are involved in the metabolism of a large number of drugs. For a review on this enzyme superfamily see *Pharmacogenetics* (1997) 7, 255-269. The presence of at least nine UDP-glucuronosyl transferase isoenzymes has been described in International patent application WO 92/12987.

One of these enzymes, the human bilirubin glucuronosyl transferase gene encoded at the UGT1 locus has been associated with gene defects by Ritter et al., *J. Clin. Invest.* (1992), 90, 150-155; Aomoo et al., *Biochem. Biophys. Res. Commun.* (1993), 197, 1239-1244; Moghrabi et al., *Am. J. Hum. Genet.* (1993), 53, 722-729; Labrune et al., *Hum. Genet.* (1994), 94, 693-697 and Seppen et al. *J. Clin. Invest.* (1994), 268, 2385-2391.

The term polymorphism relates to the observation that different nucleotides can occur at a given position in a specific DNA sequence. Genetic polymorphisms occur at random throughout the genome. Genetic polymorphisms may affect the function of a gene by altering the structure of the protein that the gene codes or by affecting the level of expression of that gene.

Genetic variations or polymorphisms among individuals are responsible to a great extent for the observable biological differences between individuals. Genetic variations are also responsible for the differences on how individuals respond to a drug.

*In vitro* experiments have shown that variations in *UGT1A6* and *UGT1A7* genes affect the enzymatic activity on specific substrates of the enzymes coded by these genes.

Genetic polymorphisms in the human *UGT1A6* (plasma phenol) UDP-glucuronosyl transferase and the pharmacological implications thereof have been described by Ciotti et al., *Pharmacogenetics* (1997), 7, 485-495. The cloned and isolated *UGT1A6\*2* allelic

variant (which contains the Thr181Ala and Arg 184Ser mutations) when expressed in COS cells metabolised, at pH 6.4, the substrates 4-nitrophenol, 4-tert-butylphenol, 3-ethylphenol, 4-ethylphenol, 4-hydroxycoumarin, butylated hydroxy anisole and butylated hydroxy toluene at only 27-75% of the rate of the wild-type isoenzyme. 1-Naphtol, 3-iodophenol, 7-hydroxycoumarin, and 7-hydroxy-4-methylcoumarin were metabolised at normal levels. 3-O-Methyl-dopa and methyl salicylate were metabolised at 41-74% and  $\beta$ -blockers at 28-69% of the rate of the wild-type isoenzyme.

Guillemete et al., Pharmacogenetics (2000), 10, 629-644, showed that three different cloned and isolated allelic variants of *UGT1A7*, when expressed in HEK cells, showed different catalytic activity towards the substrates 3-, 7- and 9-hydroxy-benzo-(a)-pyrene as compared to the wild-type enzyme. *UGT1A7*\*3 (Lys 129 Lys131 Arg208) exhibited 5.8-fold lower  $V_{max}$  relative to the wild-type *UGT1A7*\*1 (Asn129 Arg131 Trp208), whereas *UGT1A7*\*2 (Lys129 Lys131 Trp208) and *UGT1A7*\*4 (Asn129 Arg131 Arg208) had a 2.6 and 2.8-fold lower relative  $V_{max}$  than *UGT1A7*\*1. While the results mentioned above indicate that genetic variations in the *UGT1* genes, when cloned and expressed in cultured cells, can affect the enzymatic activity of the corresponding gene products on a particular substrate, they show no proof that these variations have a significant effect on the metabolism of these substrates in human individuals.

It has now been found that specific genetic variations within the *UGT1* gene complex, including *UGT1A6* and *UGT1A7*, affect the response of individual human patients to a drug which is metabolised by these enzymes.

Pharmacogenetics is an approach to use the knowledge of polymorphisms to study the role of genetic variation among individuals in variation to drug response, a variation that often results from individual differences in drug metabolism. Pharmacogenetics helps to identify patients most suited to therapy with particular pharmaceutical agents. This approach can 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. Details on pharmacogenetics and other uses of polymorphism detection can be found in 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.

As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and

the association between genetic variation at the CBS locus and plasma levels of homocysteine (De Stefano et al., *Ann. Hum. Genet.* (1998) 62, 481-90). Variation at the von Willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter  
5 (Keightley et al., *Blood* (1999) 93, 4277-83).

It has been found that in rare cases the administration of pharmaceutically active agents to human beings leads to hepatotoxicity. A typical example is the occurrence of reversible asymptomatic increase in liver transaminases activity found in certain patients with Parkinson's disease (PD) who had participated in clinical trials for tolcapone. The  
10 studies indicated that in rare circumstances, tolcapone could induce a reversible asymptomatic increase in liver transaminase activity. There was therefore a desire to establish whether there is a correlation between the occurrence of such liver abnormalities, which are indicators of liver toxicity and certain genetic pre-dispositions. It has now been found that variations in genes involved in tolcapone (TASMAR) metabolism and  
15 pharmacology cause abnormalities in metabolic activity in certain individuals, resulting in an accumulation in the liver of this drug or its metabolites to toxic levels.

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

20 The present invention therefore provides a genetic diagnostic tool for identifying the pre-disposing genotypes. Said tool consists of a method for detecting a predisposition to a hepatotoxic reaction caused by the administration of a pharmaceutically active compound to a human being based on the determination of at least one single nucleotide polymorphism in the UDP-glucuronosyltransferase (UGT1) gene in the sample of said  
25 human being, which method comprises determining the nucleotide at position 908 in exon 5 of the UGT1 gene as defined by the position in SEQ ID NO:1 and determining the status of the human being by reference to polymorphism in UGT1. Alternatively or, in addition thereto, the method comprises determining the sequence of the nucleic acid of the human being at position 528 in exon 1 of the UGT1A6 gene as defined by SEQ ID NO:2 or  
30 determining the sequence of the nucleic acid of the human being at position 197 in exon 1 of the UGT1A7 gene as defined by sequence ID NO:3 and determining the status of said human being by reference to polymorphism in UGT1.

An individual possesses a predisposition to a hepatotoxic reaction when his UGT1 gene contains variations which lead to abnormalities in its metabolic activity.

SEQ ID NO:1 refers to Genbank accession number M84124, which provides exon 5 of UGT1:

```

5      1  TAATTCCAGC TACTCTGGAG GCTGAGGCAG GAGGATGGCT TGAGCCCAGG
      51  AGTTGGAGGC TGCAGTTAGC CATGCTTGTG CCACTACACT CCAGCCCGGG
     101  CAACAGGGCA AGACTCTGTA TCTAAAAACA ACAACAACAA CAATAATAGA
     151  AACAGGTTTC CTTTCCCAAG TTTGGAAAAT CTGGTAGTCT TCTTAAGCAG
     201  CCATGAGCAT AAAGAGAGGA TTGTTCATAC CACAGGTGTT CCAGGCATAA
     251  CGAAACTGTC TTTGTGTTTA GTTACAAGGA GAACATCATG CGCCTCTCCA
     301  GCCTTCACAA GGACCGCCCG GTGGAGCCGC TGGACCTGGC CGTGTTCCTGG
     351  GTGGAGTTTG TGATGAGGCA CAAGGGCGCG CCACACCTGC GCCCCGCAGC
     401  CCACGACCTC ACCTGGTACC AGTACCATTC CTTGGACGTG ATTGGTTTCC
     451  TCTTGGCCGT CGTGCTGACA GTGGCCTTCA TCACCTTTAA ATGTTGTGCT
     501  TATGGCTACC GGAAATGCTT GGGGAAAAAA GGGCGAGTTA AGAAAGCCCA
     551  CAAATCCAAG ACCCATTGAG AAGTGGGTGG GAAATAAGGT AAAATTTTGA
     601  ACCATTCCCT AGTCATTTCC AACTTGAAA ACAGAATCAG TGTTAAATTC
     651  ATTTTATTCT TATTAAGGAA ATACTTTGCA TAAATTAATC AGCCCCAGAG
     701  TGCTTTAAAA AATTCTCTTA AATAAAAATA ATAGACTCGC TAGTCAGTAA
     751  AGATATTTGA ATATGTATCG TGCCCCCTCT GGTGTCTTTG ATCAGGATGA
     801  CATGTGCCAT TTTTCAGAGG ACGTGCAGAC AGGCTGGCAT TCTAGATTAC
     851  TTTTCTTACT CTGAAACATG GCCTGTTTGG GAGTGCGGGA TTCAAAGGTG
     901  GTCCCACGGC TGCCCCTACT GCAAATGGCA GTTTTAATCT TATCTTTTGG
     951  CTTCTGCAGA TGGTTGCAAT TGATCCTTAA CCAATAATGG TCAGTCCTCA
    1001  TCTCTGTCGT GCTTCATAGG TGCCACCTTG TGTGTTTAAA GAAGGGAAGC
    1051  TTTGTACCTT TAGAGTGTAG GTGAAATGAA TGAATGGCTT GGAGTGCACCT
    1101  GAGAACAGCA TATGATTTCT TGCTTTGGGG AAAAAAGATG ATGCTATGAA
    1151  ATTGGTGGGT GGTGTATTTG AGAAGATAAT CATTGCTTAT GTCAAATGGA
    1201  GCTGAATTTG ATAAAAACCC AAAATACAGC TATGAAGTGC TGGGCAAGTT
    1251  TACTTTTTTT CTGATGTTTC CTACAACATA AAATAAATTA ATAAATTTAT
    1301  ATAAATTCTA TTTAAGTGTT TTCACTGGTG TCGCATTTAT TTCTTGTTAA
```

1351 GTTGCATTTT CTAATTACAA AAGTAATGCA TGATTATGAC AGAAAGTTTG  
 1401 GAAAATATAG AGGTTACACAC ACACACGCCT TCATTGCGTG TGCATGCATA  
 5 1451 AATGCATGAG AAAAGAAAAA TAACCAGTAA TCACATCGCC CAGAAATAAC  
 1501 CCCAGTTACA ATTGTGGCAA ATACACATAC TTATAAATAT TGCAGATATA  
 1551 TTAAGTATAC C

The present invention is based on the discovery of novel single nucleotide polymorphisms (SNP) in the UGT1 gene locus, viz.

at position 908 of SEQ ID NO:1. The polymorphism at this position consists of the replacement of the nucleotide G at this position by a C in exon 5 of the UGT1 gene locus;

15 at position 528 of SEQ ID NO:2. The polymorphism at this position consists of the replacement of the nucleotide A at this position by a G in exon 1 of the UGT1A6 gene;

and at position 197 of SEQ ID NO:3. The polymorphism at this position consists of the replacement of the nucleotide C at this position by a G in exon 1 of the UGT1A7 gene.

20 As defined herein, the UGT1 gene includes exon coding sequences for all different UGT1A isozymes, intron sequences intervening the exon sequences and 3' and 5' untranslated region (3' UTR and 5' UTR) sequences, including the promoter element of the UGT1 gene, encoding for all UGT1A isozymes.

SEQ ID NO:2 refers to Genbank accession number M84130, which provides exon 1 of UGT1A6:

25 1 TGACACGGCC ATAGTTGGTT CATATTAACC ATGTGATTAA AATGGTTAAA  
 51 TATTAATTTG GGTCTTACA TATCAAAGGG TAAAATTCAG AGCAAGGGAG  
 30 101 AGGTAGACAG GACCTGTGAA AAGCAGTGGT TAGTTTAGGG AAAATACCTA  
 151 GGAGCCCTGT GATTTGGAGA GTGAAACTC TTTATTACCG TTGTTACTTT  
 201 AACTCTTTCC AGGATGGCCT GCCTCCTTCG CTCATTTTCAG AGAATTTCTG  
 35 251 CAGGGGTTTT CTTCTTAGCA CTTTGGGGCA TGGTTGTAGG TGACAAGCTG  
 301 CTGGTGGTCC CTCAGGACGG AAGCCACTGG CTTAGTATGA AGGATATAGT  
 40 351 TGAGGTTCTC AGTGACCGGG GTCATGAGAT TGTAGTGGTG GTGCCTGAAG  
 401 TTAATTTGCT TTTGAAAGAA TCCAAATACT ACACAAGAAA AATCTATCCA  
 451 GTGCCGTATG ACCAAGAAGA GCTGAAGAAC CGTTACCAAT CATTTGGAAA

501 CAATCACTTT GCTGAGCGAT CATTCCTAAC TGCTCCTCAG ACAGAGTACA  
551 GGAATAACAT GATTGTTATT GGCCTGTACT TCATCAACTG CCAGAGCCTC  
5 601 CTGCAGGACA GGGACACCCCT GAACTTCTTT AAGGAGAGCA AGTTTGATGC  
651 TCTTTTCACA GACCCAGCCT TACCCTGTGG GGTGATCCTG GCTGAGTATT  
10 701 TGGGCCTACC ATCTGTGTAC CTCTTCAGGG GTTTTCCGTG TTCCCTGGAG  
751 CATAcATFCA GCAGAAGCCC AGACCCTGTG TCCTACATTC CCAGGTGCTA  
801 CACAAAGTTT TCAGACCACA TGACTTTTTTC CCAACGAGTG GCCAACTTCC  
15 851 TTGTTAATTT GTTGGAGCCC TATCTATTTT ATTGTCTGTT TTCAAAGTAT  
901 GAAGAACTCG CATCAGCTGT CCTCAAGAGA GATGTGGATA TAATCACCTT  
20 951 ATATCAGAAG GTCTCTGTTT GGCTGTAAAG ATATGACTTT GTGCTTGAAT  
1001 ATCCTAGGCC GGTcATGCCc AACATGGTCT TCATTGGAGG TATCAACTGT  
1051 AAGAAGAGGA AAGACTTGTC TCAGGTTGGT GGGTTTATTT CTTTTGGACT  
25 1101 GCCTTGTTTT TTCCAGGCTC TGTCCTCCCT CACTCATTTG GCTCCTTGAG  
1151 CCGACTGTCC CTTGGAGGAT TTCCTGGAGA ACGGTGGGGG GAAGTGATAC  
30 1201 CCGGCTCGGA GCAGCGGGAA

SEQ ID NO:3 refers to Genbank accession number U39570, which provides exon 1 of UGT1A7:

35 1 TGTATTATTA TGAGTAAATC ATTGGCAGTG AATGTGAATT TTTTTTTAAA  
51 TGAATGAATA AGTACACGCC TTCTTTTGAG GGCAGGTTCT ATCTGTACTT  
40 101 CTTCCACTTA CTATATTATA GGAGCTTAGA ATCCCAGCTG CTGGCTCTGG  
151 GCTGAAGTTC TCTGATGGCT CGTGCAGGGT GGACTGGCCT CCTTCCCCTA  
201 TATGTGTGTC TACTGCTGAC CTGTGCTTTG CCAAGGTCAG GGAAGCTGCT  
45 251 GGTAGTGCCC ATGGATGGGA GCCACTGGTT CACCATGCAG TCGGTGGTGG  
301 AGAAACTCAT CCTCAGGGGG CATGAGGTGG TCGTAGTCAT GCCAGAGGTG  
50 351 AGTTGGCAAC TGGGAAGATC ACTGAATTGC ACAGTGAAGA CTTACTCAAC  
401 CTCATAcACT CTGGAGGATC AGGACCGGGA GTTCATGGTT TTTGCCGATG  
451 CTCGCTGGAC GGCACCATTG CGAAGTGCAT TTTCTCTATT AACAAGTTCA  
55 501 TCCAATGGTA TTTTTGACTT ATTTTTTTCA AATTGCAGGA GTTTGTTTTAA  
551 TGACCGAAAA TTAGTAGAAT ACTTAAAGGA GAGTTGTTTT GATGCAGTGT

5  
 10  
 15  
 20  
 25  
 30  
 35  
 40

601 TTCTCGATCC TTTTGATCGC TGTGGCTTAA TTGTTGCCAA ATATTTCTCC  
 651 CTCCCCTCTG TGGTCTTCGC CAGGGGAATA TTTTGCCACT ATCTTGAAGA  
 701 AGGTGCACAG TGCCCTGCTC CTCTTTCTTA TGTCCCCAGA CTTCTCTTAG  
 751 GGTTC TCAGA CGCCATGACT TTCAAGGAGA GAGTATGGAA CCACATCATG  
 801 CACTTGGAGG AACATTTATF TTGCCCTAT TTTTTCAAAA ATGTCTTAGA  
 851 AATAGCCTCT GAAATTCCTC AAACCCCTGT CACGGCATAT GATCTCTACA  
 901 GCCACACATC AATTTGGTTG TTGCGAACTG ACTTTGTTTT GGAGTATCCC  
 951 AAACCCGTGA TGCCCAATAT GATCTTCATT GGTGGTATCA ACTGTATCA  
 1001 GGGAAAGCCA GTGCCTATGG TAAGTTATCT CCCCTTTAGC ACATTAAGAA  
 1051 TAATCTGGCT TTGGAAATTA AAAGATTTCT TACAGAATCA TAATTTATCA  
 1101 TTTACATTTG TCCCATTTGG AATTTCTTTC TGGTTTAAGG AATTCCTTTG  
 1151 TACCAATTCA CTTAATTGTT GGGTAGCAAA TTGTATAAAG CAGCTCTTGT  
 1201 TGATATGTAA GTGTATACAA TTGATATAAT TGTAGATCAT ATCTAGGCTG  
 1251 CAATCTAAAT GCTATTTTTG GAAAAATAC

Furthermore the invention relates to a method for detecting a predisposition to liver toxicity after administration of a pharmaceutically active compound based on one or more single nucleotide polymorphism(s) in the UDP-glucuronosyltransferase (UGT1) gene locus in a human being, wherein additionally the polymorphism at one of the following positions is determined:

- 232 in exon 1 of UGT1A6 as defined by the position of SEQ ID NO:2;
- 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3; or
- 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3.

