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**(WO/2008/017038) METHODS FOR SELECTING MEDICATIONS**

Biblio. Data

Description

Claims

National Phase

Notices

Documents

**Note:** OCR Text

WO 2008017038 20080207

**METHODS FOR SELECTING MEDICATIONS****TECHNICAL FIELD**

This invention relates to methods for selecting a medication (e.g., psychotropic medication) for a patient, and more particularly to selecting a patient's medication based on the genotype of genes encoding drug-metabolizing enzymes and genes encoding products involved in, for example, neurotransmission.

**SUMMARY**

The invention is based on the identification of a set of genes with polymorphisms that are associated with psychiatric diagnosis and pharmacological response to a medication. As a result, methods of the invention allow the genotype of a patient to be determined and, based on the genotype, a suitable medication to be selected for the patient. Methods of the invention allow the output of multiple genotypic assessments to be integrated, providing important and improved clinical information on which to select and dose medications. Thus, the methods of the invention provide a rational method for the identification of a medication that will result in an optimal response in the patient.

In one aspect, the invention features a method for selecting a psychotropic medication (e.g., an antidepressant, an antipsychotic agent, an antianxiety-sedative agent, or a mood-stabilizing agent) for a patient. The method includes providing the patient's genotype for a panel of genes, wherein the panel includes at least three cytochrome P450 genes and a serotonin transporter gene and selecting the psychotropic medication based

on the genotype. The at least three cytochrome P450 genes can encode CYP2D6, 1A2, and 2C19 and providing the patient's genotype can include determining if the patient has the CYP1A2\*1A or 1A2\*3 allele, the CYP2C19\*1A, 2C19\*1B, or 2C19\*2A allele, and the CYP2D6\*1A, 2D6\*2, 2D6\*2N, 2D6\*3, 2D6\*4, 2D6\*5, 2D6\*6, 2D6\*7, 2D6\*8, 2D6\*10, 2D6\*12, or 2D6\*17 allele. The method further can include determining if the patient includes the 2D6\*41 allele.

The panel further can include a serotonin receptor 2A gene. For example, the panel can include cytochrome P450 genes encoding CYP2D6, 2C19, and 1A2, a serotonin transporter gene, and a serotonin receptor 2A gene. Selecting the psychotropic medication can include correlating genotype of the cytochrome P450 genes with capacity of each cytochrome P450 enzyme encoded by each cytochrome P450 gene to metabolize the psychotropic medication and correlating genotype of the serotonin transporter gene and the serotonin receptor gene with ability of the patient to respond to the psychotropic medication. The panel can include 4 cytochrome P450 genes (e.g., genes encoding CYP2D6,

2C19, 3A4, and 1A2). Providing the patient's genotype can include determining if said patient contains the CYP2D6\*2, 2D6\*3, 2D6\*4, 2D6\*10, or 2D6\*17 allele, the CYP2C19\*2A, 2C19\*2B, 2C19\*3, 2C19\*4, 2C19\*5A, 2C19\*5B, 2C19\*6, 2C19\*7, or 2C19\*8 allele, the CYP3A4\*1B, 3A4\*2, 3A4\*5, 3A4\*6, 3A4\*12, 3A4\*13, 3A4\*15A, 3A4\*17, 3A4\*18A allele, and the CYP1A2\*1F allele.

The panel can include at least 5 cytochrome P450 genes (e.g., CYP1A1, 1A2, 2D6, 2C19, and 3A4). Providing the patient's genotype can include determining if the patient has the CYP1A1\*1A, 1A1\*2, 1A1\*3, or 1A1\*4 allele, the CYP1A2\*1A or 1A2\*3 allele, the CYP2C19\*1A, 2C19\*1B, or 2C19\*2A allele, the CYP2D6\*1A, 2D6\*2, 2D6\*2N, 2D6\*3, 2D6\*4, 2D6\*5, 2D6\*6, 2D6\*7, 2D6\*8, 2D6\*10, 2D6\*12, 2D6\*17, or 2D6\*35 allele, and the CYP3A4\*1A or 3A4\*1B allele.

The panel further can include a plurality of dopamine receptor genes (e.g., dopamine receptor genes encoding dopamine receptors D1, D2, D3, D4, D5, and D6), a plurality of serotonin receptor genes (e.g., serotonin receptor genes encoding serotonin receptors 1A, 1B, 1D, 2A, or 2C), a tryptophan hydroxylase gene, and/or a catechol-O-methyl transferase gene.

Serotonin receptor gene 2 A is particularly useful. In some

embodiments, the panel of genes contains the CYP2D6, 2C19, 3A4, and 1A2 genes, the serotonin receptor gene, and the serotonin receptor 2A gene.

In some embodiments, the panel includes at least 10 cytochrome P450 genes (e.g., genes encoding CYP1A1, 1A2, IBI, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, and 3A5). Determining the patient's genotype can include determining if the patient has the CYP1A1 \*1A, 1A1\*2, 1A1 \*3, or 1A1 \*4 allele; the 1A2\*1A or 1A2\*3 allele; the CYP1B1, 1B1\*2, 1B1\*3, 1B1\*4, 1B1\*11, 1B1\*14, 1B1\*18, 1B1\*19, 1B1\*20, or 1B1 \*25 allele; the CYP2A6\*1A, 2A6\*1B, 2A6\*2, or 2A6\*5 allele; the CYP2B6\*1, 2B6\*2, 2B6\*3, 2B6\*4, 2B6\*5, 2B6\*6, or 2B6\*7 allele; the CYP2C8\*1A, 2C8\*1B, 2C8\*1C, 2C8\*2, 2C8\*3, or 2C8\*4 allele; the CYP2C9\*1, 2C9\*2, 2C9\*3, or 2C9\*5 allele; the CYP2C18\*ml or 2C18\*m2 allele; the CYP2C19\*1A, 2C19\*1B, or 2C19\*2A allele; the CYP2D6\*1A, 2D6\*2, 2D6\*2N, 2D6\*3, 2D6\*4, 2D6\*5, 2D6\*6, 2D6\*7, 2D6\*8, 2D6\*10, 2D6\*12, 2D6\*17, or 2D6\*35 allele; the CYP2E1 \*1A, 2E1\*1C, 2E1 \*1D, 2E1 \*2, 2E1 \*4, 2E1 \*5, or 2E1 \*7 allele; the CYP3A4\*1A or 3A4\*1B allele; and the CYP3A5\*1A, 3A5\*3, 3A5\*5, or 3A5\*6 allele.

In another aspect, the invention features an article of manufacture that includes a substrate. The substrate includes a plurality of discrete regions, wherein each region includes a different population of nucleic acid molecules, wherein the different populations of nucleic acid molecules independently include nucleic acid molecules for detecting the CYP1A2\*1A and 1A2\*3 alleles; the CYP2C19\*1A, 2C19\*1B, and

2C19\*2A alleles; the CYP2D6\*1A, 2D6\*2, 2D6\*2N, 2D6\*3, 2D6\*4, 2D6\*5, 2D6\*6, 2D6\*7, 2D6\*8, 2D6\*10, 2D6\*12, 2D6\*17, and 2D6\*35 alleles; and the 5HTT promoter repeat and exon 2 variable repeat alleles. The population of nucleic acid molecules further can include nucleic acid molecules for detecting one or more of the following: CYP1A1\*1A, 1A1 \*2, 1A1 \*3, and 1A1\*4 alleles; CYP1B1 \*1, 1B1 \*2, 1B1 \*3, 1B1 \*4, 1B1 \*11, 1B1 \*14, 1B1 \*18, 1B1 \*19, 1B1 \*20, and 1B1 \*25 alleles; the CYP2A6\*1A, 2A6\*1B, 2A6\*2, and 2A6\*5 alleles; the CYP2B6\*1, 2B6\*2, 2B6\*3, 2B6\*4, 2B6\*5, 2B6\*6, and 2B6\*7 alleles; the CYP2C8\*1A, 2C8\*1B, 2C8\*1C, 2C8\*2, 2C8\*3, and 2C8\*4 alleles; the CYP2C9\*1, 2C9\*2, 2C9\*3, and 2C9\*5 alleles; the CYP2C18\*ml and 2C18\*m2 alleles; the CYP2C19\*1A, 2C19\*1B, and 2C19\*2A alleles; the CYP2E1 \*1A, 2E1 \*1C, 2E1\*1D, 2E1 \*2, 2E1\*4, 2E1\*5, and 2E1 \*7 alleles; the CYP3A4\*1A and

3A4\*1B alleles; the DAT1 40 bp VNTR and 10 repeat alleles; and the CYP3A5\*1A, 3A5\*3, 3A5\*5, and 3A5\*6 alleles. The population further can include nucleic acid molecules for detecting the TPH A218C, A779C, G5806T, A6526G, and (CT)m(CA)n(CT)p alleles. The invention also features a method of building a database for use in selecting a medication for a patient. The method includes receiving, in a computer system, a plurality of genotypes for a panel of genes, the panel of genes including a CYP2D6 gene, a CYP2C19 gene, a CYP1A2 gene, a serotonin transporter gene, and a serotonin receptor 2A gene; receiving a plurality of medication profiles specified based on the genotypes; and storing the plurality of genotypes and the medication profiles such that each medication profile is associated with one of the genotypes. The at least one medication profile can identify a medication and the medication can be placed in one of multiple categories included in the medication profile. Such categories can be selected from the group consisting of: medications that are safe to use, medications that should be used with caution, medications that should be closely monitored when used, medications that should be avoided, and combinations thereof. The medication profile can identify a universe of possible medications for the patient's genotype.

