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(54) Title: GENETIC DIAGNOSIS FOR QT PROLONGATION RELATED ADVERSE DRUG REACTIONS

(57) Abstract: The specification is directed to a method of diagnosing whether a subject is predisposed to an adverse reaction to one or more pharmaceutical agents which may induce a prolonged QT interval or acquired LQTS in that individual. The diagnosis is genetic analysis of at least two polymorphisms or mutations which the individual may have, which are associated with an increased risk for prolonged QT intervals or Torsades de Pointes (TdP). Genetic screening for determining the predisposition of prolonged QT intervals induced by a pharmaceutical agent is performed by identifying genetic polymorphisms or mutations located in at least two classes or genes, wherein the genes are (1) *LQT* genes, (2) altered sensitivity genes (e.g., *MiRP1*) or (3) increased exposure genes (*e.g., MDR* genes or P450 cytochrome genes). The specification is also directed to compositions and kits for determining such predispositions to adverse drug reactions.

GENETIC DIAGNOSIS FOR QT PROLONGATION RELATED ADVERSE DRUG REACTIONS

CROSS REFERENCE TO RELATED APPLICATION

This application claims benefit of priority from U.S. Provisional Patent Application Serial No. 60/196,916, filed April 13, 2000, the entirety of which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods of determining a predisposition for QT interval prolongation in a subject after the administration of a pharmaceutical agent or agents. Compositions and kits for determining said predispositions to the QT interval prolongation are also described.

BACKGROUND OF THE INVENTION

The invention relates to a method of screening a subject for a predisposition to an adverse drug reaction involving prolonged QT intervals. The genetic screening of patients for said predisposition focuses on genes associated with QT interval prolongation, including *LQT* genes, P-glycoprotein membrane pump proteins (P-gp), multidrug resistance genes and cytochrome P450-mediated drug metabolism genes.

I. LQT and Cytochrome P450 Genes and Polymorphisms

1. LQT Genes

Genes associated with long QT (LQT) syndrome (LQTS) include *KVLQT1* (*LQT1*), *HERG* (*LQT2*), *SCN5A* (*LQT3*) and *MinK* (*LQT5*). A fifth gene locus exists on human chromosome 4 (*e.g., LQT4*). Recently, a sixth gene (*LQT6*) has been identified (Wang *et al.*, *Ann. Med.* 30: 58-65 (1998)). All but *LQT3* encode cardiac potassium ion (K^+) channel proteins; *LQT3* encodes a cardiac sodium ion (Na^+) channel protein (Vincent, *Annu. Rev. Med.* 49: 263-74 (1998)). At least 180 mutations have been identified among these genes (Abbott *et al.*, *Cell* 97: 175-87

(1999); Vincent, *Annu. Rev. Med.* 49: 263-74 (1998); Curran *et al., Cell* 80: 795-803 (1995); Berthet *et al., Circulation* 99: 1464-70 (1999); Dausse *et al., J. Mol. Cell Cardiol.* 28: 1609-15 (1996); Chen *et al., J. Biol. Chem.* 274: 10113-8 (1999); and Sanguinetti *et al., Proc. Natl. Acad. Sci. U.S.A.* 93: 2208-12 (1996)). Some of these mutations cause altered ion channel function resulting in non-drug induced prolonged QT intervals and a propensity for Torsades de Pointes (TdP) (See, *e.g.*, Berthet *et al.*, *Circulation* 99: 1464-70 (1999)). Accordingly, genetic screening can be performed on subjects suspected of having long QT syndrome, as well as other patients (see, *e.g.*, Satler *et al., Hum. Genet.* 102: 265-72 (1998)). Larson *et al., Hum. Mutat.* 13: 318-27 (1999) reported a high-throughout single strand polymorphism (SSCP) analysis for detecting point mutations associated with LQTS.

U.S. Patent No. 5,599,673 claims two (*e.g.*, *HERG* and *SCN5A*) of the six *LQT* genes. Two *HERG*-related genes have also been claimed (U.S. Patent No. 5,986,081). International PCT Application WO 97/23598 describes a method of assessing a patient's risk for long QT syndrome (LQTS) by screening for genetic mutations in the MinK gene. However, these patents do not disclose methods of diagnosing a patient's predisposition to an adverse drug reaction involving elongation of the QT interval due to mutations in any of the LQT genes.