The polymorphism at position 232 in exon1 of UGT1A6 of SEQ ID NO:2 consists of a replacement of the nucleotide T at this position by a G (which results in a Ser to Ala amino acid exchange at position 7 in the corresponding protein).

The polymorphism at position 754 of SEQ ID NO:2 consists of a replacement of the nucleotide A at this position by a G in exon 1 of UGT1A6 (which results in a Thr to Ala amino acid exchange at position 181 in the corresponding protein).

5 The polymorphism at position 765 of SEQ ID NO:2 consists of a replacement of the nucleotide A at this position by a C in exon 1 of UGT1A6 (which corresponds to a Arg to Ser amino acid exchange at position 184 of the corresponding protein).

The polymorphism at position 551 of SEQ ID NO:3 consists of the replacement of the nucleotide T at this position by a G in exon 1 of the UGT1A7 gene and results in an amino acid exchange from Asn to Lys at position 129 in the corresponding protein.

10 The polymorphism at position 555 of SEQ ID NO:3 consists of the replacement of the nucleotide C at this position by an A in exon 1 of the UGT1A7 gene. This may result in a silent mutation (if the nucleotide at position 556 of SEQ ID NO:3 consists of a G) or result in an amino acid exchange from Arg to Lys at position 131 in the corresponding protein (if the nucleotide at position 556 of SEQ ID NO:3 consists of an A).

15 The polymorphism at position 556 of SEQ ID NO:3 consists of the replacement of the nucleotide G at this position by an A in exon 1 of the UGT1A7 gene and results in an amino acid exchange from Arg to Gln at position 131 in the corresponding protein in case the nucleotide at position 555 is a C or in an amino acid exchange from Arg to Lys at position 131 in case the nucleotide at position 555 is an A.

20 The polymorphism at position 786 of SEQ ID NO:3 consists of a replacement of the nucleotide T at this position by a C in exon 1 of UGT1A7 (which results in a Trp to Arg exchange at position 208 in the corresponding protein).

Thus, the invention relates to a method of detecting a predisposition to liver toxicity after administration of a pharmaceutically active compound based on the determination of  
25 at least one single nucleotide polymorphism, in which the single nucleotide polymorphism at position 908 in exon 5 of the UGT1 gene locus consists of the presence of a C or a G, the single nucleotide polymorphism at position 528 in exon 1 of UGT1A6 consists of the presence of a G or an A, the single nucleotide polymorphism at position 197 in exon 1 of UGT1A7 consists of the presence of a G or a C, the single nucleotide polymorphism at  
30 position 232 in exon 1 of UGT1A6 consists of the presence of a G or a T, the single nucleotide polymorphism at position 754 in exon 1 of UGT1A6 consists of the presence of an A or a G, the single nucleotide polymorphism at position 765 in exon 1 of UGT1A6 consists of the presence of an A or a C, the single nucleotide polymorphism at position 551

in exon 1 of UGT1A7 consists of the presence of an G or a T, the single nucleotide polymorphism at position 555 in exon 1 of UGT1A7 consists of the presence of an A or a C, the single nucleotide polymorphism at position 556 in exon 1 of UGT1A7 consists of the presence of an A or a C, and the single nucleotide polymorphism at position 786 in exon 1  
5 of UGT1A7 consists of the presence of a C or a T.

A number of pharmaceutically active compounds are known which cause a hepatotoxic reaction. Examples of such compounds are nitrocatechol derivatives like entacapone, nitecapone or tolcapone. The main metabolic pathway for these drugs is glucuronidation.

10 Glucuronidation is an important pathway of elimination of many xenobiotics including drugs. The UGT1 enzymes are well-known to catalyze the glucuronidation of many endogeneous and exogeneous substrates, including many drugs. Pharmacokinetic experiments in human subjects have shown that the main pathway of tolcapone elimination from the body is glucuronidation (Jorga et al., Br. J. Clin. Pharmacol. (1999),  
15 4, 513-20. Deficiencies in the elimination pathway of a drug can be the cause of adverse effects. The present invention shows a first example of genetic variations, some of which known from in vitro experiments to affect the glucuronidation activity of a number of substrates including drugs, which are significantly associated with the development of adverse effects in human patients treated with a drug. From these results it can be  
20 concluded that the method described herein can be applied to predict the predisposition to adverse effects of any drug that is metabolised by UGT1 enzymes.

Drug glucuronidation by UGTs is a major phase II conjugation reaction in the mammalian detoxification system (Burchell et al., Life Sci. (1995), 57, 1819-31). Polymorphisms in UGTs can markedly affect binding of a substrate, which can be  
25 manifested either as a clinical syndrome (if an endogenous substrate is affected) or as a change of response to a drug and/or as a adverse event (if a drug is affected). Therefore it is important to identify genetic sequence polymorphisms in the UGT1 gene in general. Nucleid acids comprising the polymorphic sequences can be used in screening assays, and for genotyping individuals. The genotyping information can be used to predict an  
30 individual's rate of metabolism for UGT1 substrates, potential drug-drug interactions, and adverse/side effects as well as diseases that result from environmental or occupational exposure to toxins. The nucleic acids can be used to establish animal, cell and in vitro models for drug metabolism. All the following identified polymorphisms are amenable to be associated with an individual's rate of metabolism for UGT1 substrates, potential drug-

drug interactions, adverse/side effects and diseases that result from environmental or occupational exposure to toxins.

Table 1. SNP positions

gene	acc.no.	SNP	SNP pos.	substit.	AA subst.
UGT1A6	M84130 SEQ ID NO:2	exon 1	232	T/G	Ser7Ala
		exon 1	318	C/T	silent
		exon 1	528	A/G	silent
		exon 1	754	A/G	Thr181Ala
		exon 1	765	A/C	Arg184Ser
UGT1 A7	U39570 SEQ ID NO:3	5'UTR	108	T/G	silent
		exon 1	197	C/G	silent
		exon 1	551	T/G	Asn129Lys
		exon 1	555	C/A	silent or Arg131Lys
		exon 1	556	G/A	Arg131Gln or Lys
		exon 1	786	T/C	Trp208Arg
		exon 1	920	G/A	silent
		exon 1	824	C/T	silent
UGT1 A10	U39550 SEQ ID NO:4	exon 1	959	C/T	Asn/Lys
					silent
UGT1 A8	U42604 SEQ ID NO:5	prom	245	C/A	
UGT1 A9	AF056188 SEQ ID NO:6	exon1	214	C/T	silent
UGT ex2-5	M84122 SEQ ID NO:7	intron	117	C/T	
common to all UGT1As	M84123 SEQ ID NO:8 M84124 SEQ ID NO:1	intron	379	C/T	
		exon4	473	G/T	Gly/Val
		3'UTR	423	T/G	
		3'UTR	780	T/C	
		3'UTR	908	G/C	
		3'UTR	1012	C/G	

- 5 The SNP positions in Table 1 always refer to the position in the sequence with the specified accession number in the public domain and the corresponding SEQ ID NO. given

in this application. Primer sequences for genotyping assays are given in the method section. For nucleotide substitution the nucleotide of the wildtype allele is given first, same for the amino acid substitutions. SEQ ID NOs 1-3 are given above, SEQ ID NOs 4-8 are following below.

5 SEQ ID NO:4 refers to Genbank accession number U39550, which provides exon 1 of UGT1A10.

```

1   CTCTCCCTCC AAGGCGAAGA CCATAATCTA CTCTTGCTCTG AAATCATACA
10  51  AGTAGGTATC TCAGCAAATG ATACTCGTGT GTTATCGTTC TTATGAGTAA
    101  ATCATTGGCA GTGAGTGTGA TTTTTTTTTT TTTTATGAAA GGATAAATAC
    151  ACGCCCTCTA TTGGGGTCAG GTTTTGTGCC TGTACTTCTT CCGCCTACTG
15  201  TATCATAGCA GCTTAGAATC CCAGCTGCTG GCTCGGGCTG CAGTTCTCTC
    251  ATCGTCGCGC AGGGTGATGG CTCGCGCAGG GTGGACCAGC CCCGTTCCCT
20  301  TATGTGTGTG TCTACTGCTG ACCTGTGGCT TTGCCGAGGC AGGGAAGCTG
    351  CTGGTAGTGC CCATGGATGG GAGTCACTGG TTCACCATGC AGTCGGTGGT
    401  GGAGAAACTT ATCCTCAGGG GGCATGAGGT GGTGTAGTGC ATGCCAGAGG
25  451  TGAGTTGGCA ACTGGAAAGA TCACTGAATT GCACAGTGAA GACTTACTCA
    501  ACCTCGTACA CTCTGGAAGA TCAGAACCGG GAATTCATGG TTTTCGCCCA
30  551  TGCTCAATGG AAAGCACAGG CACAAAGTAT ATTTTCTCTA TTAATGAGTT
    601  CATCCAGTGG TTTTCTTGAC TTATTTTTTT CGCATGTCAG GAGTTTGTTT
    651  AATGACCGAA AATTAGTAGA ATACTTAAAG GAGAGTTCTT TTGATGCAGT
35  701  GTTCTGGAT CCTTTTGATA CCTGTGGCTT AATTGTTGCT AAATATTTCT
    751  CCCTCCCCTC TGTGGTCTTC ACCAGGGGAA TATTTTGCCA CCATCTTGAA
40  801  GAAGGTGCAC AGTGCCCTGC TCCTCTTTCC TATGTCCCCA ATGATCTCTT
    851  AGGGTTCTCA GATGCCATGA CTTTCAAGGA GAGAGTATGG AACCACATCG
    901  TGCACTTGGA GGACCATTTA TTTTGCCAGT ATCTTTTLAG AAATGCCCTA
45  951  GAAATAGCCT CTGAAATTCT CCAAACCCCT GTCACGGCAT ATGATCTCTA
1001 CAGTCACACA TCAATTTGGT TGTTGCGAAC GGACTTTGTT TTGGACTATC
50 1051 CCAAACCCGT GATGCCCAAC ATGATCTTCA TTGGTGGTAT CAACTGTCAT
    1101 CAGGGAAAGC CATTGCCTAT GGTAAGTCAC CTCTCCTTTA GCACATTAAG
    1151 AATAATCTGG CTTTGAATT AAAAAAGGAT TCCTTACTGA ACTGTGATTT
55 1201 GACATTTCGT TGTGGCATT C AATTTCTTTC CAGTTTAACA AATTATTTTG
    1251 TGCGAATTCA TGTACTCATC AATTATCAAA TTTTATAAAA CTGCCCTTCT
60 1301 TGAAAGTATA TGTAATAATT TAAAAATTAT AGATCATATT CAGGCTACAT

```

- 12 -

1351 TTTAAAATAC GATGTTTAGA AAAGTACCAA AAAACCACAG CAAGAAATGA  
 1401 AACTTCCGTT TTTTTGTTAT TCTATGTGAC CCCGTAGTTG AAAATGCTCT  
 5  
 1451 TA

SEQ ID NO:5 refers to Genbank accession number U42604, which provides exon 1 of UGT1A8:

10  
 1 GGGCATGATC TGTCCAAGGC AGAGACTATA AGCTACTCTT ATAGTACTCT  
 51 TATGAGATAC ATACAAGTAG GTATCTCAA AAATGATACT CATGTATTCC  
 15 101 TGTTCTTATG AGTAAATCAT TGGCAGTGAG TGTGATTTTT TTTTTTTTTTA  
 151 TGACAGGATC CCTACACGCC CTCTATTGGG GTCAGGTTTT GTGCCTGTAG  
 20 201 TTCTTCCGCC TACGTATCAT AGCAGTTAGA ATCCCAGCTG CTGGCTCGGG  
 251 CTGCAGTTCT CTCATGGCTC GCACAGGGTG GACCAGCCCC ATTCCCCTAT  
 301 GTGTTTCTCT GCTGCTGACC TGTGGCTTTG CTGAGGCAGG GAAGCTGCTG  
 25 351 GTAGTGCCCA TGGATGGGAG TCACTGGTTC ACCATGCAGT CGGTGGTGGA  
 401 GAAACTTATC CTCAGGGGGC ATGAGGTGGT TGTAGTCATG CCAGAGGTGA  
 451 GTTGGCAACT GGGAAAATCA CTGAATTGCA CAGTGAAGAC TTA CTCAACC  
 30 501 TCATACACTC TGGAGGATCT GGACCGGGAA TTCATGGATT TCGCCGATGC  
 551 TCAATGGAAA GCACAAGTAC GAAGTTTGT TCTCTATTT CTGAGTTCAT  
 35 601 CCAATGGTTT TTTTAACTTA TTTTTTTCGC ATTGCAGGAG TTTGTTTAAT  
 651 GACCGAAAAT TAGTAGAATA CTTAAAGGAG AGTCTTTTTG ATGCGGTGTT  
 701 TCTTGATCCT TTTGATGCCT GTGCGTTAAT TGTGCCAAA TATTTCTCCC  
 40 751 TCCCCTCTGT GGTCTTCGCC AGGGGAATAG GTTGCCACTA TCTTGAAGAA  
 801 GGTGCACAGT GCCCTGCTCC TCTTTCCTAT GTCCCCAGAA TTCTCTTAGG  
 45 851 GTTCTCAGAT GCCATGACTT TCAAGGAGAG AGTACGGAAC CACATCATGC  
 901 ACTTGGAGGA ACATTTATTT TGCCAGTATT TTTCCAAAAA TGCCCTAGAA  
 50 951 ATAGCCTCTG AAATTCTCCA AACACCTGTC ACAGCATATG ATCTCTACAG  
 1001 CCACACATCA ATTTGGTTGT TGCGAACAGA CTTTGTTTTG GACTATCCCA  
 1051 AACCCGTGAT GCCCAATATG ATCTTCATTG GTGGTATCAA CTGCCATCAG  
 55 1101 GGAAAGCCAT TGCCTATGGT AAGTCACCTC TCCTTTAGCA CATTAGGAAT  
 1151 AATCTTGGCT TTGGAAATTA AAAAAAGATT CCTTACTGAA TTGTGATTTG  
 1201 ACATTTTCAT TTGTTGCATT TCAAATTTCT TTCCAGTTTA CAGA  
 60