In another aspect, the invention features a computer program product containing executable instructions that when executed cause a processor to perform operations. The operations can include: receive a plurality of genotypes for a panel of genes, wherein the panel of genes includes a CYP2D6 gene, a CYP2C19 gene, a CYP1A2 gene, a serotonin transporter gene, and a serotonin receptor 2A gene; receive a plurality of medication profiles specified based on the genotypes; and store the genotypes and the medication profiles such that each medication profile is associated with one of the genotypes. The invention also features a method of selecting a medication for a patient. The method includes receiving, in a computer system, a patient's genotype for a panel of genes, wherein the panel of genes includes a CYP2D6 gene, a CYP2C19 gene, a CYP1A2 gene, a serotonin transporter gene, and a serotonin receptor 2A gene; identifying, in a database comprising a plurality of medication profiles associated with genotypes, a medication profile that is associated with the patient's genotype; and outputting the identified medication profile in response to receiving the patient's

genotype. A user can enter the patient's genotype in the computer system or the patient's genotype can be received directly from equipment used in determining the patient's genotype.

The medication profile can include a ranking of several medications, e.g., based on specific co-factors. The method can include adjusting the ranking before outputting the identified medication profile (e.g., based on receiving a genotypic polymorphism carried by the patient or based on receiving a clinical response relating to the patient). The clinical response can be by a family member of the patient.

In yet another aspect, the invention features a computer program product containing executable instructions that when executed cause a processor to perform operations that include receiving a patient's genotype for a panel of genes, wherein the panel of genes includes a CYP2D6 gene, a CYP2C 19 gene, a CYP 1A2 gene, a serotonin transporter gene, and a serotonin receptor 2A gene; identify, in a database including a plurality of medication profiles associated with genotypes, a medication profile that is associated with the patient's genotype; and output the identified medication profile in response to receiving the patient's genotype.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

## DESCRIPTION OF THE DRAWINGS

Figure 1 is a block diagram of a computer system 100, according to one embodiment.

Figure 2 is a flow chart of a method 200 for building a database for use in selecting a medication for a patient.

Figure 3 is a flow chart of a method 300 of selecting a medication for a patient.

**DETAILED DESCRIPTION** In general, the invention features a method for selecting a medication (e.g., psychotropic medication) for a patient based on the genotype of genes that are useful for medication selection. Genes to be genotyped typically encode products that influence the metabolism of a medication or that are associated with a better treatment response. An algorithm can be used that initially excludes medications based on problematic genotypes of drug metabolizing enzyme genes (e.g., cytochrome P450 genes). A second stage of such an algorithm can begin with a list of potentially appropriate medications (e.g., antidepressants) and result in further classification of optimal treatments based on the genotype of target genes. For example, for antidepressants, further classification of the potential antidepressants can be based on the genotype of the serotonin transporter (5HTT) and serotonin receptor 2A (HTR2A).

Psychotropic medications include antipsychotics or neuroleptics, antianxiety- sedative agents, antidepressants or mood elevating agents, and mood-stabilizing drugs such as lithium salts or valproic acid. Non-limiting examples of antipsychotics include the tricyclic phenothiazines (e.g., chlorpromazine, triflupromazine, thioridazine, and mesoridazine, fluphenazine, and trifluoperazine), thioxanthenes (e.g., chlorprothixene, clopenthixol, flupenthixol, piflutixol, and thiothixene), and dibenzepines (e.g., loxapine, clozapine, clothiapine, metiapine, zotapine, ICI-204,636, fluperlapine, and olanzapine); butyrophenones such as haloperidol, diphenylbutylpiperidines such as fluspirilene, penfluridol, and pimozide, haloperidol decanoate, and indolones. Non-limiting examples of antianxiety-sedative agents include benzodiazepines such as chlordiazepoxide, diazepam, oxazepam, clorazepate, lorazepam, prazepam, alprazolam, and halazepam). Antidepressants or mood elevating agents include norepinephrine-reuptake inhibitors such as the tertiary amine tricyclics (e.g., amitriptyline, clomipramine, doxepin, and imipramine) and secondary amine tricyclics (e.g., amoxapine, desipramine, maprotiline, protriptyline, and nortriptyline); selective serotonin-reuptake inhibitors (SSRIs) such as

fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram, escitalopram, and venlafaxine; atypical antidepressants such as bupropion, nefazodone, and trazodone; noradrenergic and specific serotonergic antidepressants such as mirtazapine; and monoamine oxidase inhibitors such as phenelzine, tranylcypromine, and selegiline. In certain ethnic groups as many as 10% of the adolescent population have a 2D6 haplotype that is associated with poor metabolism of many antidepressant medications. See Wong et al. (2001) Ann. Acad. Med. Singapore 29:401-406. Clinical genomic testing of these individuals has clear implications for their treatment and prognosis. In extreme cases, children who were poor metabolizers and who were not identified have had tragic outcomes. These negative case reports have included a reported death of a nine-year-old boy who was not recognized to be a poor 2D6 metabolizer. The treatment of this child with fluoxetine continued despite the development of multiple symptoms because these symptoms were not recognized as being related to his extremely high serum levels of fluoxetine. Sallee et al. (2000) J. Child Adol. Psychiatry 10(l):27-34. While careful clinical surveillance to identify unexpected side effects during the administration of low doses of medication is an alternative clinical strategy to genotyping, genomic testing of a plurality of genes encoding drug metabolizing enzymes (e.g. cytochrome P450 genes) and other target genes (e.g., genes involved in neurotransmission for psychotropic drugs) provides a safe method by which potentially dangerous side effects can be avoided in an affected patient.

## Panels of Genes

The method includes obtaining a biological sample from a patient and obtaining the patient's genotype for a panel of

genes. Typically, the panel of genes that are genotyped includes at least three cytochrome P450 genes. The cytochrome P450 genes can be selected from the P450 genes listed in Table 1. For example, the at least three cytochrome P450 genes can encode CYP2D6, 1A2, and 2C19. In embodiments for selecting antidepressant medications, the genotype of four cytochrome P450 genes (e.g., genes encoding CYP2D6, 2C19, 3A4, and 1A2) can be obtained. In other embodiments, the genotype of at least 5 cytochrome P450 genes (e.g., genes encoding CYP1A1, 1A2, 2D6, 2C19, and 3A4) can be obtained. In still other embodiments, the genotype of at

least 10 cytochrome P450 genes (e.g., genes encoding CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, and 3A5) can be obtained. Alleles for each of these cytochrome P450 genes are set forth in Table 1.

Substrates of CYP2D6 typically are weak bases with the cationic binding site located away from the carbon atom to be oxidized. In particular, substrates of CYP2D6 include amitriptyline, nortriptyline, haloperidol, and desipramine. Venlafaxine is another substrate of CYP2D6 that can be biotransformed to an active metabolite, O-desmethylenlafaxine, primarily by the CYP2D6 enzyme. Venlafaxine also can be metabolized to an inactive metabolite, N-desmethy venlafaxine, which requires the CYP2D6 enzyme. Some individuals have altered CYP2D6 gene sequences that result in synthesis of enzymes devoid of catalytic activity or in enzymes with diminished catalytic activity. These individuals metabolize SSRIs (e.g., venlafaxine) and tricyclic antidepressants (TCAs) poorly. Duplication of the functional CYP2D6 gene also has been observed and results in ultrarapid metabolism of SSRIs and other drugs. Individuals without inactivating polymorphisms, deletions, or duplications have the phenotype of an extensive drug metabolizer and are designated as CYP2D6\*1. The CYP2D6\*2 allele has decreased enzymatic activity resulting from amino acid substitutions. The CYP2D6\*3 and \*4 alleles account for nearly 70% of the total deficiencies that result in the poor metabolizer phenotype. The polymorphism responsible for CYP2D6\*3 (2549A>del) produces a frame-shift in the mRNA. A polymorphism involved with the CYP2D6\*4 allele (1846G>A) disrupts mRNA splicing. These changes produce truncated forms of CYP2D6 devoid of catalytic activity. Other poor metabolizers are CYP2D6\*5, \*10, and \*17. CYP2D6\*5 is due to complete gene deletion. The polymorphisms in CYP2D6\*10 and \*17 produce amino acid substitutions in the CYP2D6 enzyme which have decreased enzyme activity. All of these polymorphisms are autosomal recessive. Consequently, only individuals who are homozygous or who are compound heterozygous for these polymorphisms are poor metabolizers. Individuals who are heterozygous, with one normal gene and one polymorphic gene, will have metabolism intermediate between the extensive (normal) and poor metabolizers.