Drugs have been identified that cause QT interval prolongation, and thereby adverse drug reactions. Certain antihistamines, such as terfenadine (*e.g.*, Seldane®) and astemizole (*e.g.*, Hismanal®), reportedly block potassium channels (Woosley, *Annu. Rev. Pharmacol. Toxicol.* 36: 233-52 (1996)) and inhibit the HERG protein, and thereby were postulated to induce Torsades de Pointes (Wang *et al.*, 1998). All antiarrhythmic drugs that lengthen repolarization reportedly can cause Torsades de Pointes (Drici *et al., Circulation* 94: 1471-4 (1996)). Additional non-cardiac and cardiac drugs capable of inducing QT prolongation including many that were identified by the inventor were released on March 27, 1998 at the following web site: www.qtdrugs.org. However, Wei *et al.*, *Circulation* 92: I-125 (1995) could not identify *HERG* or *SCN5A* gene mutations that were linked to acquired LQTS in patients treated with an anti-arrhythmic agent. To the best knowledge of the inventor,

no one has described diagnosing a predisposition towards an adverse drug or drug-drug reaction which causes QT interval elongation by screening patients for one or more polymorphisms in one or more *LQT* genes.

1. Cytochrome P450 Genes

The cytochrome P450 enzymes have also been linked to adverse drug reactions. CYP2D6 was the first cytochrome P450 isoform found to be genetically polymorphic in its distribution (Eichelbaum *et al., Eur. J. Clin. Pharmacol.* 16: 183-7 (1979); and Mahgoub *et al., Lancet* 2: 584-6 (1977)), and it is now clear that this enzyme metabolizes a large number of drugs (Inaba *et al., Can. J. Physiol. Pharmacol.* 73: 331-8 (1995); and Buchert *et al., Pharmacogenetics* 2: 2-11 (1992)). At least 30 mutations exist which alter the activity or specificity of CYP2D6 (Jordan *et al., Endocr. Rev.* 20: 253-78 (1999)). These include alleles that contain single point mutations resulting in no activity (*e.g.,* CYP2D6*4), alleles in which the *CYP2D6* gene has been deleted (*e.g.,* CYP2D6*5) and alleles in which it has been duplicated (*e.g.,* CYP2D6*2_n) (Aklillu *et al., J. Pharmacol. Exp. Ther.* 278: 441-6 (1996)).

There are numerous cytochrome P450 genes which are involved in the metabolism of drugs and drug metabolites. Several of them include *CYP1A2*, *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP2E1*, *CYP3A4*, *CYP3A5* and *CYP3A7*. Allelic variations exist amongst these genes. Certain of these allelic variations combine to produce a poor metabolizer phenotype in 7% of Caucasians, but smaller percentages of Africans and Asians and the "ultrarapid" phenotype in ~5% of Caucasian and up to 30% Africans. As ethnic-specific alleles for both Asians (Yokoi *et al., Pharm. Res.* 15: 517-24 (1998)) and Africans (Aklillu *et al., J. Pharmacol. Exp. Ther.* 278: 441-6 (1996); and Oscarson *et al., Mol. Pharmacol.* 52: 1034-40 (1997)) have been identified, that may alter the mean activity of the enzymes in these populations (see Table 1 below), it is also important to test for these alleles in studies of the relationship between genotype and phenotype.

Table 1

Chromosome Distribution of Cytochrome P450 Gene

Chr. 15	Chr. 10 Polymorphic 3-5% Caucasian PMs 15-20% Asian PMs	Chr. 10 Polymorphic 1-3% Caucasian PMs	Chr. 22 Polymorphic 5-10% Caucasian PMs	Chr. 10	Chr. 7

In fact, due to the metabolic differences, methods have been reported which identify a drug which interacts with the *CYP2C19* gene product, S-mephenytoin 4'-hydroxylase (U.S. Patent No. 5,786,191).

Methods for detecting the presence or absence of mutations in certain of the cytochrome P450 genes have been described. For example, U.S. Patent No. 5,891,633 relates to a method of identifying mutations in the cytochrome P450 genes *CYP2C9* and *CYP2A6*.

International PCT Application WO 95/30772 reportedly describes a CYP2D6 gene polymorphisms involving a 9 bp insertion in exon 9, which is associated with a slower than normal rate of drug metabolism in individuals bearing it and may be therefore useful diagnostically. PCR primers have been described for detecting mutations in drug metabolism enzymes, including detection of the debrisoquine polymorphism, mephenytoin polymorphism and the acetylation polymorphism (U.S. Patent Nos. 5,648,484 and 5,844,108). Additional mutations have been identified in CYP2D6 bufuralol-1'-hydroxylase, including mutations at positions 271, 281, 294, and 506 which result in metabolizer/poor metabolizer phenotypes as described in International PCT Application WO 91/10745 and U.S. Patent No. 5,981,174.

Japanese Patent No. 8168400 provides a method of determining mutations in exons 6 and 7 of the *CYP2C19* gene. Japanese Patent No. 10014585 describes

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