SEQ ID NO:6 refers to Genbank accession number AF056188, which provides exon 1 of UGT1A9:

```

5      1  CTCAGCTGCA GTTCTCTGAT GGCTTGCACA GGGTGGACCA GCCCCCTTCC
      51  TCTATGTGTG TGTCTGCTGC TGACCTGTGG CTTTGCCGAG GCAGGGAAGC
     101  TACTGGTAGT GCCCATGGAT1 GGGAGCCACT GGTTCACCAT GAGGTCGGTG
     151  GTGGAGAAAC TCATTCTCAG GGGGCATGAG GTGGTTGTAG TCATGCCAGA
     201  GGTGAGTTGG CAACTGGGAA GATCACTGAA TTGCACAGTG AAGACTTATT
     251  CAACTTCATA TACCCTGGAG GATCTGGACC GGGAGTTCAA GGCTTTTGCC
     301  CATGCTCAAT GGAAAGCACA AGTACGAAGT ATATATTCTC TATTAATGGG
     351  TTCATACAAT GACATTTTTG ACTTATTTTT TTCAAATTGC AGGAGTTTGT
     401  TTAAGACAAA AAAATTAGTA GAATACTTAA AGGAGAGTTC TTTTGATGCA
     451  GTGTTTCTCG ATCCTTTTGA TAACTGTGGC TTAATTGTTG CCAAATATTT
     501  CTCCCTCCCC TCCGTGGTCT TCGCCAGGGG AATACTTTGC CACTATCTTG
     551  AAGAAGGTGC ACAGTGCCCT GCTCCTCTTT CCTATGTCCC CAGAATTCTC
     601  TTAGGGTTCT CAGATGCCAT GACTTTCAAG GAGAGAGTAC GGAACCACAT
     651  CATGCACTTG GAGGAACATT TATTATGCCA CCGTTTTTTC AAAAATGCCC
     701  TAGAAATAGC CTCTGAAATT CTCCAAACAC CTGTTACGGA GTATGATCTC
     751  TACAGCCACA CATCAATTTG GTTGTGCGA ACGGACTTTG TTTTGGACTA
     801  TCCCAAACCC GTGATGCCCA ACATGATCTT CATTGGTGGT ATCAACTGCC
     851  ATCAGGGAAA GCCGTTGCCT ATGGAATTTG AAGCCTACAT TAATGCTTCT
     901  GGAGAACATG GAATTGTGGT TTTCTCTTTG GGATCAATGG TCTCAGAAAT
     951  TCCAGAGAAG AAAGCTATGG CAATTGCTGA TGCTTTGGGC AAAATCCCTC
    1001  AGACAGTCCT GTGGCGGTAC ACTGGAACCC GACCATCGAA TCTTGCGAAC
    1051  AACACGATAC TTGTTAAGTG GCTACCCCAA AACGATCTGC TTGGTCACCC
    1101  GATGACCCGT GCCTTTATCA CCCATGCTGG TTCCCATGGT GTTTATGAAA
    1151  GCATATGCAA TGGCGTTCCC ATGGTGATGA TGCCCTTGTT TGGTGATCAG
    1201  ATGGACAATG CAAAGCGCAT GGAGACTAAG GGAGCTGGAG TGACCCTGAA
    1251  TGTTC2TGGAA ATGACTTCTG AAGATTTAGA AAATGCTCTA AAAGCAGTCA
    1301  TCAATGACAA AAGTTACAAG GAGAACATCA TCGCCTCTC CAGCCTTCAC
    1351  AAGGACCGCC CGGTGGAGCC GCTGGACCTG GCCGTGTTCT GGGTGGAGTT
    1401  TGTGATGAGG CACAAGGGCG CGCCACACCT GCGCCCCGCA GCCCAGACC
    1451  TCACCTGGTA CCAGTACCAT TCCTTGACG TGATTGGTTT CCTCTTGCC
    1501  GTCGTGCTGA CAGTGGCCTT CATCACCTTT AAATGTTGTG CTTATGGCTA
65
```

- 14 -

1551 CCGGAAATGC TTGGGGAAAA AAGGGCGAGT TAAGAAAGCC CACAAATCCA  
 1601 AGACCCATTG AGAAGTGGGT GGGAAATAAG GTAAAATTTT GAACCATTCC  
 5 1651 CTAGTCATTT CCAAACCTGA AAACAGAATC AGTGTTAAAT TCATTTTATT  
 1701 CTTATTAAGG AAATACTTTG CATAAATTAA TCAGCCCCAG AGTGCTTTAA  
 1751 AAAATTCTCT TAAATAAAAA TAATAGACTC GCTAGTCAGT AAAGATATTT  
 10 1801 GAATATGTAT CGTGCCCCCT CCGGTGTCTT TGATCAGGAT GACATGTGCC  
 1851 ATTTTTCAGA GGACGTGCAG ACAGGCTGGC ATTCTAGATT ACTTTTCTTA  
 1901 CTCTGAAACA TGGCCTGTTT GGGAGTGC GG GATCAAAGG TGGTCCCACC  
 1951 GCTGCCCTTA CTGCAAATGG CAGTTTTAAT CTTATCTTTT GGCTTCTGCA  
 2001 GATGGTTGCA ATTGATCCTT AACCAATAAT GGTCAATCCT CATCTCTGTC  
 20 2051 CTGCTTCATA GGTGCCACCT TGTGTGTTTA AAGAAGGGAA GCTTTGTACC  
 2101 TTTAGAGTGT AGGTGAAATG AATGAATGGC TTGGAGTGCA CTGAGAACAG  
 2151 CATATGATTT CTTGCTTTGG GGAAAAAGAA TGATGCTATG AAATTGGTGG  
 2201 GTGGTGTATT TGAGAAGATA ATCATTGCTT ATGTCAAATG GAGCTGAATT  
 2251 TGATAAAAAAC CCAAATACA GCTATGAAGT GCTGGGCAAG TTTACTTTTT  
 30 2301 TTCTGATGTT TCCTACAAC

SEQ ID NO:7 refers to Genbank accession number M84122, which provides the intron of UGT1A:

35 1 TTTGCATCTC AAGGATAATT CTGTAAGCAG GAACCCTTCC TCCTTTAGAA  
 51 GGAAGTAAAG GAGAGGAAAA TGCTGTAAAA CTTACATATT AATAATTTTT  
 40 101 TACTCTATCT CAAACACGCA TGCCTTTAAT CATAGTCTTA AGAGGAAGAT  
 151 ATCTAATTCA TAACTTACTG TATGTAGTCA TCAAAGAATA TGAGAAAAAA  
 201 TTAAGTGAAG ATTTTCTTTC TGGCTCTAGG AATTTGAAGC CTACATTAAT  
 45 251 GCTTCTGGAG AACATGGAAT TGTGGTTTTT TCTTTGGGAT CAATGGTCTC  
 301 AGAAATCCA GAGAAGAAAG CTATGGCAAT TGCTGATGCT TTGGGCAAAA  
 50 351 TCCCTCAGAC AGTAAGAAGA TTCTATACCA TGGCCTCATA TCTATTTTCA  
 401 CAGGAGCGCT AATCCCAGAC TTCCAGCTTC CAGATTAATT CTCTTAATTG  
 451 GAACCTTAGA TTTGGCTTTT CCCTGCCACT TCCCAACTAT TAATCCAAAG  
 55 501 GTTTTTTTTG TT

SEQ ID NO:8 refers to Genbank accession number M84123, which provides exon 4 of UGT1A:

60 1 AAAGATGTCC TCAAGGGACC CTGTTTTCTA GTTAGTATAG CAGATTTGTT

51 TTCTAATCAT ATTATGTCTT TCTTTACGTT CTGCTCTTTT GCCCCTCCCA  
 101 GGTCTGTGG CGGTACACTG GAACCCGACC ATCGAATCTT GCGAACAACA  
 5 151 CGATACTTGT TAAGTGGCTA CCCCAAACG ATCTGCTTGG TATGTTGGGC  
 201 GGATTGGATG TATAGGTCAA ACCAGGGTCA AATTAAGAAA ATGGCTTAAG  
 10 251 CACAGCTATT CTAAAGGATT GTTGAGCTTG AAAATATTAT GGCCAACATA  
 301 TCCTACATTG CTTTTTATCT AGTGGGGTAT CTCAACCCAC ATTTTCTTCT  
 351 GCAAATTTCT GCAAGGGCAT GTGAGTAACA CTGAGTCTTT GGAGTGTTTT  
 15 401 CAGAACCTAG ATGTGTCCAG CTGTGAACT CAGAGATGTA ACTGCTGACA  
 451 TCCTCCCTAT TTTGCATCTC AGGTCACCCG ATGACCCGTG CCTTTATCAC  
 20 501 CCATGCTGGT TCCCATGGTG TTTATGAAAG CATATGCAAT GGCCTTCCCA  
 551 TGGTGATGAT GCCCTTGTTT GGTGATCAGA TGGACAATGC AAAGCGCATG  
 601 GAGACTAAGG GAGCTGGAGT GACCCTGAAT GTTCTGGAAA TGACTTCTGA  
 25 651 AGATTTAGAA AATGCTCTAA AAGCAGTCAT CAATGACAAA AGGTAAGAAA  
 701 GAAGATACAG AAGAATACTT TGGTCATGGC ATTCATGATA AAATTGTTTC  
 30 751 AAATATGAAA ACATTTACGT AGCATTTAAT ACGT

The method in accordance with the present invention can be performed using any  
 suitable method for detecting single nucleotide variations, such as e.g. allele specific  
 amplification (i.e. ARMS<sup>TM</sup>-allele specific amplification; ARMS referring to amplification  
 35 refractory mutation system), allele specific hybridisation (ASH), oligonucleotide ligation  
 assay (OLA) and restriction fragment length polymorphism (RFLP).

The status of a human being may be determined by reference to allelic variation at  
 position 908 in exon 5 as defined by the position in SEQ ID NO:1 and, if necessary, at one  
 or more additional positions displaying a polymorphism.

40 The test sample of the nucleic acid carrying the said polymorphism is conveniently a  
 sample of blood, bronchoalveolar lavage fluid, sputum, urine 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  
 45 convenient technique, e.g. polymerase chain reaction (PCR) or ligase chain reaction (LCR),  
 before analysis of allelic 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 polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. International patent application WO 00/06768 lists a number of amplification techniques and mutation  
5 detection techniques, some based on PCR. These may be used in combination with a number of signal generation systems, a selection of which is also listed in WO 00/06768. 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  
10 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

The invention also relates to diagnostic nucleic acids comprising within their sequence the polymorphism at position 908 of exon 5 of UGT1 (SEQ ID NO:1), the polymorphism at position 754 in exon 1 (SEQ ID NO:2) or the polymorphism at position 765 of exon 1.

15 The term "diagnostic nucleic acid" refers to a nucleotide sequence of at least 17 nucleotides in length which corresponds to part or all of the human UGT1 gene. The diagnostic nucleic acid is preferably a part of the human UGT1 gene which part expresses the polymorphism. A length of 17 to 100 nucleotides is preferred.

20 Furthermore the invention relates to allele specific primers which can be used as diagnostic primers for detecting a polymorphism in the UGT1 gene capable of hybridizing to nucleic acids comprising within their sequence the polymorphisms as defined above.

An allele specific primer is 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  
25 used for ARMS<sup>TM</sup> assays. The length of the allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, most preferably about 17-30 nucleotides.

30 Preferably the allele specific primer corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected 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 primer. Often the nucleotide at the -2 and/or -3 position (relative to the 3' terminus) is mismatched in order to optimize differential primer binding and preferential extension from the correct allele discriminatory primer only.

Suitable examples of such diagnostic allele specific primers are the following:

UGT1A6 T181S:	CGTGTTCCCTGGAGCATA	(SEQ ID NO:24)
UGT1A6 T181S:	CGTGTTCCCTGGAGCATG	(SEQ ID NO:25)
UGT1A6 R184S:	GACACAGGGTCTGGGCTT	(SEQ ID NO:27)
5 UGT1A6 R184S:	GACACAGGGTCTGGGCTG	(SEQ ID NO:28)
UGT1A-3' 908-2:	TGCAGTAGGGGCAGCG	(SEQ ID NO:30)
UGT1A-3' 908-2	TGCAGTAGGGGCAGCC	(SEQ ID NO:31).

Any convenient method of synthesis may be used to manufacture primers. Examples of such methods may be found in standard textbooks, for example "Protocols for  
10 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 primers may be labelled to facilitate detection.

Furthermore the invention relates to allele-specific oligonucleotide probes for detecting a polymorphism in the UGT1 gene capable of hybridizing to diagnostic nucleic  
15 acids comprising within their sequence the polymorphisms as defined above.

The length of the allele-specific oligonucleotide probes are preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, most preferably about 17-30 nucleotides.

The design of such probes will be apparent to the person skilled in the art. In general  
20 such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the 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.

25 Furthermore the invention relates to diagnostic kits comprising one or more allele-specific oligonucleotide primers or allele-specific oligonucleotide probes for detecting a polymorphism in the UGT1 gene.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise one or more appropriate  
30 buffers and one or more polymerases such as thermostable polymerases, for example Taq polymerase. Such kits may also comprise companion/constant primers and/or control primers or probes. A companion/constant primer is one that is part of the pair of primers used to perform PCR. Such primer usually complements the template strand precisely.

Furthermore the invention relates to a pharmaceutical pack comprising a pharmaceutically active compound like Tolcapone and instructions for administration of the drug to human beings diagnostically tested for a single nucleotide polymorphism according to a method of the present invention.

5 Furthermore the invention relates to a computer readable medium having stored thereon a sequence information for the polymorphism at position 908 of exon 5 of UGT1.

This invention further relates to a method for performing sequence identification, which methods comprise the steps of providing a nucleic acid sequence carrying e. g. the polymorphic site of position 908 of exon 5 or a complementary strand thereof or a  
10 fragment thereof of at least 20 bases; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity.

The invention is further illustrated by the following figures:

Figure 1 shows the primary metabolic routes of tolcapone in the liver. Tolcapone is oxidized by cytochrome P450 3A4 (CYP3A4), the nitro group is reduced and acetylated by  
15 N-acetyltransferase (NAT). The phenolic hydroxy group can be sulfated by sulfotransferase (ST) or methylated by catechol-O-methyl transferase (COMT). Glucuronidation of the hydroxy group, a major reaction of detoxification in the liver, is catalyzed by UDP-glucuronosyltransferase (UGT). Subsequent oxidation or conjugation with glucuronate, sulphate and acetate further modifies primary metabolites.

20 Figure 2 represents the UGT1A gene structure. The UGT1A gene spans more than 500kb, and consists of at least 12 promoters and first exons which can be spliced with the common exons to result in 12 different UGT1A enzymes. The structure of the UGT1A6 transcript is shown below. The arrows indicate the relative position of the polymorphic markers used in this study. UGT-3'\_908 represents the polymorphism at position 908 in  
25 exon 5 as defined by the position in SEQ ID NO:1. This polymorphism in the 3'UTR (untranslated region) can potentially affect the expression of all nine functional UGT1A enzymes. The other two polymorphisms in exon 1A6 affect the protein structure of UGT1A6.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by the person skilled in the art to which this invention belongs.