CYP1A2 metabolizes many aromatic and heterocyclic amines including clozapine and imipramine. The CYP 1A2 \*1F allele can result in a product with higher inducibility or increased activity. See Sachse et al. (1999) Br. J. Clin. Pharmacol. 47: 445-449. CYP2C19 also metabolizes many substrates including imipramine, citalopram, and diazepam. The CYP2C19 \*2A, \*2B, \*3, \*4, \*5A, \*5B, \*6, \*7, and \*8 alleles encode products with little or no activity. See Ibeanu et al. (1999) J. Pharmacol. Exp. Ther. 290: 635-640.

CYP1A1 can be associated with toxic or allergic reactions by extrahepatic generation of reactive metabolites. CYP3A4 metabolizes a variety of substrates including alprazolam. CYP1B1 can be associated with toxic or allergic reactions by extrahepatic generation of reactive metabolites and also metabolizes steroid hormones (e.g., 17 $\beta$ -estradiol). Substrates for CYP2A6 and CYP2B6 include valproic acid and bupropion, respectively. Substrates for CYP2C9 include Tylenol and antabuse (disulfuram). Substrates for CYP2E1 include phenytoin and carbamazepine. Decreases in activity in one or more of the cytochrome P450 enzymes can impact one or more of the other cytochrome P450 enzymes.

#### Table 1 Cytochrome P450 Genes

Typically, to select a medication, the genotype of other target genes also is obtained in addition to the genotype of the genes encoding drug-metabolizing enzymes. Target genes can encode products that relate to the ability of the patient to respond to a particular class of medication. For example, to select an antidepressant, the target genes can be the serotonin transporter gene and serotonin receptor 2A gene. As such, in one embodiment, the panel of genes can be the CYP2D6 gene, 1A2 gene, and 2C19 gene, the

serotonin transporter gene, and serotonin receptor 2A gene. To select an antipsychotic, the target gene can be a dopamine transporter gene.

Table 2 sets forth the alleles for target genes that can be genotyped in addition to the genes encoding drug-metabolizing enzymes. For example, the panel of genes to be genotyped can include one or more dopamine receptor genes (e.g., genes encoding dopamine receptors D1, D2, D3, D4, D5, and/or D6), a dopamine transporter gene, one or more serotonin receptor genes (e.g., genes encoding serotonin receptors 1A, 1B, 1D, 2A, or 2C), a catechol-O-methyl transferase (COMT) gene, or a tryptophan hydroxylase gene. In one embodiment, a COMT gene and a tryptophan hydroxylase gene are included on the panel with cytochrome P450 genes. In other embodiments, one or more dopamine receptor genes, one or more serotonin receptor genes, a COMT gene, and a tryptophan hydroxylase gene are assessed in combination with the cytochrome P450 genes.

Table 2

For example, to select an antidepressant, the genotype of three (e.g., genes encoding 2D6, 2C19, and 1A2) or four cytochrome P450 genes (e.g., genes encoding 2D6, 2C19, 3A4, and 1A2), the serotonin transporter (5HTT) gene, and serotonin receptor 2A (HTR2A) gene can be assessed in a patient. In particular, it can be determined if the patient contains the CYP2D6 \*2, \*3, \*4, \*10, \*17, or \*5 del allele, the CYP2C19 \*2(A,B), \*3, \*4, \*5 (A,B), \*6, \*7, \*8 alleles, the CYP1A2 \*1F allele, the short or long form of the serotonin transporter gene, and the HTR2A T102C polymorphism. In embodiments in which the 3A4 gene is included in the panel, it can be determined if the patient contains the CYP3A4 \*1B, \*2, \*5, \*6, \*12, \*13, \*15A, \*17, or \*18A alleles.

Each of these genes influences the metabolism of at least one antidepressant medication or is associated with a better treatment response. As described herein, an algorithm has been created based on a set of six rules relating to the genotype of the six genes (e.g., 2D6 gene, 2C19 gene, 3A4 gene, 1A2 gene, the 5HTT gene, and HTR2A gene). Similarly, an algorithm can be created based on a set of five rules relating to the five genes (e.g., 2D6 gene, 2C19 gene, 1A2 gene, the 5HTT gene, and HTR2A gene). Based on these algorithms, medication profiles are provided for a given patient based on the patient's

genotype, allowing a clinician to select an acceptable antidepressant without the trial and error of determining if the patient will respond or tolerate a particular antidepressant.

Determining Genotype Genomic DNA generally is used to determine genotype, although mRNA also can be used. Genomic DNA is typically extracted from a biological sample such as a peripheral blood sample, but can be extracted from other biological samples, including tissues (e.g., mucosal scrapings of the lining of the mouth or from renal or hepatic tissue). Routine methods can be used to extract genomic DNA from a blood or tissue sample, including, for example, phenol extraction. Alternatively, genomic DNA can be extracted with kits such as the QIAamp<sup>®</sup> Tissue Kit (Qiagen, Chatsworth, CA), Wizard<sup>®</sup> Genomic DNA purification kit (Promega) and the A.S.A.P.<sup>™</sup> Genomic DNA isolation kit (Boehringer Mannheim, Indianapolis, IN).

Typically, an amplification step is performed before proceeding with the genotyping. For example, polymerase chain reaction (PCR) techniques can be used to obtain amplification products from the patient. PCR refers to a procedure or technique in which target nucleic acids are enzymatically amplified. Sequence information from the ends of the region of interest or beyond typically is employed to design oligonucleotide primers that are identical in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. Primers are typically 14 to 40 nucleotides in length, but can range from 10 nucleotides to hundreds of nucleotides in length. General PCR techniques are described, for example in PCR Primer: A Laboratory Manual, Ed. by Dieffenbach, C. and Dveksler, G, Cold Spring Harbor Laboratory Press, 1995. When using RNA as a source of template, reverse transcriptase can be used to synthesize complementary DNA (cDNA) strands. Ligase chain reaction, strand displacement amplification, self-sustained sequence replication or nucleic acid sequence-based amplification also can be used to obtain isolated nucleic acids. See, for example, Lewis (1992) Genetic Engineering News 12(9): 1; Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87: 1874-1878; and Weiss (1991) Science 254: 1292-1293.

Primers typically are single-stranded or double-stranded oligonucleotides that are 10 to 50 nucleotides in length, and when combined with mammalian genomic DNA and subjected to PCR conditions, is capable of being extended to produce a nucleic acid product corresponding to a region of interest within a gene. Typically, PCR products are at least 30 nucleotides in length (e.g., 30, 35, 50, 100, 250, 500, 1000, 1500, or 2000 or more nucleotides in length). Primers such as those listed in Table 7 are particularly useful for producing PCR products for the genes encoding the dopamine transporter, dopamine receptors, tryptophan hydroxylase, serotonin transporter, serotonin receptors, and COMT. Specific regions of mammalian DNA can be amplified (i.e., replicated such that multiple exact copies are produced) when a pair of oligonucleotide primers is used in the same

PCR reaction, wherein one primer contains a nucleotide sequence from the coding strand of a nucleic acid and the other primer contains a nucleotide sequence from the non-coding strand of the nucleic acid. The "coding strand" of a nucleic acid is the nontranscribed strand, which has the same nucleotide sequence as the specified RNA transcript (with the exception that the RNA transcript contains uracil in place of thymidine residues), while the "non-coding strand" of a nucleic acid is the strand that serves as the template for transcription.

A single PCR reaction mixture may contain one pair of oligonucleotide primers. Alternatively, a single reaction mixture may contain a plurality of oligonucleotide primer pairs, in which case multiple PCR products can be generated (e.g., 5, 10, 15, or 20 primer pairs). Each primer pair can amplify, for example, one exon or a portion of one exon. Intron sequences also can be amplified.

Exons or introns of a gene of interest can be amplified then directly sequenced. Dye primer sequencing can be used to increase the accuracy of detecting heterozygous samples. Alternatively, one or more of the techniques described below can be used to determine genotype.