The following examples are provided for illustration of the invention, but are not  
5 intended to limit the scope of what is regarded as the invention.

### Examples

#### Analysis of tolcapone induced liver toxicity

##### Selection of patients

The study protocol and the informed consent form were submitted for approval to  
10 the local ethical committees in the respective countries. All patients provided written informed consent for their blood sample to be used for genotyping. The consent could be withdrawn up to a month later, if the patients changed their mind.

All the samples were assigned new independent codes and a month after sample  
15 collection the link between the new and original codes was deleted. This was an added measure to ensure patient confidentiality; however, as a consequence it is not possible to retrieve genotype information based on the patient's name or number used in the original clinical trial. In approximately 15 years time, all blood and DNA samples will be destroyed.

Initially, 645 patients who had received tolcapone in previous clinical trials were  
20 considered for inclusion in this retrospective genetic analysis. This included 215 patients who had displayed liver enzyme levels of  $\geq 1.5$ x the upper limit of normal (ULN) and 430 patients who had normal liver enzyme levels. Each patient with elevated liver transaminases (ELT) was matched with at least two control patients from the same study for gender and age (ethnic matching was not necessary since the great majority of the target patients were Caucasians). Disease severity had already been controlled for by the original  
25 study inclusion criteria. Of the 215 ELT patients, 135 ELT patients were enrolled in the study, 31 patients did not participate because their respective investigators could not obtain ethical approval to conduct the study, and 49 patients were either lost to follow-up or deceased. In total, 409 patients participated in this pharmacogenetic analysis. The distribution of the patients across the different sites and countries is shown in Table 2.

Table 2: patient distribution

Country	No of Sites	No of ELT patients	No of Controls patients	Total
Austria	2	2	7	9
Australia	1	2	4	6
Canada	14	31	74	106
Switzerland	5	4	10	14
Germany	9	11	29	40
Denmark	4	8	13	21
Spain	4	4	6	10
UK	4	8	10	18
Italy	5	12	23	35
Norway	2	5	4	9
USA	20	48	94	142
Total	70	135	274	410

#### Preparation of the samples

Single blood samples (9ml) were collected in EDTA tubes. These were frozen and stored between -20 and -70°C, before being sent to the Roche Central Sample Office (CSO) in Basel, Switzerland, where they were aliquoted into three tubes and assigned new, independent codes on bar code labels to assure patient anonymity. Two samples of blood (1ml and 4mls) were sent to the Roche Sample Repository (RSR) at Roche Molecular Systems (RMS) in Alameda, California. The remaining 4ml aliquot was stored at -80 °C in the CSO in Basel, Switzerland. All procedures performed on the samples at the RSR were done according to established standard operating procedures using GCP guidelines.

DNA was extracted from 400 µl of the whole blood using a silica gel-based extraction method (QiaAmp DNA Blood kit, Valencia, CA). Controls included 10 mM Tris pH 8.0, 1 mM EDTA (TE) buffer and whole blood from a blood unit with a known yield of DNA.

Samples were genotyped for eight different single nucleotide polymorphisms (SNPs) using a combination of the amplification refractory mutation system (ARMS) that relies on 3' terminal mismatches between the PCR primers and the template being amplified according to Newton et al., *Nucleic Acids Res.* (1989), 17(7), 2503-16.

Analysis of any point mutation in DNA was transformed by using the amplification refractory mutation system (ARMS, *Nucleic Acids Res.* (1989), 17(7), 2503-16) and using the kinetic thermal cycler (KTC) format of the polymerase chain reaction. This method

allows discrimination of single nucleotide polymorphisms (SNP) in a single-tube without the use of fluorescent probes (Higuchi et al., *Biotechnology* (1993), 11, 1026-1030).

In the KTC format, the generation of double-stranded amplification product is monitored using a DNA intercalating dye and a thermal cycler which has a fluorescence-  
5 detecting CCD camera attached (PE-Biosystems GeneAmp 5700 Sequence Detection System). Fluorescence in each well of the PCR amplification plate is measured at each cycle of annealing and denaturation. The cycle at which the relative fluorescence reached a threshold of 0.5 using the SDS software from PE-Biosystems was defined as the  $C_t$ .

The amplification reactions were designed to be allele-specific, so that the  
10 amplification reaction was positive if the polymorphism was present and the amplification reaction was negative if the polymorphism was absent. For each bi-allelic polymorphism, one well of the amplification plate was set up to be specific for allele 1 and a second well was set up to be specific for allele 2. For each polymorphism to be detected, three primers were designed – two allele-specific primers and one common primer (Table 3). Reactions  
15 for allele 1 contained allele 1-specific primer and the common primer and reactions for allele 2 contained allele 2-specific primer and the common primer.

Table 3: list of oligonucleotide primers used for polymorphism detection

<u>Marker</u>	<u>Primer type</u>	<u>Nucleotide sequence</u>	<u>SEQ ID NO</u>	<u>Primer concentration (in <math>\mu</math>M)</u>	<u>Annealing temperature</u>
COMT V158M	AS1	GCACACCTTGTCTTCAT	9	0.4	58
COMT V158M	AS2	GCACACCTTGTCTTCAC	10	0.4	58
COMT V158M	common	CATCACCATCGAGATCAAC	11	0.4	58
CYP3A4 A/G	AS1	CTATTAAATCGCCTCTCTCT	12	0.4	56
CYP3A4 A/G	AS2	CTATTAAATCGCCTCTCTCC	13	0.4	56
CYP3A4 A/G	common	GGATGAATTTCAAGTATTT	14	0.4	56
MnSOD V-9A	AS1	AGCCCAGATACCCCAAAG	15	0.4	58
MnSOD V-9A	AS2	AGCCCAGATACCCCAAAA	16	0.4	58
MnSOD V-9A	common	TGTGCTTTCTCGTCTTCA	17	0.4	58
NAT2 I114T	AS1	TGTAATTCCTGCCGTCAG	18	0.2	58
NAT2 I114T	AS2	TGTAATTCCTGCCGTCAG	19	0.2	58
NAT2 I114T	common	ATACAGCACTGGCATGG	20	0.2	58
SULT1A1 R213H	AS1	CCTGGAGTTTGTGGGGCG	21	0.2	58
SULT1A1 R213H	AS2	CCTGGAGTTTGTGGGGCA	22	0.2	58
SULT1A1 R213H	common	TGAACCATGAAGTCCACG	23	0.2	58
UGT1A6 T181S	AS1	CGTGTTCCTGGAGCATA	24	0.2	58
UGT1A6 T181S	AS2	CGTGTTCCTGGAGCATG	25	0.2	58

UGT1A6 T181S	common	GAATGTAGGACACAGGGTCT	26	0.2	58
UGT1A6 R184S	AS1	GACACAGGGTCTGGGCTT	27	0.2	58
UGT1A6 R184S	AS2	GACACAGGGTCTGGGCTG	28	0.2	58
UGT1A6 R184S	common	TACCTCTTCAGGGGTTTTTC	29	0.2	58
UGT1A-3' 908-2	AS1	TGCAGTAGGGGCAGCG	30	0.2	58
UGT1A-3' 908-2	AS2	TGCAGTAGGGGCAGCC	31	0.2	58
UGT1A-3' 908-2	common	GGAGTGCGGGATTCAA	32	0.2	58

The amplification conditions were as follows: 10 mM Tris pH 8.0, 40 mM KCl, 2 mM MgCl<sub>2</sub>, 50 μm each of dATP, dCTP, and dGTP, 25 μm of TTP and 75 μm of dUTP, 4% DMSO, 0.2X SyBr Green (Molecular Probes, Eugene, OR), 2% glycerol, uracil N-glycosylase (UNG, 2 units), Stoffel Gold DNA polymerase (15 units, for reference see Nature (1996), 381, 445-6) and primers in an 85 μl volume for each well. The concentration of the primers used for each assay are listed in Table 2. 30 ng of DNA in a 15 μl volume was then added to each well.

To reduce the possibility of contamination by pre-existing amplification product, the assay procedure included the incorporation of dUTP into the amplification product and an incubation step for UNG degradation of pre-existing U-containing products (Longo et al, Gene (1990), 93, 125-128).

Amplification reactions were prepared using an aliquoting robot (Packard Multiprobe II, Meriden, CT) in 96-well amplification plates identified by barcode labels generated by the experiment management database. Parameters for procedures performed by the robot were set to minimize the possibility of cross-contamination. For each plate of 81 samples, 5 samples were run in duplicate and the duplicate results were analysed to determine that they matched.

The thermal cycling conditions were as follows: 5 minutes at 50 °C for UNG degradation of any previously contaminating PCR products, 12 minutes at 95 °C for Stoffel Gold polymerase activation, 55 cycles of denaturation at 95 °C and annealing at the annealing temperature indicated in Table 2, followed by a dissociation step of 1 minute at 1 degree increments from 60 °C to 95 °C. The amplification reactions were run in PE Biosystems GeneAmp 5700 Sequence Detection Systems (SDS) instruments (Foster City, CA). The first derivatives of the dissociation curves were produced by the SDS software and examined as needed to confirm that the fluorescence in a given reaction was due to amplification of a specific product with a well-defined dissociation peak rather than non-

specific primer-dimer. Product differentiation was done by Analysis of DNA Melting Curves during PCR following the method of K.M. Ririe et al., *Anal. Biochem.* (1997), 245, 154 – 160.

The  $C_t$  of each amplification reaction was determined and the difference between the  $C_t$  for allele 1 and allele 2 (delta  $C_t$ ) was used as the assay result. Samples with delta  $C_t$ s between -3.0 and 3.0 were considered heterozygous (A1/A2). Samples with delta  $C_t$ s below -3.0 were considered homozygous for A1 (A1/A1); samples with delta  $C_t$ s above 3.0 were considered homozygous for A2 (A2/A2). In most cases, the delta  $C_t$  differences between the three groups of genotypes were well-defined and samples with  $C_t$  values close to 3.0 were re-tested as discrepant.

Each assay was run on a panel of 14 cell line DNAs to identify cell lines with the appropriate genotypes for use as controls on each assay plate (A1/A1, A1/A2, and A2/A2). The cell line DNA was obtained from the Human Genetics Department, Roche Molecular Systems (RMS) Alameda, CA and was extracted using the Qiagen extraction kits (QiaAmp DNA Blood kits, Valencia, CA). The genotypes of the cell line DNAs were confirmed by DNA sequencing. Three cell line DNAs (A1/A1, A1/A2, and A2/A2) were run as controls on each plate of clinical trial samples and used to determine the between-plate variability. In addition, DNA from two cell lines were run in quadruplicate for each assay to determine the within-plate assay variability. The  $C_t$  values obtained for the control cell lines were analyzed to determine the cutoff for the delta  $C_t$  values obtained for the clinical trial samples.

A data file containing the  $C_t$  values for each well was generated by the SDS software and entered into the experiment management database. A data file with the final genotypes identified by the independent code was extracted from the database and matched to the clinical data also identified by the independent code for the statistical analysis.

For all other single nucleotide polymorphisms (SNPs) discovery and genotyping was done by double-stranded DNA sequencing using an ABI capillary sequencer and Big Dye chemistry (ABI). The primers used to amplify all exons are shown below and were also used as sequencing primers. Publicly available genomic sequences were used as references for primer design. All polymorphisms were targeted with these pairs-of-primer sets:

UGT1A6-1 fragment:	UGT1A6-F1	ACACGGCCATAGTTGGTTCA	(SEQ ID NO:33)
	UGT1A6-R1	CAGTTGATGAAGTACAGGCC	(SEQ ID NO:34)

- 24 -

	UGT1A6-2 fragment:	UGT1A6-F2	TGTAGTGGTGGTGCCTGAAG	(SEQ ID NO:35)
		UGT1A6-R2	GACAGCTGATGCGAGTTCTTC	(SEQ ID NO:36)
	UGT1A7-1 fragment:	UGT1A7-F1	GAGGGCAGGTTCTATCGTAC	(SEQ ID NO:37)
		UGT1A7-R1	GGGCACTGTGCACCTTCTTC	(SEQ ID NO:38)
5	UGT1A7-2 fragment:	UGT1A7-F2	ACGGCACCATTGCGAAGTGC	(SEQ ID NO:39)
		UGT1A7-R2	ACTTACATATCAACAAGTGCTGC	(SEQ ID NO:40)
	UGT1A8 fragment:	UGT1A8-F	GGGCATGATCTGTCCAAGGC	(SEQ ID NO:41)
		UGT1A8-R	GGTTGAGTAAGTCTTCACTGTG	(SEQ ID NO:42)
	UGT1A9 fragment:	UGT1A9-F	CTCAGCTGCAGTTCTCTG	(SEQ ID NO:43)
10		UGT1A9-R	CCAGATCCTCCAGGGTATATG	(SEQ ID NO:44)
	UGT1A10 fragment:	UGT1A10-F	GAGTTCATCCAGTGGTTTTTC	(SEQ ID NO:45)
		UGT1A10-R	CAGTTCAGTAAGGAATCC	(SEQ ID NO:46)
	UGT1in fragment:	UGT1Ain-F	CAAGGATAATTCTGTAAGCAGG	(SEQ ID NO:47)
		UGT1Ain-R	GGATTAATAGTTGGGAAGTGGC	(SEQ ID NO:48)
15	UGT1ex4 fragment:	UGT1ex4-F	GGCCAACATATCCTACATTG	(SEQ ID NO:49)
		UGT1ex4-R	CGTATTAAATGCTACGTAAATGT	(SEQ ID NO:50)
	UGT1-ex5-1 fragment:	UGT1ex5-1-F	CAGTTAGCCATGCTTGTGCC	(SEQ ID NO:51)
		UGT1ex5-1-R	GCACTCTGGGGCTGATTAAT	(SEQ ID NO:52)
	UGT1-ex5-2 fragment:	UGT1ex5-2-F	CGTGCTGACAGTGGCCTTC	(SEQ ID NO:53)
20		UGT1ex5-2-R	CAGTGCACTCCAAGCCATTC	(SEQ ID NO:54)
	UGT1-ex5-3 fragment:	UGT1ex5-3-F	GATGGTTGCAATTGATCC	(SEQ ID NO:55)
		UGT1ex5-3-R	TTAGTTGTAGGAAACATCAG	(SEQ ID NO:56)

Primer UGT1A6-F1 corresponds to positions 3 to 22 in exon 1 of UGT1A6 as defined by the positions in SEQ ID NO:2. Primer UGT1A6-R1 corresponds to the complementary strand and hybridizes to positions 571 to 590 as defined by the positions in SEQ ID NO:2. Primer UGT1A6-F2 refers to positions 381 to 400 in exon 1 of UGT1A6 as defined by the positions in SEQ ID NO:2. Primer UGT1A6-R2 corresponds to the complementary strand and hybridizes to positions 901 to 921 as defined by the positions in SEQ ID NO:2.

Primer UGT1A7-F1 corresponds to positions 78 to 98 in exon 1 of UGT1A7 as defined by the positions in SEQ ID NO:3. Primer UGT1A7-R1 corresponds to the complementary strand and hybridizes to positions 696 to 715 as defined by the positions in SEQ ID NO:3. Primer UGT1A7-F2 corresponds to positions 459 to 478 in exon 1 of UGT1A7 as defined by the positions in SEQ ID NO:3. Primer UGT1A7-R2 corresponds to the complementary strand and hybridizes to positions 1190 to 1212 as defined by the positions in SEQ ID NO:3.

Primer UGT1A8-F corresponds to positions 1 to 20 in exon 1 of UGT1A8 as defined by the positions in SEQ ID NO:5. Primer UGT1A8-R hybridizes to positions 479 to 500 as defined by the positions in SEQ ID NO:5.