For example, allele specific hybridization can be used to detect sequence variants, including complete haplotypes of a mammal. See, Stoneking et al., 1991, *Am. J. Hum. Genet.* 48:370-382; and Prince et al., 2001, *Genome Res.* 11(1): 152-162. In practice, samples of DNA or RNA from one or more mammals can be amplified using pairs of primers and the resulting amplification products can be immobilized on a substrate (e.g.,

in discrete regions). Hybridization conditions are selected such that a nucleic acid probe can specifically bind to the sequence of interest, e.g., the variant nucleic acid sequence. Such hybridizations typically are performed under high stringency as some sequence variants include only a single nucleotide difference. High stringency conditions can include the use of low ionic strength solutions and high temperatures for washing. For example, nucleic acid molecules can be hybridized at 42°C in 2X SSC (0.3M NaCl/0.03 M sodium citrate/0.1% sodium dodecyl sulfate (SDS) and washed in 0.1X SSC (0.015M NaCl/0.0015 M sodium citrate), 0.1% SDS at 65°C. Hybridization conditions can be adjusted to account for unique features of the nucleic acid molecule, including length and sequence composition. Probes can be labeled (e.g., fluorescently) to facilitate detection. In some embodiments, one of the primers used in the amplification reaction is biotinylated (e.g., 5' end of reverse primer) and the resulting biotinylated amplification product is immobilized on an avidin or streptavidin coated substrate (e.g., in discrete regions). Allele-specific restriction digests can be performed in the following manner. For nucleotide sequence variants that introduce a restriction site, restriction digest with the particular restriction enzyme can differentiate the alleles. For sequence variants that do not alter a common restriction site, mutagenic primers can be designed that introduce a restriction site when the variant allele is present or when the wild type allele is present. A portion of the nucleic acid of interest can be amplified using the mutagenic primer and a wild type primer, followed by digest with the appropriate restriction endonuclease. Certain variants, such as insertions or deletions of one or more nucleotides, change the size of the DNA fragment encompassing the variant. The insertion or deletion of nucleotides can be assessed by amplifying the region encompassing the variant and determining the size of the amplified products in comparison with size standards. For example, a region of a gene of interest can be amplified using a primer set from either side of the variant. One of the primers is typically labeled, for example, with a fluorescent moiety, to facilitate sizing. The amplified products can be electrophoresed through acrylamide gels with a set of size standards that are labeled with a fluorescent moiety that differs from the primer.

PCR conditions and primers can be developed that amplify a product only when the variant allele is present or only when the wild type allele is present (MSPCR or allele-specific PCR). For example, patient DNA and a control can be amplified separately using either a wild type primer or a primer specific for the variant allele. Each set of reactions is then examined for the presence of amplification products using standard methods to visualize the DNA. For example, the reactions can be electrophoresed through an agarose gel and the DNA visualized by staining with ethidium bromide or other DNA intercalating dye. In DNA samples from heterozygous patients, reaction products would be detected in each reaction. Patient samples containing solely the wild type allele would have amplification products only in the reaction using the wild type primer. Similarly, patient samples containing solely the variant allele would have amplification products only in the reaction using the variant primer. Allele-specific PCR also can be performed using allele-specific primers that introduce priming sites for two universal energy-transfer-labeled primers (e.g., one primer labeled with a green dye such as fluoroscein and one primer labeled with a red dye such as sulforhodamine). Amplification products can be analyzed for green and red fluorescence in a plate reader. See, Myakishev et al., 2001, *Genome* 11(11): 163-169.

Mismatch cleavage methods also can be used to detect differing sequences by PCR amplification, followed by hybridization with the wild type sequence and cleavage at points of mismatch. Chemical reagents, such as carbodiimide or hydroxylamine and osmium tetroxide can be used to modify mismatched nucleotides to facilitate cleavage.

Kits also are available commercially to detect many of the cytochrome P450 variants. For example, TAG-IT™ kits are available from Tm Biosciences Corporation (Toronto, Ontario).

### Selecting Medications

After the genotype is determined for each gene on the panel, the medication can be selected. Typically, selecting includes correlating genotype of the cytochrome P450 genes with capacity of each cytochrome P450 enzyme encoded by each cytochrome P450 gene to metabolize the medication. The genotype of other target genes on the panel, e.g.,

the serotonin transporter and serotonin receptor 2A, can be correlated with the ability of the patient to respond to the medication.

An algorithm can be used to select the most appropriate medications for an individual patient. The design of the algorithm requires the initial identification of the phenotype, which provides a preliminary identification of the universe of possible

medications. At the next step of the algorithm, the results of the target gene analyses can be sequentially entered. The potential list of appropriate medications can be subsequently rank-ordered based on specific co-factors in the algorithmic equation, which assign a positive, negative, or neutral probability score to each of the identified medications in the originally identified set of possible choices. This process adjusts the rank ordering based on the genotypic polymorphism carried by the patient. At the next entry point in the algorithmic equation, the results of the CYP gene analyses can be introduced. This algorithmic analysis is designed to place the medications in three categories: 1) medications that are acceptable for use, i.e., the medication has a high probability of normal metabolism within an individual having a particular genotype, 2) medications that can be used with caution (e.g., medication may require some dosing adjustment based on atypical metabolism); and 3) medications that should be avoided or used with caution and monitoring, e.g., due to potential difficulties in dosing. At this point in the selection process, data related to the medication response of first- and second-degree relatives of the patient can be entered into the algorithmic equation, which pertains to the medication selection of drugs in the first category that has been identified. An adjustment of the rank-ordered, appropriate medications then can be calculated based on clinical responses by family members.

The selection of an appropriate medication further can be enhanced by including both target data and data related to drug metabolism. This can determine the impact of the CYP products on the clinical response of a particular patient. For example, inclusion of target data and data related to drug metabolism provides the amount of available drug, the ability of the patient to utilize the drug, and information about the quality of the receptor target of the drug, providing a rational approach to selection of medication. An example of this process would be the selection of an appropriate antidepressant for a given patient. Once the phenotype of depression is established, the

initial universe of antidepressant medications is identified. For example, citalopram, fluvoxamine, bupropion, escitalopram, sertraline, mirtazapine, fluoxetine, venlafaxine, amitriptyline, imipramine, paroxetine, and nortriptyline can be the initial universe of antidepressant medications. It is understood that this initial universe of medications can change as additional antidepressants become available or as additional pharmacogenomic data are available. Subsequently, the data from the target genotyping is entered. If during the first components of the algorithm, it is revealed that the genotype reflects poor metabolism for 2D6 and normal metabolism for 1A2, the rank ordering of medication would be to identify fluvoxamine and bupropion as medications that are likely to be both effective and appropriately managed at recommended doses. At the next stage of the algorithm, if an individual is homozygous for the long form of the serotonin transporter gene, fluvoxamine would be confirmed as being acceptable for use. Output from the algorithm then can be integrated with historical data. For example, if a family member had responded well to fluvoxamine, this would confirm that the medication is acceptable for use, or, if a first or second degree relative had a problematic response to this medication, an alternative could be chosen.

### Computer Systems

Techniques described herein can be implemented in a computer system having a processor that executes specific instructions in a computer program. The computer system may be arranged to output a medication profile based on receiving a patient's genotype. Particularly, the computer program may include instructions for the system to select the most appropriate medication (e.g., a psychotropic medication) for an individual patient. The following are examples of features that may be included in a system. The computer program may be configured such that the computer system can identify the phenotype based on received data and provide a preliminary identification of the universe of possible medications. The system may be able to rank-order the identified medications based on specific co-factors in the algorithmic equation. The system may be able to adjust the rank ordering based on the genotypic polymorphism(s) carried by the patient.

The system may be able to adjust the rank ordering based on clinical responses, such as by family members of the patient.

Figure 1 is a block diagram of a computer system 100 that can be used in the operations described above, according to one embodiment. The system 100 includes a processor 110, a memory 120, a storage device 130 and an input/output device 140. Each of the components 110, 120, 130 and 140 are interconnected using a system bus 150. The system may include analyzing equipment 160 for determining the patient's genotype. The processor 110 is capable of processing instructions for execution within the system 100. In one embodiment, the processor 110 is a single-threaded processor. In another embodiment, the processor 110 is a multi-threaded processor. The processor 110 is capable of processing instructions stored in the memory 120 or on the storage device

130, including for receiving or sending information through the input/output device 140. The memory 120 stores information within the system 100. In one embodiment, the memory 120 is a computer-readable medium. In one embodiment, the memory 120 is a volatile memory unit. In another embodiment, the memory 120 is a non-volatile memory unit.

The storage device 130 is capable of providing mass storage for the system 100.

In one embodiment, the storage device 130 is a computer-readable medium. In various different embodiments, the storage device 130 may be a floppy disk device, a hard disk device, an optical disk device, or a tape device.

The input/output device 140 provides input/output operations for the system 100.

In one embodiment, the input/output device 140 includes a keyboard and/or pointing device. In one embodiment, the input/output device 140 includes a display unit for displaying graphical user interfaces. The system 100 can be used to build a database. Figure 2 shows a flow chart of a method 200 for building a database for use in selecting a medication for a patient.

Preferably, the method 200 is performed in the system 100. For example, a computer program product can include instructions that cause the processor 110 to perform the steps of the method 200. The method 200 includes the following steps. Receiving, in step 210, a plurality of genotypes 170 for a panel of genes. A computer program in the system 100 may include instructions for presenting a suitable

graphical user interface on input/output device 140, and the graphical user interface may prompt the user to enter the genotypes 170 using the input/output device 140, such as a keyboard.

Receiving, in step 220, a plurality of medication profiles 180. The medication profiles 180 are specified based on the genotypes 170. The user may enter the medication profiles 180 using the input/output device 140, such as a keyboard. For example, the medication profile 180 may include information 190 regarding at least one medication. Storing, in step 230, the received genotypes 170 and the medication profiles 180 such that each medication profile 180 is associated with one of the genotypes 170. The system 100 may store the medication profiles 180 and the genotypes 170 in the storage device 130. For example, when the storing is complete, the system 100 can identify a particular one of the medication profiles 180 that is associated with a specific genotype 170. Having identified the medication profile 180, the system 100 can access the information 190 contained within the identified medication profile 180, as will be described in the following example.

The system 100 may be used for selecting a medication. Figure 3 shows a flow chart of a method 300 of selecting a medication for a patient. Preferably, the method 300 is performed in the system 100. For example, a computer program product can include instructions that cause the processor 110 to perform the steps of the method 300. The method 300 includes the following steps.

Receiving, in step 310, a patient's genotype for a panel of genes. The genotype may be entered by a user via input/output device 140. For example, the user may obtain the patient's genotype for a panel of genes using the analyzing equipment 160 (which may or may not be connected to the system 100). The user may type the patient's genotype on input/output device 140, such as a keyboard, for receipt by the system 100. The genotype may be received directly from the analyzing equipment 160. For example, analyzing equipment 160 may include a processor and suitable software such that it can communicate over a network. The system 100 may be connected to the analyzing equipment 160 through input/output device 140, such as a network adapter, and directly receive the patient's genotype.

Identifying, in step 320, one of the medication profiles 180 that is associated with the patient's genotype. For example, the system 100 may perform a database search in the storage device 130. Particularly, the system 100 may access the genotype 170 for individual medication profiles 180 until a match is found. Optional step 325 will be described below.

Outputting, in step 330, the identified medication profile 180 in response to receiving the patient's genotype. The system may output the identified medication profile 180 through input/output device 140. For example, the identified medication profile may be printed or displayed in a suitable graphical user interface on a display device. As another example, the system 100 may transmit the identified medication profile over a network, such as a local area network or the Internet, to which the input/output device 140 is connected.

The medication profiles 180 can be created such that there is flexibility in how the system 100 outputs them. For example, the information 190 in one or more of the medication profiles 180 may include a ranking of several medications. The program may include instructions for applying rules to the received patient's genotype and adjust the ranking accordingly. In such implementations, the method 300 may include optional step 325 of adjusting the ranking before outputting the identified medication profile. For example, the system 100 may receive a genotypic polymorphism carried by the patient (optionally in the same way the patient's genotype was received) and adjust the ranking accordingly in step 325. As another example, step 325 may involve adjusting the ranking based on a clinical response. The clinical response may be received by the system 100 in the same way as the patient's genotype. For example, the ranking can be adjusted based on a clinical response by a member of the patient's family. The medication profiles 180 may be updated as necessary. For example, the introduction of a new medication on the market may prompt a revision of one or more existing medication profiles. A new medication may also be the basis for creating a new medication profile. The adjustment or creation of



medication profiles may be done substantially as described above. The medication profiles 180 may be used for medication selection in the same system where they were created, or in a different system. That is, the system 100 may

first be used for building a database of the medication profiles 180, and the system 100 may thereafter be used to select a medication profile for the genotype of a specific patient. As another example, one or more medication profiles 180 may be transmitted within a computer readable medium such as a global computer network for remote processing according to the invention.

#### Articles of Manufacture

In one embodiment, the article of manufacture is a composition containing oligonucleotide primers. Typically, such a composition will contain a first oligonucleotide primer and a second oligonucleotide primer, each 10 to 50 nucleotides in length, which can be combined with genomic DNA from a mammal and subjected to PCR conditions, to produce a nucleic acid product that corresponds to a region of interest within a target gene. A composition also may contain buffers and other reagents necessary for PCR (e.g., DNA polymerase or nucleotides). Furthermore, a composition may contain one or more additional pairs of oligonucleotide primers (e.g., 5, 10, 15, or 20 primer pairs), such that multiple nucleic acid products can be generated.

In other embodiments, articles of manufacture include populations of nucleic acid molecules immobilized on a substrate. Suitable substrates provide a base for the immobilization of the nucleic acids, and in some embodiments, allow immobilization of nucleic acids into discrete regions. In embodiments in which the substrate includes a plurality of discrete regions, different populations of isolated nucleic acids can be immobilized in each discrete region. The different populations of nucleic acid molecules independently can include nucleic acid molecules for detecting one or more of the alleles set forth in Tables 1 and 2. Suitable substrates can be of any shape or form and can be constructed from, for example, glass, silicon, metal, plastic, cellulose or a composite. For example, a suitable substrate can include a multiwell plate or membrane, a glass slide, a chip, or polystyrene or magnetic beads. Nucleic acid molecules or polypeptides can be synthesized in situ, immobilized directly on the substrate, or immobilized via a linker, including by covalent, ionic, or physical linkage. Linkers for immobilizing nucleic acids and polypeptides, including reversible or cleavable linkers, are known in the art. See, for example, U.S.

Patent No. 5,451,683 and WO98/20019. Immobilized nucleic acid molecules typically are about 20 nucleotides in length, but can vary from about 10 nucleotides to about 1000 or more nucleotides in length.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

#### EXAMPLES Example 1

##### CYP450 Genotype of Patients

This experiment highlights the results of an assessment of adolescents with atypical responses to selective serotonin reuptake inhibitors. The following CYP2D6 alleles that produce deficiencies were detected: \*2 (1661G>C and 2850OT), \*3 (2549A>del), \*4 (1661OC and 1846OA), \*10 (100OT, 1661OC and 1846G), \*17 (1023OT and 2850OT). Deletion of CYP2D6 (\*5 del) was detected by electrophoresis following PCR amplification. Tandem duplication of the CYP2D6 gene was detected by amplification of a duplication-specific sequence with detection following electrophoresis.

Genomic DNA was amplified by the polymerase chain reaction in an Applied Biosystems Thermal Cycler using the primers listed below. The lower case letters "f" and "r" imply the direction of synthesis ("f" indicates a forward/sense synthesis reaction and "r" indicates a reverse/antisense synthesis reaction). The amplified DNA was hybridized with oligonucleotide probes (described below) specific for the normal and polymorphic variants and detected using the Nanogen Molecular Biology Workstation. The oligonucleotide probes were fluorescently labeled (e.g., CY3 or CY5).

##### PreNest Oligonucleotides (Primary Amplification)

Amplification Primers: CYP2D6 Prenest B F 5'-CCA GAA GGC TTT GCA GGC TTC A-S' (Bases

1281 -1302, SEQ ID NO: 1)

CYP2D6 Prenest B R 5'-ACT GAG CCC TGG GAG GTA GGT A-3<sup>1</sup> (Bases 6357-6378, SEQ ID NO:2)

##### Nest Oligonucleotides (Secondary Amplification)

CYPIDO (C I OOT)

## Amplification Primers:

■ CYP2D6 (100)B f 5'GGC CTA CCC TGG GTA AGG GCC TGG AGC AGG A -3' (Bases 1579-1594, SEQ ID NO:3)

■ CYP2D6 (100)B r 5'-/5Bio/ CCT GGT CGA AGC AGT AT-3' (Bases 2394- 241 1 , SEQ ID NO:4)

Probes and Stabilizers: CYP2D6 CIOOT wild type 5'-/5Cy3/GCA CGC TAC C -3' (Bases 1700-1710, SEQ ID NO: 5)

CYP2D6 CIOOT polymorphism 5'-/5Cy5/GCA CGC TAC T -3' (Bases 1700-1710, SEQ ID NO:6)

CYP2D6 CIOOT stabilizer 5'CAC CAG GCC CCC TGC CAC TG -3' (Bases 171 1 -1731 , SEQ ID NO:7)

CYP2D6 (C1023T)|

## Amplification Primers:

■ CYP2D6 (1023)B f 5'-CCT GCT CAC TCC TGG TAG CC -3' (Bases 2394- 2413, SEQ ID NO:8)

■ CYP2D6(1023) B r 5'-/5Bio/ CTG TTT CAT GTC CAC GAC C-3<sup>1</sup> (Bases 2760- 2779, SEQ ID NO:9)

Probes and Stabilizers: CYP2D6 (1023) wild type 5'-/5Cy3/TGC CCA TCA C -3' (Bases 2632-2642, SEQ ID NO: 10)

CYP2D6 (1023) polymorphism 5'-/5Cy3/TGC CCA TCA T -3' (Bases 2632- 2642, SEQ ID NO: 1 1 )

CYP2D6 (1023) stabilizer 5'CCA GAT CCT GGG TTT CGG GCC GCG -3'

(Bases 2643-2667, SEQ ID NO: 12)

CYP2D6 (G 1661C and G1846A)| Note: There are 2 polymorphisms on this amplicon.