Primer UGT1A9-F corresponds to positions 1 to 18 in exon 1 of UGT1A9 as defined by the positions in SEQ ID NO:6. Primer UGT1A9-R hybridizes to positions 257 to 277 as defined by the positions in SEQ ID NO:6.

Primer UGT1A10-F corresponds to positions 596 to 615 in exon 1 of UGT1A7 as defined by the positions in SEQ ID NO:4. Primer UGT1A10-R hybridizes to positions 1177 to 1194 as defined by the positions in SEQ ID NO:4.

Primer UGT1Ain-F corresponds to positions 10 to 31 in the intron of UGT1A as defined by the positions in SEQ ID NO:7. Primer UGT1Ain-R hybridizes to positions 475 to 496 as defined by the positions in SEQ ID NO:7.

Primer UGT1ex4-F corresponds to positions 291 to 310 in exon 4 of UGT1A as defined by the positions in SEQ ID NO:8. Primer UGT1ex4-R hybridizes to positions 761 to 784 as defined by the positions in SEQ ID NO:8.

Primer UGT1ex5-1-F corresponds to positions 63 to 82 in exon 5 of UGT1 as defined by the positions in SEQ ID NO:1. Primer UGT1ex5-1-R hybridizes to positions 684 to 703 as defined by the positions in SEQ ID NO:1. Primer UGT1ex5-2-F corresponds to positions 461 to 480 in exon 5 of UGT1 as defined by the positions in SEQ ID NO:1.

Primer UGT1ex5-2-R hybridizes to positions 1082 to 1101 as defined by the positions in SEQ ID NO:1. Primer UGT1ex5-3-F corresponds to positions 959 to 976 in exon 5 of UGT1 as defined by the positions in SEQ ID NO:1 and primer UGT1ex5-3-R hybridizes to positions 1261 to 1280 as defined by the positions in SEQ ID NO:1.

5           Fourty nanograms of genomic DNA were PCR-amplified in 50  $\mu$ l reactions using an automated PCR machine. Reaction conditions varied as follows. For the amplification of the UGT1A6-fragment conditions were as follows: 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.4  $\mu$ M of each primer and 1.5U Boehringer Taq Polymerase. The thermocycling protocol consisted of an initial incubation of 95 °C for 10 15 min. followed by 35 cycles of 94 °C for 1 min., 57 °C for 30 sec., 72 °C for 1 min., and one final extension step of 72 °C for 10 min. The UGT1A7-1 fragment was amplified using Qiagen PCR buffer with 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.4  $\mu$ M of each primer and 1.5U Boehringer Taq Polymerase. The thermocycling protocol was the same as for UGT1A6-fragment with one exception: the annealing temperature was 61 °C. For 15 UGT1A7-2 fragment PCR conditions were as follows: 150 mM Tris pH 8.5, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.4  $\mu$ M of each primer and 1.5U Qiagen Hot Start Taq Polymerase. Thermocycling was done using a touch-down PCR protocol. After an iniatial amplification of 95 °C for 10 min. followed 5 cycles of 95 °C for 1min., 62 °C for 30 sec. (minus 0.5 °C per cycle), 72 °C for 1 min. and thirty cycles of 95 °C 20 for 1 min., 60 °C for 30 sec., 72 °C for 1 min. and a final extension step of 72 °C for 10 min. After PCR amplification fragments were purified using the Qiaquick PCR purification kit on a Biorobot 9600. Cycle sequencing was performed on an automated PCR machine using ABI Big Dye terminator chemistry according to the manufacturer's intruction with the following changes: 2.5-5 ng/100 bp of PCR product were mixed with 2  $\mu$ l Big Dye 25 terminator mix, oligonucleotide primer concentration was 10 pmol, if necessary 5% DMSO was added to the reaction; the final reaction volume was 10  $\mu$ l. Sequencing reactions were subjected to 28 cycles at 93 °C for 30 sec, 48 °C for 30 sec, and 58 °C for 120 sec., followed by an ethanol/NaOAc precipitation. After decanting the ethanol, samples were evaporated to dryness using a SpeedVac for 2 min. and were resuspended in 45  $\mu$ l 30 ultrapure water (MERCK, HPLC grade). 2.5 ml were loaded on an ABI 3700 capillary sequencer using POP5 as a polymer. After sequencing, the polymorphism analyses were done using Polyphred software (licenced from University of Washington).

#### Selection and discovery of genetic markers

The genetic markers were selected based on the known pharmacology of tolcapone 35 and knowledge from the literature of genetic polymorphisms that could affect the activity

of corresponding and relevant gene products. The main metabolic pathway for tolcapone elimination is glucuronidation by UGT1 enzymes.

In addition to polymorphisms in the UGT1 enzyme, genetic polymorphisms in genes encoding the following enzymes involved in tolcapone metabolism (Figure 1) were  
5 selected: catechol-*O*-methyl transferase (COMT) according to Lachman et al.,  
Pharmacogenetics (1996), 6, 243-250; N-acetyl transferase (NAT2, for reference see Vatsis  
et al., Proc. Natl. Acad. Sci. (1991), 88, 6333-6337; Bandmann et al., Lancet (1997), 350,  
1136-1139); liver sulfotransferase (SULT1A1) according to Ozawa et al., Chem. Biol.  
Interact. (1998), 109, 237-248 and Cytochrome P450 enzyme (CYP3A4, Rebbeck et al., J.  
10 Natl. Cancer Inst. (1998), 50, 1225-1229). The genotype for manganese superoxide  
dismutase (MnSOD, Shimoda-Matsubayashi et al., Biochem. Biophys. Res. Commun.  
(1996), 226, 561-565), involved in oxidative stress response, was also investigated.

UDP-glucuronosyltransferase 1A6 (UGT1A6) was selected as it potentially  
metabolises tolcapone via glucuronidation. The alleles Thr181Ala and Arg184Ser are  
15 described as showing reduced activity for levodopa and other substrates (Ciotti et al.,  
Pharmacogenetics (1997), 7, 485-495). The known genetic polymorphisms in the UGT1A  
gene affect only single members of this gene cluster of twelve genes (Figure 2). Therefore, it  
was reasoned that genetic variations in the potentially common regulatory region, that is  
the 3'-end of the gene, could have an effect on the expression of any of the twelve UGT1A  
20 genes. Moreover, it was suspected that UGT1A7 may be involved in the elimination of  
tolcapone. In order to identify new genetic polymorphisms common exons 2-5 and the 3'  
untranslated region of UGT1A and exon 1 of the UGT1A6, UGT1A7, UGT1A8, UGT1A9  
and UGT1A10 genes were sequenced in 47 different DNA samples from ethnically diverse  
individuals. The 300-700 bp fragments were column purified with the Qiaquick PCR  
25 purification kit on a Biorobot 9600 and both strands were sequenced on an ABI3700  
capillary sequencer using dye-terminator chemistry and the PCR amplification primers as  
sequencing primers as described in detail above. A G/C variation was identified designated  
as UGT1A-3'\_908, which occurred in the following frequencies: CC: 0.63; GC:0.33;  
GG:0.04. The number 908 refers to the position of the SNP relative to the DNA sequence  
30 with Genbank accession number M84124 from the public data bases. Moreover, the  
following polymorphisms have been identified in UGT1A6, UGT1A7, UGT1A8, UGT1A9  
and UGT1A10 genes:

UGT1A6exon1\_318 and UGT1A6exon1-528. The number refers to the position of  
the SNP relative to the DNA sequence with Genbank accession number M84130 from the  
35 public database.

UGT1A7 exon1\_197, UGT1A7exon1\_824, UGT1A7exon1\_920, and UGT1A7exon1\_992. The numbers refer to the position of the SNP relative to the DNA sequence with Genbank accession number U39570 from the public database.

5 UGT1A8promoter\_245. The number refers to the position of the SNP relative to the DNA sequence with Genbank accession number U42604 from the public database.

UGT1A9exon1\_214. The number refers to the position of the SNP relative to the DNA sequence with Genbank accession number AF056188 from the public database.

UGT1A10exon1\_959. The number refers to the position of the SNP relative to the DNA sequence with Genbank accession number U39550 from the public database.

10 UGT1Aintron\_117 and UGT1Aintron\_379. The numbers refer to the position of the SNPs relative to the DNA sequence with Genbank accession number M84122 from the public database.

UGT1Aexon4\_473. The number refers to the position of the SNP relative to the DNA sequence with Genbank accession number M84123 from the public database.

15 UGT1Aexon5\_423, UGT1Aexon5\_780, UGT1Aexon5\_908 (described above in detail) and UGT1Aexon5\_1012. The numbers refer to the position of the SNPs relative to the DNA sequence with Genbank accession number M84124 from the public domain.

20 The patient samples were divided into two groups. Group 1 contained samples from case patients whose aspartate aminotransferase (AST:SGOT), alanine aminotransferase (ALT: SGPT), or bilirubin values were  $\geq 1.5x$  ULN of the investigators range while taking tolcapone treatment. Group 2 contained samples from control patients whose SGOT, SGPT, and bilirubin values were below  $1x$  ULN when measured while taking tolcapone treatment.

For each genotype, the following analyses were performed:

25 a) Analysis of the entire genotype: patients were classified according to the following three categories: homozygous 1/1 (two copies of allele 1 and no copies of allele 2), heterozygous 1/2 (one copy of allele 1 and one copy of allele 2) or homozygous 2/2 (no copies of allele 1 and two copies of allele 2). Analysis was conducted to assess whether there were differences in the proportion of case patients in each of these groups, compared with the proportion of  
30 control patients. The Cochran-Maentel-Hanszel (CMH) test was applied to the data

presented in a 2-by-3 table (the two columns indicating presence or absence of liver function abnormality and the three rows indicating the three categories of the genotype).

b) Analysis of alleles: the presence of allele 1 or 2 in case patients was compared to the presence of the respective allele in the control patients. For each allele, the CMH test was applied using a 2-by-2 table (the two columns indicating presence or absence of liver function abnormality and the two rows indicating the presence or absence of the respective allele), and the case-control odds ratio and 95% confidence interval were calculated. An odds-ratio of greater than 1.0, together with a confidence interval that does not include 1.0, indicated a positive association between the presence of the allele and the occurrence of liver function abnormality.

c) Analysis of allele counts: this analysis was conducted to compare the distribution of the alleles in patients with liver function abnormality to that of patients without abnormality. The total number of copies of allele 1 among patients with liver function abnormality was compared with the total number of copies of allele 2 among these case patients. The CMH test was applied to the data using a 2-by-2 table (the two columns indicating presence or absence of liver function abnormality and the two rows indicating the two allele counts). Again case-control odds ratio and 95% confidence intervals were obtained.

## Results

A total of 409 patients treated with tolcapone, of which 135 had liver enzyme elevation of 1.5 times or more above the upper limit and 274 were matched controls, were genotyped for different genetic markers from genes encoding enzymes involved in the metabolism of tolcapone, including the UGT1 genes. The results from the analysis of the genetic markers that resulted in a significant association are presented in tables 4 to 13. All markers showing significant association to elevated liver transaminases corresponded to SNPs in the UGT1 genes.

Table 4. UGT1A6\_765 Arg184Ser

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous Arg/Arg	128 ( 46.7%)	45 ( 33.3%)
	Heterozygous Arg/Ser	122 ( 44.5%)	64 ( 47.4%)
	Homozygous Ser/Ser	24 ( 8.8%)	26 ( 19.3%)
	P-value	0.0023	
Allele Arg	Absent	24 ( 8.8%)	26 ( 19.3%)
	Present	250 ( 91.2%)	109 ( 80.7%)
	Relative Risk	0.58	
	Odds Ratio	0.40	
	95% CI	( 0.22, 0.72)	
	P-value	0.0023	
Allele Ser	Absent	128 ( 46.7%)	45 ( 33.3%)
	Present	146 ( 53.3%)	90 ( 66.7%)
	Relative Risk	1.47	
	Odds Ratio	1.75	
	95% CI	( 1.14, 2.69)	
	P-value	0.0101	
Count	Allele Arg	378 ( 69.0%)	154 ( 57.0%)
	Allele Ser	170 ( 31.0%)	116 ( 43.0%)
	Odds Ratio	1.67	
	95% CI	( 1.24, 2.26)	
	P-value	0.0008	

Table 5. UGT1A6\_754 Thr181Ala

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous Thr/Thr	144 ( 52.6%)	51 ( 37.8%)
	Heterozygous Thr/Ala	108 ( 39.4%)	59 ( 43.7%)
	Homozygous Ala/Ala	22 ( 8.0%)	25 ( 18.5%)
	P-value	0.0014	
Allele Thr	Absent	22 ( 8.0%)	25 ( 18.5%)
	Present	252 ( 92.0%)	110 ( 81.5%)
	Relative Risk	0.57	
	Odds Ratio	0.38	
	95% CI	( 0.21, 0.70)	
	P-value	0.0018	
Allele Ala	Absent	144 ( 52.6%)	51 ( 37.8%)
	Present	130 ( 47.4%)	84 ( 62.2%)
	Relative Risk	1.50	
	Odds Ratio	1.82	
	95% CI	( 1.20, 2.77)	
	P-value	0.0050	
Count	Allele Thr	396 ( 72.3%)	161 ( 59.6%)
	Allele Ala	152 ( 27.7%)	109 ( 40.4%)
	Odds Ratio	1.76	
	95% CI	( 1.30, 2.39)	
	P-value	0.0003	

Table 6. UGT1A-3' \_908

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous C/C	163 ( 59.5%)	101 ( 74.8%)
	Heterozygous G/C	97 ( 35.4%)	30 ( 22.2%)
	Homozygous G/G	14 ( 5.1%)	4 ( 3.0%)
	P-value	0.0097	
Allele C	Absent	14 ( 5.1%)	4 ( 3.0%)
	Present	260 ( 94.9%)	131 ( 97.0%)
	Relative Risk	1.51	
	Odds Ratio	1.76	
	95% CI	( 0.58, 5.40)	
	P-value	0.3202	
Allele G	Absent	163 ( 59.5%)	101 ( 74.8%)
	Present	111 ( 40.5%)	34 ( 25.2%)
	Relative Risk	0.61	
	Odds Ratio	0.49	
	95% CI	( 0.31, 0.78)	
	P-value	0.0023	
Count	Allele C	423 ( 77.2%)	232 ( 85.9%)
	Allele G	125 ( 22.8%)	38 ( 14.1%)
	Odds Ratio	0.55	
	95% CI	( 0.37, 0.82)	
	P-value	0.0033	

Table 7. UGT1A6\_232 Ser7Ala

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous T/T	112 ( 42.1%)	40 ( 30.5%)
	Heterozygous T/G	123 ( 46.2%)	63 ( 48.1%)
	Homozygous G/G	31 ( 11.7%)	28 ( 21.4%)
	P-value	0.0130	
Allele T	Absent	31 ( 11.7%)	28 ( 21.4%)
	Present	235 ( 88.3%)	103 ( 78.6%)
	Relative Risk	0.64	
	Odds Ratio	0.49	
	95% CI	( 0.28, 0.84)	
P-value	0.0106		
Allele G	Absent	112 ( 42.1%)	40 ( 30.5%)
	Present	154 ( 57.9%)	91 ( 69.5%)
	Relative Risk	1.41	
	Odds Ratio	1.65	
	95% CI	( 1.06, 2.58)	
P-value	0.0259		
Count	Allele T	347 ( 65.2%)	143 ( 54.6%)
	Allele G	185 ( 34.8%)	119 ( 45.4%)
	Odds Ratio	1.56	
	95% CI	( 1.16, 2.11)	
P-value	0.0037		

Table 8. UGT1A6\_528 A/G

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous A/A	129 ( 48.7%)	44 ( 33.8%)
	Heterozygous A/G	114 ( 43.0%)	60 ( 46.2%)
	Homozygous G/G	22 ( 8.3%)	26 ( 20.0%)
	P-value	0.0008	
Allele A	Absent	22 ( 8.3%)	26 ( 20.0%)
	Present	243 ( 91.7%)	104 ( 80.0%)
	Relative Risk	0.55	
	Odds Ratio	0.36	
	95% CI	( 0.20, 0.66)	
	P-value	0.008	
Allele G	Absent	129 ( 48.7%)	44 ( 33.8%)
	Present	136 ( 51.3%)	86 ( 66.2%)
	Relative Risk	1.52	
	Odds Ratio	1.85	
	95% CI	( 1.20, 2.68)	
	P-value	0.0053	
Count	Allele A	372 ( 70.2%)	148 ( 56.9%)
	Allele G	158 ( 29.8%)	112 ( 43.1%)
	Odds Ratio	1.78	
	95% CI	( 0.31, 2.42)	
	P-value	0.0002	

Table 9: UGT1A7\_197 C/G

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous A/A	32 ( 11.9%)	25 ( 18.8%)
	Heterozygous A/C	112 ( 41.6%)	62 ( 46.6%)
	Homozygous C/C	125 ( 46.5%)	46 ( 34.6%)
	P-value	0.0400	
Allele A	Absent	125 ( 46.5%)	46 ( 34.6%)
	Present	144 ( 53.5%)	87 ( 65.4%)
	Relative Risk	1.40	
	Odds Ratio	1.64	
	95% CI	( 1.07, 2.52)	
	P-value	0.0235	
Allele C	Absent	32 ( 11.9%)	25 ( 18.8%)
	Present	237 ( 88.1%)	108 ( 81.2%)
	Relative Risk	0.71	
	Odds Ratio	0.58	
	95% CI	( 0.33, 1.03)	
	P-value	0.0623	
Count	Allele A	176 ( 32.7%)	112 ( 42.1%)
	Allele G	362 ( 67.3%)	154 ( 57.9%)
	Odds Ratio	0.67	
	95% CI	( 0.49, 0.90)	
	P-value	0.0090	

Table 10: UGT1A7\_551 Asn129Lys

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous G/G	96 ( 35.7%)	59 ( 44.7%)
	Heterozygous G/T	124 ( 46.1%)	59 ( 44.7%)
	Homozygous T/T	49 ( 18.2%)	14 ( 10.6%)
	P-value	0.0762	
Allele G	Absent	49 ( 18.2%)	14 ( 10.6%)
	Present	220 ( 81.8%)	118 ( 89.4%)
	Relative Risk	1.57	
	Odds Ratio	1.88	
	95% CI	( 1.00, 3.52)	
	P-value	0.0494	
Allele T	Absent	96 ( 35.7%)	59 ( 44.7%)
	Present	173 ( 64.3%)	7 ( 55.3%)
	Relative Risk	0.78	
	Odds Ratio	0.69	
	95% CI	( 0.45, 1.05)	
	P-value	0.0821	
Count	Allele G	116 ( 58.7%)	177 ( 67.0%)
	Allele T	222 ( 41.3%)	87 ( 33.0%)
	Odds Ratio	0.70	
	95% CI	( 0.51, 0.95)	
	P-value	0.0232	

Table 11: UGT1A7\_555 silent or Arg131Lys

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous A/A	96 ( 35.7%)	58 ( 43.9%)
	Heterozygous A/C	124 ( 46.1%)	60 ( 45.5%)
	Homozygous G/G	49 ( 18.2%)	14 ( 10.6%)
	P-value	0.0894	
Allele A	Absent	49 ( 18.2%)	14 ( 10.6%)
	Present	220 ( 81.8%)	118 ( 89.4%)
	Relative Risk	1.57	
	Odds Ratio	1.88	
	95% CI	( 1.00, 3.52)	
	P-value	0.0494	
Allele C	Absent	96 ( 35.7%)	58 ( 43.9%)
	Present	173 ( 64.3%)	74 ( 56.1%)
	Relative Risk	0.80	
	Odds Ratio	0.71	
	95% CI	( 0.46, 1.08)	
	P-value	0.1108	
Count	Allele A	316 ( 58.7%)	176 ( 66.7%)
	Allele C	222 ( 41.3%)	88 ( 33.3%)
	Odds Ratio	0.71	
	95% CI	( 0.52, 0.97)	
	P-value	0.0303	

Table 12: UGT1A7\_556 Arg131Lys or Gln

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous A/A	96 ( 35.8%)	44 ( 33.8%)
	Heterozygous A/G	123 ( 45.9%)	59 ( 44.7%)
	Homozygous G/G	49 ( 18.3%)	14 ( 10.6%)
	P-value	0.0772	
Allele A	Absent	49 ( 18.3%)	14 ( 10.6%)
	Present	219 ( 81.7%)	118 ( 89.4%)
	Relative Risk	1.58	
	Odds Ratio	1.89	
	95% CI	( 1.01, 3.53)	
	P-value	0.0477	
Allele G	Absent	96 ( 35.8%)	59 ( 44.7%)
	Present	172 ( 64.2%)	73 ( 55.3%)
	Relative Risk	0.78	
	Odds Ratio	0.69	
	95% CI	( 0.45, 1.06)	
	P-value	0.0870	
Count	Allele A	315 ( 58.8%)	177 ( 67.0%)
	Allele G	221 ( 41.2%)	87 ( 33.0%)
	Odds Ratio	0.70	
	95% CI	( 0.51, 0.95)	
	P-value	0.0238	

Table 13: UGT1A7\_786 Trp208Arg

		Liver Function Abnormality	
		Absent	Present
Genotype	Homozygous T/T	124 ( 46.3%)	46 ( 34.3%)
	Heterozygous T/C	114 ( 42.5%)	62 ( 46.3%)
	Homozygous C/C	30 ( 11.2%)	26 ( 19.4%)
	P-value	0.0224	
Allele T	Absent	30 ( 11.2%)	26 ( 19.4%)
	Present	238 ( 88.8%)	108 ( 80.6%)
	Relative Risk	0.67	
	Odds Ratio	0.52	
	95% CI	( 0.30, 0.92)	
	P-value	0.0252	
Allele G	Absent	124 ( 46.3%)	46 ( 34.3%)
	Present	144 ( 53.7%)	86 ( 65.7%)
	Relative Risk	1.40	
	Odds Ratio	1.65	
	95% CI	( 1.07, 2.53)	
	P-value	0.0225	
Count	Allele T	362 ( 67.5%)	154 ( 57.5%)
	Allele C	174 ( 32.5%)	114 ( 42.5%)
	Odds Ratio	1.54	
	95% CI	( 0.14, 2.08)	
	P-value	0.0050	

The main metabolic pathway for tolcapone elimination is glucuronidation. The results from the current retrospective analysis have shown a significant association between three genetic polymorphisms in the UDP-glucuronosyltransferase gene and liver function abnormality. These findings support the hypothesis that impaired elimination of tolcapone may be a cause for liver toxicity. *In vitro* studies in rat hepatocyte cultures have shown that inhibition of glucuronidation and oxidation increase cytotoxicity of tolcapone. Moreover, the UGT1A6 Ala181/Ser184 variant was shown to have reduced activity *in vitro* compared with the Thr181/Arg184 variant (Ciotti et. al., Pharmacogenetics 1997, 7, 485-495). This concurs with the findings from the current analysis whereby the presence of Ala181 and the absence of Ser184 were associated with an incrementally higher risk of liver abnormality. The polymorphism located in the 3'UTR of the UGT1A gene (Figure 2) may affect the expression of all UGT1A genes involved in metabolism of Tolcapone. Alternatively, the polymorphism may be in linkage disequilibrium with another mutation that affects either the structure of the UGT1A proteins, or the expression of the gene.

No significant association was found with the other markers tested. These results do not rule out the potential contribution of other polymorphisms within the gene tested.

The relatively low odd ratios that result from these associations, are due to the multifactorial nature of drug induced liver toxicity. It was clear throughout this study that the occurrence of liver enzyme elevation upon treatment with tolcapone was the result of multiple factors including external influences such as co-medication, and or the combination of several genetic factors in different individuals.

SEQUENCE LISTING

<110> F. Hoffmann- La Roche AG

5 <120> Method for detecting a pre-disposition to drug induced  
hepatotoxicity

<130> 20707 ELMS

10 <140>  
<141>

<150> EP00115353.5  
<151> 2000-07-14

15 <160> 56

<170> PatentIn Ver. 2.1

20 <210> 1  
<211> 1561  
<212> DNA  
<213> Homo sapiens

25 <300>  
<308> GENBANK M84124

<400> 1

30	taattccagc	tactctggag	gctgaggcag	gaggatggct	tgagcccagg	agttggaggc	60
	tgcagtttagc	catgcttgtg	ccactacact	ccagcccggg	caacagggca	agactctgta	120
	tctaaaaaca	acaacaacaa	caataataga	aacagggttc	ctttcccaag	tttggaaaat	180
	ctggtagtct	tcttaagcag	ccatgagcat	aaagagagga	ttgttcatac	cacagggtgtt	240
	ccaggcataa	cgaaactgtc	tttgtgttta	gttacaagga	gaacatcatg	cgctctcca	300
	gccttcacaa	ggaccgccc	gtggagccgc	tggacctggc	cgtgttctgg	gtggagtttg	360
35	tgatgaggca	caagggcgcg	ccacacctgc	gccccgcagc	ccacgacctc	acctggtacc	420
	agtaccattc	cttggacgtg	attggtttcc	tcttggccgt	cgtgctgaca	gtggccttca	480
	tcacctttaa	atggttgtgt	tatggctacc	ggaaatgctt	ggggaaaaa	gggagagtta	540
	agaaagccca	caaatccaag	accattgag	aagtgggtgg	gaaataaggt	aaaattttga	600
	accattccct	agtcatttcc	aaacttgaaa	acagaatcag	tgtaaattc	attttattct	660
40	tattaaggaa	atactttgca	taaattaatc	agccccagag	tgctttaaaa	aattctctta	720
	aataaaaata	atagactcgc	tagtcagtaa	agatatttga	atatgtatcg	tgccccctct	780
	ggtgtctttg	atcaggatga	catgtgccat	ttttcagagg	acgtgcagac	aggctggcat	840
	tctagattac	ttttcttact	ctgaaacatg	gcctgtttgg	gagtgcggga	ttcaaagggtg	900
	gtcccacggc	tgcccctact	gcaaatggca	gttttaatct	tatcttttgg	cttctgcaga	960
45	tggttgcaat	tgatccttaa	ccaataatgg	tcagtcctca	tctctgtcgt	gcttcatagg	1020
	tgccaccttg	tgtgtttaaa	gaaggggaagc	tttgtacctt	tagagtgtag	gtgaaatgaa	1080
	tgaatggctt	ggagtgcact	gagaacagca	tatgatttct	tgctttgggg	aaaaagaatg	1140
	atgctatgaa	attggtgggt	ggtgtatattg	agaagataat	cattgcttat	gtcaaattgga	1200
	gctgaatttg	ataaaaaacc	aaaatacagc	tatgaagtgc	tgggcaagtt	tacttttttt	1260
50	ctgatgtttc	ctacaactaa	aaataaatta	ataaatttat	ataaattcta	tttaagtgtt	1320
	ttcactgggtg	tcgcatttat	ttcttgttaa	gttgcatttt	ctaattacaa	aagtaatgca	1380
	tgattatgac	agaaagtgtg	gaaaatatag	aggttcacac	acacacgcct	tcattgcgtg	1440
	tccatgcata	aatgcatgag	aaaagaaaaa	taaccagtaa	tcacatcgcc	cagaaataac	1500
	cccagttaca	attgtggcaa	atacacatac	ttataaatat	tgcatatata	ttaagtatac	1560
55	c						1561

<210> 2  
<211> 1220

<212> DNA  
 <213> Homo sapiens

<300>  
 5 <308> GENBANK M84130

<400> 2

	tgacacggcc	atagttgggt	catattaacc	atgtgattaa	aatgggttaa	tattaatttg	60
	ggttcttaca	tatcaaaggg	taaaattcag	agcaagggag	aggtagacag	gacctgtgaa	120
10	aagcagtgg	tagtttaggg	aaaataccta	ggagccctgt	gatttgaga	gtgaaaactc	180
	tttattaccg	ttgttacttt	aactctttcc	aggatggcct	gcctccttcg	ctcatttcag	240
	agaatttctg	caggggtttt	cttcttagca	ctttggggca	tggttgtagg	tgacaagctg	300
	ctgggtggcc	ctcaggacgg	aagccactgg	cttagtatga	aggatatagt	tgaggttctc	360
	agtgaccggg	gtcatgagat	tgtagtgggt	gtgcctgaag	ttaatttgct	tttгаааgaa	420
15	tccaaatact	acacaagaaa	aatctatcca	gtgccgtatg	accaagaaga	gctgaagaac	480
	cgttaccaat	catttggaag	caatcacttt	gctgagcgat	cattcctaac	tgctcctcag	540
	acagagtaca	ggaataacat	gattgttatt	ggcctgtact	tcatcaactg	ccagagcctc	600
	ctgcaggaca	gggacaccct	gaacttcttt	aaggagagca	agtttgatgc	tcttttcaca	660
	gaccagcct	taccctgtgg	ggtgatcctg	gctgagtatt	tgggcctacc	atctgtgtac	720
20	ctcttcaggg	gttttccgtg	ttccctggag	catacattca	gcagaagccc	agaccctgtg	780
	tcctacattc	ccaggtgcta	cacaaagttt	tcagaccaca	tgactttttc	ccaacgagtg	840
	gccaaactcc	ttgttaattt	gttggagccc	tatctatttt	attgtctgtt	ttcaaagtat	900
	gaagaactcg	catcagctgt	cctcaagaga	gatgtggata	taatcacctt	atatcagaag	960
	gtctctgttt	ggctgttaag	atatgacttt	gtgcttgaat	atcctaggcc	ggtcatgccc	1020
25	aacatggctc	tcattggagg	tatcaactgt	aagaagagga	aagacttgct	tcaggttggg	1080
	gggtttat	cttttggact	gccttgtttc	ttccaggctc	tgctctccct	cactcatttg	1140
	gctccttgag	ccgactgtcc	cttggaggat	ttcctggaga	acgggtgggg	gaagtgatac	1200
	ccggctcgga	gcagcgggaa					1220