## Amplification Primers:

■ CYP2D6 (1661,1846)B f 5'- CAG AGG CGC TTC TCC G -3' (Bases 3266-3282,

SEQ ID NO: 13) ■ CYP2D6 (1661,1846)B r 5'- /5BIOZCTC GGT CTC TCG CTC CGC AC -S'

(Bases 3630-3650, SEQ ID NO: 14)

## Probes and Stabilizers:

CYP2D6(1661) wild type 5'-/5Cy3/CTT CTC CGT G -3' (Bases 3266-3276, SEQ ID NO: 15) CYP2D6(1661) polymorphism 5'-/5Cy3/CTT CTC CGT C -3' (Bases 3266-

3276, SEQ ID NO: 16)

CYP2D6(1661) stabilizer 5'-TCC ACC TTG CGC AAC TTG GGC CTG GG -3' (Bases 3277-3303, SEQ ID NO: 17)

CYP2D6(1846) wild type 5'-/5Cy3/CCA CCC CCA G -3' (Bases 3460-3470, SEQ ID NO: 18)

CYP2D6(1846) polymorphism 5'-/5Cy3/CCA CCC CCA A -3' (Bases 3460- 3470, SEQ ID NO: 19)

CYP2D6(1846) stabilizer 5'-GAC GCC CCT TTC GCC CCA ACG -3' (Bases 3471 -3492, SEQ ID NO:20)

[CYP2D6 (A2549del and C2850T)]

## Amplification Primers:

CYP2D6 (A2549del and C2850T)B f 5'- TGA GAC TTG TCC AGG TGA AC -3' (Bases 4001-4022, SEQ ID NO:21)

■ CYP2D6(A2549del and C2850T)B r 5'-/5Bio/ CCC AGA TGG GCT CAC GCT GC-3' (Bases 4558-4578, SEQ ID NO:22)

Probes and Stabilizers: CYP2D6 (2549) wild type 5'-/5Cy3/ACT GAG CAC A -3' (Bases 4161-4171, SEQ ID NO:23)

CYP2D6 (2549) polymorphism 5<sup>1</sup>-/5Cy3/ ACT GAG CAC G-3<sup>1</sup> (Bases 4161- 4171, SEQ ID NO:24)

CYP2D6 (2549) stabilizer 5'-GGA TGA CCT GGG ACC CAG CCC AGC C3' (Bases 4172-4199, SEQ ID NO:25)

CYP2D6 (2850) wild type 5'-/5Cy3/GAG AAC CTG C -3' (Bases 4462-4472, SEQ ID NO:26)

CYP2D6 (2850) polymorphism 5'-/5Cy3/ GAG AAC CTG T-3' (Bases 4462- 4472, SEQ ID NO:27) CYP2D6 (2850) stabilizer 5'-GCA TAG TGG TGG CTG ACC TGT TCT CTG

-3' (Bases 4473-4500, SEQ ID NO:28)

PCR Amplification.

A. PreNest (Initial Amplification):

[CYP2D6 PreNest B The initial PreNest PCR for the CYP2D6(C1661G),

CYP2D6 (G 1846A), CYP2D6(A2549Del), CYP2D6(C100T), CYP2D6(C2850T), and CYP2D6 (C 1023T) polymorphisms was performed with the following reagents per sample: 0.6 µL 25µM PreNest B F, 0.6 µL 25µM PreNestB R, 2.5 µL 10 mM dNTP's, 39.55 µL dH<sub>2</sub>O, 5.0 µL Expand Buffer 3 (Roche), 0.75 µL Expand Enzyme Mix (Roche), and 1.0 µL genomic DNA in a total volume of 50 µL. PCR was performed by holding at 94<sup>0</sup>C for 2 minutes then 30 cycles of denaturation at 94<sup>0</sup>C for 30 seconds, annealing at 67°C for 30 seconds, and extension at 68<sup>0</sup>C for 7 minutes, followed by a final extension at 72<sup>0</sup>C for 10 minutes. The expected PCR product was « 4.8 - 5Kb.

All subsequent PCR' s were performed using the PreNest B product as the template for the amplification.

CYP2D6(100) B

The (C100T) nested PCR was performed using 1 µl of the initial PreNest B amplicon as template. The product of this PCR was used to detect the CYP2D6(C100T) mutation and uses the following reagents (25 µL total volume): 1.0 µl 25µM

CYP2D6(100) B f, 1.0 µl 25µM CYP2D6(100) B r, 12.5µl Qiagen Hot Start Master Mix, and 9.5µl dF<sup>o</sup>. PCR was performed by holding at 95°C for 15 minutes, then 30 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

[CYP2D6(1023) B

A (C 1023 T) nested PCR was performed using 1 µl of PreNest B product as template. The product of this PCR was used to detect the CYP2D6 (C1023T) mutation and uses the following reagents (25 µL total volume): 1.0 µl 25µM CYP2D6 (1023) B f, 1.0µl 25µM CYP2D6 (1023)B r, 12.5 µl Amplitaq Gold Master Mix, and 9.5 µl dH<sub>2</sub>O. PCR was performed by holding at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 30 sec, annealing at 59°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

[CYP2D6(C1661G and G1846A) B]

The (C 1661 G and G 1846A) nested PCR was performed using 1 µl of PreNest B product as template. The product of this PCR was used to detect the CYP2D6 (C 166 IG) and CYP2D6 (G 1846A) mutations and uses the following reagents (25 µL total volume): 1.0 µl 25µM CYP2D6 (1661 ,1846)B f, 1.0 µl 25µM CYP2D6 (1661 ,1846)B r, 12.5µl 2X Amplitaq Gold Master Mix, and 9.5µl dH<sub>2</sub>O. PCR was performed by holding at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 30 sec, annealing at 63°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

[CYP2D6 (A2549Del and CYP2D6C2850T) B

The (A2549Del and C2850T) nested PCR was performed using 1 µl of PreNest B product as template. The product of this

PCR was used to detect the CYP2D6 (A2549Del) and CYP2D6 (C2850T) mutations and uses the following reagents (25 µL total volume): 1.0 µL 25µM CYP2D6 (2549,285O)B f, 1.0 µL 25µM CYP2D6 (2549,285O)B r, 12.5 µL 2X Amplitaq Gold Master Mix, and 9.5 µL dH<sub>2</sub>O. PCR was performed by holding at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min.

Polymorphisms were detected using the Nanogen Molecular Biology Workstation according to the Nanogens User's manual. Samples were desalted on Millipore desalting plates. In a 1.5 ml centrifuge tube, the following reagents were added: 2.0 µL of wild type probe, 2.0 µL of polymorphism probe, 2.0 µL of stabilizer, and 44 µL high salt buffer. Each assay had probes and stabilizers that were labeled and specific to it. The reagents were vortexed, spun briefly, and dispensed over the Nanochip array for 5 minutes and placed in a dark container. The chips were read with a trending method from 24° C to 50° C, with readings every two degrees. It was confirmed that the fluorescent probe hybridized at 24°C and that all probes had de-hybridized at 50°C. If the relative fluorescence units (RFUs) were above 80 on any chip at 50°C, the chips were stripped in 200 µL of 1.0 M NaOH for 10 minutes and washed three times with dH<sub>2</sub>O and once with high salt buffer. A heterozygote control (known heterozygote, one to "normalize" or scale the RFUs and the other to confirm the correcting trending pattern observed in previously genotyped heterozygotes) and a negative control (no DNA) were included for each polymorphism. For background subtraction, Z-histidine was used.

Tables 3-5 summarize the total number of participants for different age groups and the CYP2D6 inferred metabolic phenotype detected using the above procedure. Table 6 provides the particular 2D6 haplotypes that were observed in the patients that had antidepressant medication resistant subtypes of depression.