30  
 <210> 3  
 <211> 1279  
 <212> DNA  
 <213> Homo sapiens

35  
 <300>  
 <308> GENBANK U39570

<400> 3

40	tgtattatta	tgagtaaate	attggcagtg	aatgtgaatt	ttttttttaa	tgaatgaata	60
	agtacacgcc	ttcttttgag	ggcaggttct	atctgtactt	cttccactta	ctatattata	120
	ggagcttaga	atcccagctg	ctggctctgg	gctgaagttc	tctgatggct	cgtgcagggg	180
	ggactggcct	ccttccccta	tatgtgtgtc	tactgctgac	ctgtgctttg	ccaaggtcag	240
	ggaagctgct	ggtagtggcc	atggatggga	gccactgggt	caccatgcag	tcgggtgggg	300
45	agaaactcat	cctcaggggg	catgaggtgg	tcgtagtcat	gccagaggtg	agttggcaac	360
	tgggaagatc	actgaattgc	acagtgaaga	cttactcaac	ctcatacact	ctggaggatc	420
	aggaccggga	gttcatgggt	tttgccgatg	ctcgctggac	ggcaccattg	cgaagtgcac	480
	tttctctatt	aacaagttca	tccaatggta	tttttgactt	atttttttca	aattgcagga	540
	gtttgtttaa	tgaccgaaaa	ttagtagaat	acttaaagga	gagttgtttt	gatgcagtgt	600
50	ttctcgatcc	ttttgatcgc	tgtggcttaa	ttggtgccaa	atatttctcc	ctccccctctg	660
	tggtcttcgc	caggggaata	ttttgccact	atcttgaaga	aggtgcacag	tgccctgctc	720
	ctcttttcta	tgctcccaga	cttctcttag	ggttctcaga	cgccatgact	ttcaaggata	780
	gagtatggaa	ccacatcatg	cacttggagg	aacatttatt	ttgcccctat	tttttcaaaa	840
	atgtcttaga	aatagcctct	gaaatttctc	aaaccctgtg	cacggcatac	gatctctaca	900
55	gccacacatc	aatttgggtg	ttgcgaactg	actttgtttt	ggagtatccc	aaaccctgta	960
	tgcccaatat	gatcttcatt	ggtgggtatca	actgtcatca	gggaaagcca	gtgcctatgg	1020
	taagttatct	ccccttttagc	acattaagaa	taatctggct	ttggaaatta	aaagatttct	1080
	tacagaatca	taatttatca	tttacatttg	tcccatttgg	aatttctttc	tggtttaagg	1140
	aattcttttg	taccaattca	cttaattggt	gggtagcaaa	ttgtataaag	cagctcttgt	1200

tgatatgtaa gtgtatacaa ttgatataat tgtagatcat atctaggctg caatctaaat 1260  
 gctatTTTTTg gaaaaatac 1279

5 <210> 4  
 <211> 1452  
 <212> DNA  
 <213> Homo sapiens

10 <300>  
 <308> GENBANK U39550

<400> 4  
 ctctccctcc aaggcgaaga ccataatcta ctcttgtctg aaatcataca agtaggtatc 60  
 15 tcagcaaatg atactcgtgt gttatcgttc ttatgagtaa atcattggca gtgagtgtga 120  
 tttttttttt ttttatgaaa ggataaatac acgccctcta ttggggtcag gttttgtgcc 180  
 tgtacttctt cgcctactg tatcatagca gcttagaalc ccagctgctg gctcgggctg 240  
 cagttctctc atcgtcgcgc aggggtgatgg ctgcgcgagg gtggaccagc cccgttcctt 300  
 tatgtgtgtg tctactgctg acctgtggct ttgccgaggc agggaaagctg ctggtagtgc 360  
 20 ccatggatgg gagtcaactg ttcacatgc agtcggtggg ggagaaactt atcctcaggg 420  
 ggcatgaggg ggtttagtgc atgccagagg tgagttggca actggaaaga tcaactgaatt 480  
 gcacagtga gacttactca acctcgtaca ctctggaaga tcagaaccgg gaattcatgg 540  
 ttttcgcca tgctcaatgg aaagcacagg cacaagat attttctcta ttaatgagtt 600  
 catccagtgg ttttcttgac ttatTTTTTt cgcattgcag gagtttggtt aatgaccgaa 660  
 25 aattagtaga atacttaaag gagagttctt ttgatgcagt gtttctggat ccttttgata 720  
 cctgtggctt aattgttgc aaatatttct cctccctc tgtggtcttc accaggggaa 780  
 tattttgcca ccatcttgaa gaaggtgcac agtgcctcgc tcctctttcc tatgtcccca 840  
 atgatctctt agggttctca gatgccatga ctttcaagga gagagtatgg aaccacatcg 900  
 tgcacttggg ggaccattta ttttgccagt atcttttttag aaatgcccta gaaatagcct 960  
 30 ctgaaattct ccaaaccct gtcacggcat atgatctcta cagtcacaca tcaatttggg 1020  
 tgttgcaaac ggactttggt ttggactatc ccaaaccctg gatgcccaac atgatcttca 1080  
 ttggtggtat caactgtcat cagggaaagc cattgcctat ggtaagtcac ctctccttca 1140  
 gcacattaag aataatctgg ctttgggaatt aaaaaaggat tccttactga actgtgattt 1200  
 gacatttctg tgtggcattc aatttctttc cagtttaaca aattatTTTg tgcaattca 1260  
 35 tgtactcatc aattatcaaa ttttataaaa ctgcccttct tgaaagtata tgtaataatt 1320  
 taaaaattat agatcatatt caggctacat tttaaaatac gatgttttaga aaagtaccaa 1380  
 aaaaccacag caagaaatga aacttccgtt tttttgttat tctatgtgac cccgtagttg 1440  
 aaaatgctct ta 1452

40 <210> 5  
 <211> 1244  
 <212> DNA  
 <213> Homo sapiens

45 <300>  
 <308> GENBANK U42604

<400> 5  
 50 gggcatgatc tgtccaaggc agagactata agctactctt atagtactct tatgagatac 60  
 atacaagtag gtatctcaaa aaatgatact catgtattcc tgttcttatg agtaaatcat 120  
 tggcagtgag tgtgattttt ttttttttta tgacaggatc cctacacgcc ctctattggg 180  
 gtcaggTTTT gtgcctgtag ttcttccgcc tacgtatcat agcagttaga atcccagctg 240  
 ctggctcggg ctgcagttct ctcatggctc gcacagggtg gaccagcccc attcccctat 300  
 55 gtgtttctct cctgctgacc tgtggctttg ctgagggcagg gaagctgctg gtagtgccca 360  
 tggatgggag tcaactggtc accatgcagt cgggtggtgga gaaacttatc ctcagggggc 420  
 atgaggtggg tgtagtcatg ccagaggtga gttggcaact gggaaaatca ctgaattgca 480  
 cagtgaagac ttactcaacc tcatacactc tggaggatct ggaccgggaa ttcatggatt 540  
 tcgccgatgc tcaatggaaa gcacaagtac gaagtttgtt ttctctattt ctgagttcat 600

```

ccaatggttt ttttaactta tttttttcgc attgcaggag tttgtttaat gaccgaaaat 660
tagtagaata cttaaaggag agttcttttg atgcggtggt tcttgatcct tttgatgcct 720
gtgcgttaat tgttgccaaa tatttctccc tcccctctgt ggtcttcgcc aggggaatag 780
gttgccacta tcttgaagaa ggtgcacagt gcctgctcc tctttcctat gtccccagaa 840
5 tttctcttagg gttctcagat gccatgactt tcaaggagag agtacggaac cacatcatgc 900
acttggagga acatttattt tgccagtatt tttccaaaaa tgccctagaa atagcctctg 960
aaattctoca aacacctgtc acagcatatg atctctacag ccacacatca atttggttgt 1020
tgccaacaga ctttgttttg gactatccca aaccctgtat gcccaatatg atcttcattg 1080
gtggtatcaa ctgccatcag ggaaagccat tgccatgggt aagtcacctc tccttttagca 1140
10 cattaggaat aatcttggct ttggaaatta aaaaaagatt ccttactgaa ttgtgatttg 1200
acattttcat ttggtgcatt tcaaatttct ttccagttta caga 1244

```

```

<210> 6
15 <211> 2320
    <212> DNA
    <213> Homo sapiens

```

```

<300>
20 <308> GENBANK AF056188

```

```

<400> 6
ctcagctgca gttctctgat ggcttgcaca ggggtggacca gcccccttcc tctatgtgtg 60
tgtctgctgc tgacctgtgg ctttgccgag gcagggaagc tactggtagt gcccatggat 120
25 gggagccact ggttcaccat gaggtcggtg gtggagaaac tcattctcag ggggcatgag 180
gtggttgtag tcatgccaga ggtgagttgg caactgggaa gatcactgaa ttgcacagtg 240
aagacttatt caacttcata taccctggag gatctggacc gggagttaa ggcttttggc 300
catgctcaat ggaaagcaca agtacgaagt atatatctc tattaatggg ttcatacaat 360
gacatttttg acttattttt ttcaaattgc aggagtttgt ttaaagacaa aaaattagta 420
30 gaatacttaa aggagagttc ttttgatgca gtgtttctcg atccttttga taactgtggc 480
ttaattggtg ccaaataatt ctcccctccc tccgtggctc tcgccagggg aatactttgc 540
cactatcttg aagaaggtgc acagtgccct gctcctcttt cctatgtccc cagaattctc 600
ttagggttct cagatgccat gactttcaag gagagagtac ggaaccacat catgcacttg 660
gaggaacatt tattatgcca ccgttttttc aaaaatgcc tagaaatagc ctctgaaatt 720
35 ctccaaacac ctgttacgga gtatgatctc tacagccaca catcaatttg gttgttgca 780
acggactttg ttttgacta tcccaaacc gtgatgccca acatgatctt cattggtggg 840
atcaactgcc atcagggaaa gcggttgcc atggaatttg aagcctacat taatgcttct 900
ggagaacatg gaattgtgtg tttctcttgg ggatcaatgg tctcagaaat tccagagaag 960
aagctatagg caattgctga tgctttgggc aaaaatccctc agacagtcct gtggcgttac 1020
40 actggaacc caccatcgaa tcttgcgaac aacacgatac ttgttaagt gctaccccaa 1080
aacgatctgc ttggtcacc gatgaccgt gcctttatca cccatgctgg ttcccatggg 1140
gtttatgaaa gcatatgcaa tggcgttccc atgggtgatga tgcccttgtt tgggtgatcag 1200
atggacaatg caaagcgc atggagactaag ggagctggag tgaccctgaa tgttctggaa 1260
atgacttctg aagatttaga aaatgctcta aaagcagtca tcaatgaca aagttacaag 1320
45 gagaacatca tgccctctc cagccttcac aaggaccgcc cgggtggagcc gctggacctg 1380
gccgtgttct ggggtggagt tgtgatgagg cacaagggcg cccacacct gcgcccgcga 1440
gccacgacc tcacctgta ccagtaccat tccttggacg tgattgggtt cctcttggcc 1500
gtcgtgctga cagtggcctt catcaccttt aaatgttgtg cttatggcta ccggaaatgc 1560
ttggggaaaa aagggcgagt taagaaagcc cacaaatcca agaccattg agaagtgggt 1620
50 gggaaataag gtaaaattt gaaccattcc ctagtcatct ccaaacttga aaacagaatc 1680
agtgttaaat tcattttatt cttattaagg aaatactttg cataaattaa tcagccccag 1740
agtgtcttaa aaaattctct taataaaaa taatagactc gctagtcatg aaagatattt 1800
gaatatgtat cgtgccccct ccggtgtctt tgatcaggat gacatgtgcc atttttcaga 1860
ggacgtgcag acaggctggc attctagatt acttttctta ctctgaaaca tggcctgttt 1920
55 gggagtgcgg gattcaaagg tggcccacc gctgccccta ctgcaaatgg cagttttaat 1980
cttatctttt ggcttctgca gatggttgca attgatcctt aaccaataat ggtcagtcct 2040
catctctgtc ctgcttcata ggtgccacct tgtgtgttta aagaagggaa gctttgtacc 2100
tttagagtgt aggtgaaatg aatgaatggc ttggagtgca ctgagaacag catatgattt 2160
cttgcttttg ggaaaaagaa tgatgctatg aaattgggtg gtggtgtatt tgagaagata 2220

```

atcattgctt atgtcaaag gagctgaatt tgataaaaac ccaaaataca gctatgaagt 2280  
 gctgggcaag tttacttttt ttctgatggt tcctacaact 2320

5 <210> 7  
 <211> 512  
 <212> DNA  
 <213> Homo sapiens

10 <300>  
 <308> GENBANK M84122

<400> 7  
 tttgcatctc aaggataatt ctgtaagcag gaacccttcc tccttttagaa ggaagtaaag 60  
 15 gagaggaaaa tgctgtaaaa cttacatatt aataatTTTT tactctatct caaacacgca 120  
 tgcctttaat catagtctta agaggaagat atctaattca taacttactg tatgtagtca 180  
 tcaaagaata tgagaaaaaa ttaactgaaa atttttcttc tggctctagg aatttgaagc 240  
 ctacattaat gcttctggag aacatggaat tgtggttttc tctttgggat caatggctctc 300  
 agaaattcca gagaagaaag ctatggcaat tgctgatgct ttggggcaaaa tccctcagac 360  
 20 agtaagaaga ttctatacca tggcctcata tctatTTTca caggagcgct aatcccagac 420  
 ttccagcttc cagattaatt ctcttaattg gaacccttaga tttggctttt ccttgccact 480  
 tcccaactat taatccaaag gttttttttg tt 512

25 <210> 8  
 <211> 784  
 <212> DNA  
 <213> Homo sapiens

30 <300>  
 <308> GENBANK M84123

<400> 8  
 aaagatgtcc tcaagggacc ctgttttcta gttagtatag cagatttggt ttctaactcat 60  
 35 attatgtctt tctttacggt ctgctctttt gccctccca ggtcctgtgg cggtaactctg 120  
 gaaccgacc atcgaatctt gcgaacaaca cgatacttgt taagtggcta ccccaaacg 180  
 atctgcttgg tatgttgggc ggattggatg tatagggtcaa accagggtca aattaagaaa 240  
 atggcttaag cacagctatt ctaaaggatt gttgagcttg aaaatattat ggccaacata 300  
 tcctacattg ctttttatct agtggggtat ctcaaccac attttcttct gcaaatttct 360  
 40 gcaagggcat gtgagtaaca ctgagctctt ggagtgtttt cagaacctag atgtgtccag 420  
 ctgtgaaact cagagatgta actgctgaca tcctccctat tttgcatctc aggtcaccctg 480  
 atgaccctgt cctttatcac ccatgctggt tcccatgggtg tttatgaaag catatgcaat 540  
 ggcgttccca tggatgatgat gcccttgttt ggtgatcaga tggacaatgc aaagcgcctg 600  
 gagactaagg gagctggagt gaccctgaat gttctggaaa tgacttctga agatttagaa 660  
 45 aatgctctaa aagcagtcac caatgacaaa aggtaagaaa gaagatacag aagaatactt 720  
 tggatcatggc attcatgata aaattgTTTT aaatatgaaa acatttacgt agcatttaac 780  
 acgt 784