Table 3

Total Number of Participants in Each Grouping (All Ages; N = 86) CYP2D6 Inferred Metabolic Phenotype

Table 4

Total Number of Participants in Each Grouping (Ages: <19; N = 12) CYP2D6 Inferred Metabolic Phenotype

Table 5

Total Number of Participants in Each Grouping (Ages: >18; N = 74) CYP2D6 Inferred Metabolic Phenotype

Table 6

#### Antidepressant Medication Resistant Subtypes of Depression<sup>1</sup>

P=poor, N=normal, I=intermediate, HI = Higher inducibility, WT = wild type

All of these adolescents were found to have atypical 2D6 haplotypes (see Tables 3-5). The initial results of our analysis of the cytochrome P450 gene alleles in an adult sample also demonstrated a high degree of variability in the polymorphisms of the 2D6 gene (see Tables 3-5). Adolescents with polymorphisms associated with poor 2D6 metabolism were noted to have clinical histories marked by either a poor response to the

medication or by a high frequency of side effects when they had been treated with the selective serotonin reuptake inhibitors that are metabolized by the 2D6 enzyme.

#### Example 2 Determining Genotype of the Dopamine Transporter, Dopamine Receptors,

Tryptophan Hydroxylase, Serotonin Receptors, and COMT genes

The following probes and primers of Table 7 can be generated to detect the genotype of the dopamine transporter (DAT1, SLC6A3), dopamine receptors (DRD1, DRD2, DRD3, DRD4, and DRD5), tryptophan hydroxylase (TPH), serotonin transporter (5-HTT), serotonin receptors (HTR1A, HTR1B, HTR1D, HTR2A, and HTR2C), and COMT genes.

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### Example 3

The genotype of the CYP2D6, CYP2C19, and 5HTTR genes was determined in 97 patients using the methods described above. As indicated in Example 1, CYP2D6 \*2, \*3, \*4, \*10, \*17, and \*5 del alleles, CYP2C19 \*2 (A,B), \*3, \*4, \*5 (A,B), \*6, \*7, and \*8 alleles, and the short/long form of the 5HTTR gene were assessed.

Each patient that was genotyped exhibited either a poor response to at least one antidepressant medication or an intolerable side effect to at least one antidepressant medication. Based on the genotype information, 18 medication profiles were generated (Table 8). In Table 8, PM is poor metabolizer; IM is intermediate metabolizer; EM is extensive metabolizer; s is the short form of the gene; and 1 is the long form of the gene. Each medication profile provides antidepressant medications that are acceptable for use, antidepressant medications to be used with caution, and antidepressant medications to be avoided or used with caution and close monitoring.

TABLE 8

### Medication Algorithm Microarray Results from Pharmacological Study

Medication profile 1 is associated with 2D6 and 2C19 poor metabolizers and the s/s form of the 5HTTR gene. In patients with medication profile 1, bupropion is acceptable for use, mirtazapine and fluvoxamine can be used with caution, while citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, nortriptyline, and sertraline should be avoided or used with caution and close monitoring.

Medication profile 2 is associated with 2D6 and 2C19 poor metabolizers and the s/1 form of the 5HTTR gene. In patients with medication profile 2, fluvoxamine and bupropion are acceptable for use, sertraline and mirtazapine can be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

Medication profile 3 is associated with 2D6 and 2C19 poor metabolizers and the 1/1 form of the 5HTTR gene. In patients with medication profile 3, fluvoxamine and bupropion are acceptable for use, sertraline and mirtazapine can be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

Medication profile 4 is associated with 2D6 poor metabolizers, 2C 19 extensive metabolizers, and the s/s form of the 5HTTR gene. In patients with medication profile 4, bupropion is acceptable for use, mirtazapine, citalopram, fluvoxamine, and escitalopram can be used with caution, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, nortriptyline, and sertraline should be avoided or used with caution and close monitoring.

Medication profile 5 is associated with 2D6 poor metabolizers, 2C1 9 extensive metabolizers, and the s/s form of the 5HTTR gene. In patients with medication profile 5, citalopram, fluvoxamine, bupropion and escitalopram are acceptable for use, sertraline and

mirtazapine can be used with caution, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

Medication profile 6 is associated with 2D6 poor metabolizers, 2C 19 extensive metabolizers, and the 1/1 form of the 5HTTR gene. In patients with medication profile 6, citalopram, fluvoxamine, bupropion and escitalopram are acceptable for use, sertraline and mirtazapine can be used with caution, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

Medication profile 7 is associated with 2D6 intermediate metabolizers, 2C1 9 poor metabolizers, and the s/s form of the 5HTTR gene. In patients with medication profile 7, bupropion is acceptable for use, mirtazapine, venlafaxine, amitriptyline, nortriptyline, fluvoxamine, and sertraline can be used with caution, and citalopram, escitalopram, fluoxetine, imipramine, and paroxetine should be avoided or used with caution and close monitoring.

Medication profile 8 is associated with 2D6 intermediate metabolizers, 2C1 9 poor metabolizers, and the s/1 form of the 5HTTR gene. In patients with medication profile 8, fluvoxamine, bupropion, and sertraline are acceptable for use, mirtazapine, paroxetine, venlafaxine, amitriptyline, and nortriptyline can be used with caution, and citalopram, escitalopram, fluoxetine, and imipramine should be avoided or used with caution and close monitoring. Medication profile 9 is associated with 2D6 intermediate metabolizers, 2C1 9 poor metabolizers, and the 1/1 form of the 5HTTR gene. In patients with medication profile 9, fluvoxamine, bupropion, and sertraline are acceptable for use, mirtazapine, paroxetine, venlafaxine, amitriptyline, and nortriptyline can be used with caution, and citalopram, escitalopram, fluoxetine, and imipramine should be avoided or used with caution and close monitoring.

Medication profile 10 is associated with 2D6 extensive metabolizers, 2C1 9 poor metabolizers, and the s/s form of the 5HTTR gene. In patients with medication profile 10, bupropion and venlafaxine are acceptable for use, mirtazapine, nortriptyline, imipramine, amitriptyline, sertraline, paroxetine, and fluoxetine can be used with caution, and

fluvoxamine, citalopram, and escitalopram should be avoided or used with caution and close monitoring.

Medication profile 11 is associated with 2D6 extensive metabolizers, 2C1 9 poor metabolizers, and the s/1 form of the 5HTTR gene. In patients with medication profile 11, sertraline, venlafaxine, paroxetine, bupropion, and fluoxetine are acceptable for use, and mirtazapine, fluvoxamine, nortriptyline, citalopram, escitalopram, imipramine, and amitriptyline can be used with caution.

Medication profile 12 is associated with 2D6 extensive metabolizers, 2C1 9 poor metabolizers, and the 1/1 form of the 5HTTR gene. In patients with medication profile 12, sertraline, venlafaxine, paroxetine, bupropion, and fluoxetine are acceptable for use, and mirtazapine, fluvoxamine, nortriptyline, citalopram, escitalopram, imipramine, and amitriptyline can be used with caution.

Medication profile 13 is associated with 2D6 intermediate metabolizers, 2C1 9 extensive metabolizers, and the s/s form of the 5HTTR gene. In patients with medication profile 13, bupropion and mirtazapine are acceptable for use, venlafaxine, amitriptyline, imipramine, nortriptyline, citalopram, fluvoxamine, escitalopram, and sertraline can be used with caution, and paroxetine and fluoxetine should be avoided or used with caution and close monitoring.

Medication profile 14 is associated with 2D6 intermediate metabolizers, 2C1 9 extensive metabolizers, and the s/1 form of the 5HTTR gene. In patients with medication profile 14, citalopram, fluvoxamine, bupropion, escitalopram, sertraline, and mirtazapine are acceptable for use, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline can be used with caution.

Medication profile 15 is associated with 2D6 intermediate metabolizers, 2C1 9 extensive metabolizers, and the 1/1 form of the 5HTTR gene. In patients with medication profile 15, citalopram, fluvoxamine, bupropion, escitalopram, sertraline, and mirtazapine are acceptable for use, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline can be used with caution.

Medication profile 16 is associated with 2D6 extensive metabolizers, 2C19 extensive metabolizers, and the s/s form of the

5HTTR gene. In patients with medication profile 16, bupropion, mirtazapine, venlafaxine, amitriptyline, imipramine, and nortriptyline are acceptable for use, and paroxetine, fluoxetine, fluvoxamine, sertraline, escitalopram, and citalopram can be used with caution.

Medication profile 17 is associated with 2D6 extensive metabolizers, 2C19 extensive metabolizers, and the s/1 form of the 5HTTR gene. Medication profile 18 is associated with 2D6 extensive metabolizers, 2C19 extensive metabolizers, and the 1/1 form of the 5HTTR gene. In patients with medication profiles 17 or 18, citalopram, fluvoxamine, bupropion, escitalopram, sertraline, mirtazapine, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline are acceptable for use.