50 <210> 9  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

55 <400> 9  
 gcacaccttg tccttcat 18

<210> 10

- 46 -

<211> 18  
 <212> DNA  
 <213> Homo sapiens

5 <400> 10  
 gcacaccttg tccttcac 18

<210> 11  
 10 <211> 19  
 <212> DNA  
 <213> Homo sapiens

<400> 11  
 15 catcaccatc gagatcaac 19

<210> 12  
 <211> 20  
 20 <212> DNA  
 <213> Homo sapiens

<400> 12  
 25 ctattaaatc gcctctctct 20

<210> 13  
 <211> 20  
 <212> DNA  
 30 <213> Homo sapiens

<400> 13  
 ctattaaatc gcctctctcc 20

35 <210> 14  
 <211> 19  
 <212> DNA  
 <213> Homo sapiens

40 <400> 14  
 ggatgaattt caagtattt 19

45 <210> 15  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

50 <400> 15  
 agcccagata ccccaaag 18

<210> 16  
 55 <211> 18  
 <212> DNA  
 <213> Homo sapiens

<400> 16

- 47 -

agcccagata ccccaaaa 18

<210> 17  
5 <211> 18  
<212> DNA  
<213> Homo sapiens

<400> 17  
10 tgtgctttct cgtcttca 18

<210> 18  
<211> 18  
15 <212> DNA  
<213> Homo sapiens

<400> 18  
20 tgtaattcct gccgtcag 18

<210> 19  
<211> 18  
<212> DNA  
25 <213> Homo sapiens

<400> 19  
tgtaattcct gccgtcaa 18

30  
<210> 20  
<211> 17  
<212> DNA  
<213> Homo sapiens

35  
<400> 20  
atacagcact ggcattgg 17

40 <210> 21  
<211> 18  
<212> DNA  
<213> Homo sapiens

45 <400> 21  
cctggagttt gtggggcg 18

<210> 22  
50 <211> 18  
<212> DNA  
<213> Homo sapiens

<400> 22  
55 cctggagttt gtggggca 18

<210> 23  
<211> 18

- 48 -

<212> DNA  
 <213> Homo sapiens

<400> 23  
 5 tgaaccatga agtccacg 18

<210> 24  
 <211> 18  
 10 <212> DNA  
 <213> Homo sapiens

<400> 24  
 15 cgtgttcctt ggagcata 18

<210> 25  
 <211> 18  
 <212> DNA  
 20 <213> Homo sapiens

<400> 25  
 cgtgttcctt ggagcatg 18

25  
 <210> 26  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

30 <400> 26  
 gaatgtagga cacagggctt 20

35 <210> 27  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

40 <400> 27  
 gacacagggt ctgggctt 18

45 <210> 28  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

<400> 28  
 50 gacacagggt ctgggctg 18

<210> 29  
 <211> 19  
 55 <212> DNA  
 <213> Homo sapiens

<400> 29  
 tacctcttca ggggttttc 19

<210> 30  
 <211> 16  
 5 <212> DNA  
 <213> Homo sapiens

<400> 30  
 tgcagtaggg gcagcg 16  
 10

<210> 31  
 <211> 16  
 <212> DNA  
 15 <213> Homo sapiens

<400> 31  
 tgcagtaggg gcagcc 16  
 20

<210> 32  
 <211> 16  
 <212> DNA  
 <213> Homo sapiens  
 25

<400> 32  
 ggagtgcggg attcaa 16

30 <210> 33  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

35 <400> 33  
 acacggccat agttggttca 20

40 <210> 34  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

<400> 34  
 45 cagttgatga agtacaggcc 20

<210> 35  
 <211> 20  
 50 <212> DNA  
 <213> Homo sapiens

<400> 35  
 55 tgtagtgggtg gtgcctgaag 20

<210> 36  
 <211> 21  
 <212> DNA

- 50 -

<213> Homo sapiens  
 <400> 36  
 gacagctgat gcgagttctt c 21  
 5

<210> 37  
 <211> 20  
 <212> DNA  
 10 <213> Homo sapiens

<400> 37  
 gagggcaggt tctatcgtac 20  
 15

<210> 38  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens  
 20

<400> 38  
 gggcactgtg caccttcttc 20

25 <210> 39  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

30 <400> 39  
 acggcaccat tgcgaagtgc 20

35 <210> 40  
 <211> 23  
 <212> DNA  
 <213> Homo sapiens

40 <400> 40  
 acttacatat caacaagtgc tgc 23

45 <210> 41  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

<400> 41  
 gggcatgatc tgtccaaggc 20  
 50

55 <210> 42  
 <211> 22  
 <212> DNA  
 <213> Homo sapiens

<400> 42  
 ggttgagtaa gtcttcactg tg 22

- 51 -

<210> 43  
<211> 18  
<212> DNA  
5 <213> Homo sapiens

<400> 43  
ctcagctgca gttctctg 18

10  
<210> 44  
<211> 21  
<212> DNA  
<213> Homo sapiens

15  
<400> 44  
ccagatcctc cagggtatat g 21

20 <210> 45  
<211> 20  
<212> DNA  
<213> Homo sapiens

25 <400> 45  
gagttcatcc agtgggttttc 20

30 <210> 46  
<211> 18  
<212> DNA  
<213> Homo sapiens

35 <400> 46  
cagttcagta aggaatcc 18

40 <210> 47  
<211> 22  
<212> DNA  
<213> Homo sapiens

45 <400> 47  
caaggataat tctgtaagca gg 22

50 <210> 48  
<211> 22  
<212> DNA  
<213> Homo sapiens

<400> 48  
ggattaatag ttgggaagtg gc 22

55  
<210> 49  
<211> 20  
<212> DNA  
<213> Homo sapiens

<400> 49  
ggccaacata tcctacattg 20

5  
<210> 50  
<211> 23  
<212> DNA  
<213> Homo sapiens

10  
<400> 50  
cgtattaaat gctacgtaaa tgt 23

15 <210> 51  
<211> 20  
<212> DNA  
<213> Homo sapiens

20 <400> 51  
cagttagcca tgcttgtgcc 20

<210> 52  
25 <211> 20  
<212> DNA  
<213> Homo sapiens

<400> 52  
30 gcactctggg gctgattaat 20

<210> 53  
<211> 19  
35 <212> DNA  
<213> Homo sapiens

<400> 53  
40 cgtgctgaca gtggccttc 19

<210> 54  
<211> 20  
<212> DNA  
45 <213> Homo sapiens

<400> 54  
cagtgcactc caagccattc 20

50  
<210> 55  
<211> 18  
<212> DNA  
<213> Homo sapiens

55  
<400> 55  
gatggttgca attgatcc 18

<210> 56  
<211> 20  
<212> DNA  
<213> Homo sapiens

5

<400> 56  
ttagttgtag gaaacatcag

20

Claims

1. A method for detecting a predisposition to a hepatotoxic reaction of a human being caused by the administration of a pharmaceutically active compound based on the determination of at least one single nucleotide polymorphism in the UDP-  
5 glucuronosyltransferase (UGT1) gene in a sample of said human being, which method comprises  
determining the nucleotide at position 908 in exon 5 of the UGT1 gene as defined by the position in SEQ ID NO:1, and/or  
determining the nucleotide at position 528 in exon 1 of the UGT1A6 gene as defined by the  
10 position in SEQ ID NO:2, and/or  
determining the nucleotide at position 197 in exon 1 of the UGT1A7 gene as defined by the position in SEQ ID NO:3, and  
determining the status of the human being.

2. A method for detecting a predisposition to a hepatotoxic reaction of a human  
15 being caused by the administration of a pharmaceutically active compound based on the determination of at least one single nucleotide polymorphism in the UDP-  
glucuronosyltransferase (UGT1) gene in a sample of said human being, which method comprises determining the nucleotide at position 908 in exon 5 of the UGT1 gene as defined by the position in SEQ ID NO:1 and determining the status of the human being.

20 3. The method according to any one of claims 1 or 2, wherein additionally the polymorphism at one or more of the following positions is determined:  
position 232 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2, or  
position 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2, or  
position 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2, or  
25 position 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3, or  
position 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3, or  
position 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3, or  
position 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3.

4. A method according to any one of claims 1 to 3, in which the single nucleotide  
30 polymorphism at position 908 in exon 5 of the UGT1 gene locus consists of the presence of a C or a G, the single nucleotide polymorphism at position 528 in exon 1 of UGT1A6 consists of the presence of a G or an A, the single nucleotide polymorphism at position 197 in exon 1 of UGT1A7 consists of the presence of a G or a C, the single nucleotide  
polymorphism at position 232 in exon 1 of UGT1A6 consists of the presence of a G or a T,  
35 the single nucleotide polymorphism at position 754 in exon 1 of UGT1A6 consists of the

presence of an A or a G, the single nucleotide polymorphism at position 765 in exon 1 of UGT1A6 consists of the presence of an A or a C, the single nucleotide polymorphism at position 551 in exon 1 of UGT1A7 consists of the presence of an G or a T, the single nucleotide polymorphism at position 555 in exon 1 of UGT1A7 consists of the presence of an A or a C, the single nucleotide polymorphism at position 556 in exon 1 of UGT1A7 consists of the presence of an A or a C, and the single nucleotide polymorphism at position 786 in exon 1 of UGT1A7 consists of the presence of a C or a T.

5. A method as claimed in claims 1 to 4, wherein the region containing the potential polymorphism is amplified, preferably by polymerase chain reaction, prior to determining the sequence.

6. A diagnostic nucleic acid comprising the following polymorphism containing sequences:

- the nucleic acid sequence of SEQ ID NO:1 with C at position 908 in exon 5 of UGT1 as defined by the position in SEQ ID NO:1;
- 15 the nucleic acid sequence of SEQ ID NO:1 with G at position 908 in exon 5 of UGT1 as defined by the position in SEQ ID NO:1;
- the nucleic acid sequence of SEQ ID NO:2 with G at position 232 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with T at position 232 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 20 the nucleic acid sequence of SEQ ID NO:2 with G at position 528 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with A at position 528 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 25 the nucleic acid sequence of SEQ ID NO:2 with G at position 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with A at position 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with C at position 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 30 the nucleic acid sequence of SEQ ID NO:2 with A at position 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:3 with G at position 197 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 35 the nucleic acid sequence of SEQ ID NO:3 with C at position 197 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;

- the nucleic acid sequence of SEQ ID NO:3 with G at position 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with T at position 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 5 the nucleic acid sequence of SEQ ID NO:3 with A at position 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with C at position 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with A at position 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 10 the nucleic acid sequence of SEQ ID NO:3 with G at position 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with C at position 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 15 the nucleic acid sequence of SEQ ID NO:3 with T at position 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3; or
- a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

7. A diagnostic nucleic acid selected from the group consisting of
- 20 the nucleic acid sequence of SEQ ID NO:1 with C at position 908 in exon 5 as defined by the position in SEQ ID NO:1;
- the nucleic acid sequence of SEQ ID NO:2 with G at position 528 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2; or
- the nucleic acid sequence of SEQ ID NO:3 with G at position 197 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3.
- 25

8. A diagnostic nucleic acid selected from the group consisting of
- the nucleic acid sequence of SEQ ID NO:1 with G at position 908 in exon 5 of UGT1 as defined by the position in SEQ ID NO:1;
- the nucleic acid sequence of SEQ ID NO:2 with G at position 232 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 30 the nucleic acid sequence of SEQ ID NO:2 with T at position 232 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with A at position 528 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 35 the nucleic acid sequence of SEQ ID NO:2 with G at position 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;

- the nucleic acid sequence of SEQ ID NO:2 with A at position 754 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with C at position 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- 5 the nucleic acid sequence of SEQ ID NO:2 with A at position 765 in exon 1 of UGT1A6 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:3 with C at position 197 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with G at position 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 10 the nucleic acid sequence of SEQ ID NO:3 with T at position 551 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with A at position 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 15 the nucleic acid sequence of SEQ ID NO:3 with C at position 555 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with A at position 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- the nucleic acid sequence of SEQ ID NO:3 with G at position 556 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 20 the nucleic acid sequence of SEQ ID NO:3 with C at position 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3; or
- the nucleic acid sequence of SEQ ID NO:3 with T at position 786 in exon 1 of UGT1A7 as defined by the position in SEQ ID NO:3;
- 25 whenever used in combination with any one of the diagnostic nucleic acids as claimed in claim 7.

9. A set of diagnostic nucleic acids comprising the following polymorphism containing sequences:

- the nucleic acid sequence of SEQ ID NO:1 with C at position 908 in exon 5 as defined by the position in SEQ ID NO:1;
- 30 the nucleic acid sequence of SEQ ID NO:1 with G at position 908 in exon 5 as defined by the position in SEQ ID NO:1;
- the nucleic acid sequence of SEQ ID NO:2 with G at position 754 in exon 1 as defined by the position in SEQ ID NO:2;
- 35 the nucleic acid sequence of SEQ ID NO:2 with A at position 754 in exon 1 as defined by the position in SEQ ID NO:2;
- the nucleic acid sequence of SEQ ID NO:2 with C at position 765 in exon 1 as defined by

the position in SEQ ID NO:2;  
the nucleic acid sequence of SEQ ID NO:2 with A at position 765 in exon 1 as defined by  
the position in SEQ ID NO:2; or  
a complementary strand thereof or a fragment thereof of at least 20 bases comprising at  
5 least one of the polymorphisms.

10. A diagnostic nucleic acid primer for detecting a polymorphism in the UGT1 gene  
capable of hybridizing specifically to a nucleic acid having one of the polymorphisms as  
defined in claim 4.

11. A diagnostic nucleic acid primer as claimed in claim 10 which is an allele-specific  
10 nucleic acid primer having a sequence selected from the group consisting of:  
the nucleic acid sequence as defined by SEQ ID NO:24;  
the nucleic acid sequence as defined by SEQ ID NO:25;  
the nucleic acid sequence as defined by SEQ ID NO:27;  
the nucleic acid sequence as defined by SEQ ID NO:28;  
15 the nucleic acid sequence as defined by SEQ ID NO:30; or  
the nucleic acid sequence as defined by SEQ ID NO:31.

12. An allele-specific oligonucleotide probe for detecting a polymorphism in the  
UGT1 gene capable of hybridizing specifically to a nucleic acid having one of the  
polymorphisms as defined in claim 4.

20 13. A diagnostic kit comprising one or more diagnostic primer(s) as defined in claim  
10 and/or one or more allele-specific oligonucleotide probes(s) as defined in claim 12.

14. A pharmaceutical pack comprising Tolcapone and instructions for  
administration of the drug to humans diagnostically tested for a single nucleotide  
polymorphism according to a method as claimed in any one of claims 1 to 5.

25 15. A computer readable medium having stored thereon sequence information for  
the polymorphisms in UGT1 at position 908 in exon 5 of the UGT1 gene locus as defined  
by the position in SEQ ID NO:1 and/or at position 528 in exon 1 of the UGT1A6 gene as  
defined by the position in SEQ ID NO:2 and/or at position 197 in exon 1 of the UGT1A7  
gene as defined by the position in SEQ ID NO:3.

30 16. A method for performing sequence identification, said method comprising the  
steps of providing a diagnostic nucleic acid sequence as claimed in any one of claims 6 to 9  
and comparing said diagnostic nucleic acid sequence to at least one other nucleic acid or  
polypeptide sequence to identify identity.

17. A method as hereinbefore described.

\*\*\*

Fig. 1

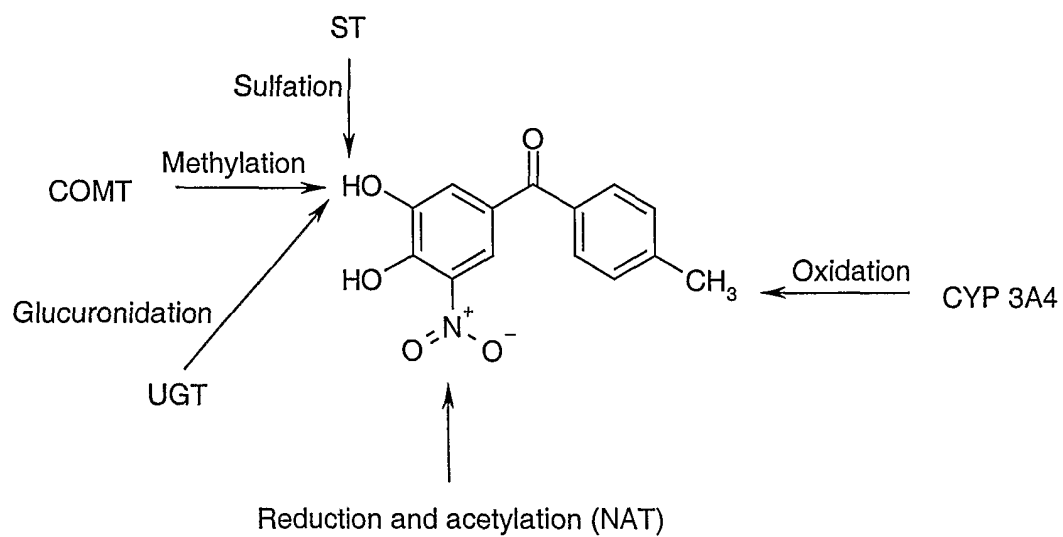


Fig. 2