#### Example 4

The genotype of the CYP2D6, CYP2C19, CYP3A4, CYP1A2, and HTR2A genes was determined in ten patients using the methods described above. The genotype of the 5HTTR gene was determined by amplifying the polymorphic region of the gene using primers flanking the region. Reaction products were sized using electrophoresis (Agilent Technologies). CYP3A4 \*1B, \*2, \*5, \*6, \*12, \*13, \*15A, \*17, and \*18A alleles, CYP1A2 \*1F allele, and the HTR2A 102 polymorphism were detected. Based on the CYP2D6 genotype, patients were classified as being poor, intermediate, extensive, or ultra rapid metabolizers. For each of the CYP2C19, CYP3A4, and CYP1A2 genotypes, patients were classified as being either extensive or not extensive (i.e., poor) metabolizers. For the 5HTTR genotype, patients were classified as having the short/short (s/s) form of the gene, the short/long (s/1) form of the gene, or the long/long (1/1) form of the gene. For the HTR2A genotype, patients were classified as having either the C/C allele or as having the T/T or T/C alleles.

The following six rules were used to sort the genotypes into 192 treatment recommendations. The first rule relates to the CYP2D6 genotype. For poor metabolizers and ultra rapid metabolizers, CYP2D6 substrates were placed in the "use very cautiously

with close monitoring" category. For intermediate metabolizers, exclusive CYP2D6 substrates were placed in the "avoid or use very cautiously with close monitoring" category. For intermediate metabolizers, substrates that are partially metabolized by CYP2D6 were placed in the "use cautiously" category. For extensive metabolizers, CYP2D6 substrates were placed in the "acceptable for use" category.

After applying the first rule, the second rule relating to the 2C19 genotype was applied. For poor metabolizers based on their CYP2C19 genotype, exclusive CYP2C19 substrates were placed in the "avoid or use very cautiously with close monitoring" category. For extensive or intermediate metabolizers based on their CYP2C19 genotype, CYP2C19 substrates were placed in the "acceptable for use" category. Substrates that are primarily metabolized by both CYP2D6 and CYP2C19 were placed in the "avoid or use very cautiously with close monitoring" category if CYP2C19 was poor and CYP2D6 was either intermediate or poor, or placed in the "use cautiously" category if CYP2C19 was poor and CYP2D6 was extensive. A third rule relating to the 3A4 genotype was applied after the first and second rules were applied. For poor metabolizers based on their CYP3A4 genotype, exclusive CYP3A4 substrates were placed in the "avoid or use very cautiously with close monitoring" category. For extensive or intermediate metabolizers based on their CYP3A4 genotype, CYP3A4 substrates were placed in the "acceptable for use" category. Substrates that are primarily metabolized by any two of the following three enzymes: CYP2D6, CYP2C19, or CYP3A4, were placed in the "avoid or use very cautiously with close monitoring" category if any two of the three were intermediate or poor metabolizers or placed in the "use cautiously" category if one of the two most primary enzymes was a poor metabolizer.

The fourth rule relating to the 1A2 genotype was applied after applying the first three rules. For poor metabolizers based on CYP1A2 genotype, CYP1A2 substrates were placed in the "avoid or use very cautiously with close monitoring" category (include fluvoxamine as a primary CYP1A2 substrate). For poor metabolizers based on the CYP1A2 genotype, substrates having the CYP1A2 pathway as one of their primary pathways were placed in the "use with caution" category.

After applying the first four rules, the fifth rule relating to the 5HTTR genotype was applied. For the homozygous short genotype (s/s) of the serotonin transporter gene, SSRTs that were categorized as "acceptable for use" were shifted to the "use with caution" category. For the homozygous short genotype (s/s) of the serotonin transporter gene, SSRI's that were categorized as "use with caution" were shifted to the "avoid or use very cautiously with close monitoring" category. Antidepressants, outside the SSRI class, do not shift category based on 5HTTR genotype.

Rule six, which relates to the HTR2A genotype, was applied after applying the first five rules. For the "mutant form" of the 5-hydroxytryptamine serotonin receptor 2A gene, if paroxetine was in the "acceptable for use" category, it was shifted to the "use with caution" category. If paroxetine was in the "use with caution" category, it was shifted to the "avoid or use very cautiously with close monitoring."

Based on the genotype of the patients and associated medication profile, medications appropriate for the patient were

selected. Table 9 summarizes the data for the 10 patients. Case studies of four of the seven patients are presented below.

The first case is a 16-year-old white female who was genotyped and found to be a poor metabolizer for CYP2D6 (\*3/\*9) as well as being a poor metabolizer for CYP2C19 (\*2/\*2). She was diagnosed at 6 years of age as having ADHD and treated unsuccessfully. She subsequently developed symptoms of depression and mood dysregulation and was placed on many medications that were metabolized by CYP2D6, such as EFFEXOR.

Additionally, she was homozygous for a short form of the serotonin transporter gene. Based on her specific genotype of these key genes, the algorithm demonstrates that there are a relatively small number of potential strategies for controlling her depression. The medication profile generated based on her genotype of HR2ATT/TC, s/s form of 5HTTR, extensive metabolizer for 1A2 and 3A4, and poor metabolizer for 2C19 and 2D6 indicated that bupropion was acceptable for use, mirtazapine and fluoxetine should be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, nortriptyline, and sertraline should be avoided or used with caution and monitoring.

Before genotyping, she had a particularly unusual side effect to citalopram in that she developed hallucinations (incidence less than 1%). This atypical and dangerous side effect could have been avoided if she had been genotyped prior to receiving this medication. The metabolic enzyme that is primarily involved in the metabolism of citalopram is CYP2C19 and a secondary metabolic pathway is CYP2D6. Both of these pathways were nonfunctional in this patient. Therefore, even on a modest dose of citalopram, this patient developed acute auditory and visual hallucinations, which were subsequently treated with an atypical antipsychotic medication. Additionally, based on an inability to develop an effective treatment plan for this patient, she required three acute hospitalizations and a partial hospitalization experience prior to being referred to a state hospital for two months. She was unsuccessfully tried on more than a dozen psychotropic medications. There is no question that her care could have been greatly improved and perhaps some of her hospitalizations could have been avoided if clinicians had been aware of her inability to use many of the drugs that had been prescribed at their standard doses. Additionally, her serotonin transporter genotype (C/C) would have suggested a poor response to many of the medications that had been prescribed. Subsequent to genotyping, she was placed on a combination of two mood stabilizers that are metabolized by an alternative pathway (LAMICTAL (lamotrigine) and TRILEPTAL (oxcarbazepine)). She is also tolerating treatment with ABILIFY (aripiprazole), which in addition to being metabolized by CYP2D6 is metabolized by CYP3A4, for which she has an internal metabolic pathway. She is now doing reasonably well.

The second case is a 42-year-old Caucasian female with a persistent depressive illness and pervasive anxiety and a long-standing history of psychotropic medication intolerance. This patient had a comprehensive neurological evaluation, which failed to provide an organic explanation for her symptoms. This patient has been treated with at least nine psychotropic medications in the past. Striking examples of CYP2D6 intolerance include the development of acute hallucinations while taking paroxetine and a very low tolerance to amitriptyline. This patient had poor CYP2D6 metabolism and intermediate CYP2C19 metabolism. This represents a rare genotype that would suggest difficulties in tolerating treatment with citalopram. Indeed, she did have irritability and lethargy when taking citalopram. To

compound this problem, she also was homozygous for the short form of the serotonin transporter, which is associated with a poor response to serotonin receptive inhibitors. She had experienced multiple outpatient visits for her psychiatric problems. Awareness of her genotype earlier in her history would clearly have allowed her clinician to avoid a number of medications that she could not tolerate. The medication profile generated based on her genotype of HR2A TT/TC, s/s form of 5HTTR, extensive metabolizer for 1A2 and 3A4, and poor metabolizer for 2C19 and 2D6 indicated that bupropion was acceptable for use, mirtazapine and fluoxetine should be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, nortriptyline, and sertraline should be avoided or used with caution and monitoring.

The third case history is a 67-year-old woman with a diagnosis of major depressive disorder, who had been treated with seven different medications in the past. These had not been effective, and she had required two psychiatric hospitalizations. It was determined that she was a poor metabolizer for both CYP2D6 (\*4/\*9) and CYP2C19 (\*2/\*2). Additionally, she was homozygous for the long form of the serotonin transporter gene. Based on her genotype of HR2A C/C, 1/1 form of 5HTTR, extensive metabolizer for 1A2 and 3A4, and poor metabolizer for 2C19 and 2D6, the associated medication profile indicated that fluoxetine and bupropion were acceptable for use, sertraline and mirtazapine should be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and monitoring. If her genotype had been known, it would have been possible to anticipate that she would have had side effects to both EFFEXOR (Venlafaxine) and LEXAPRO (escitalopram oxalate). This could have been predicted, given that two of the three metabolic pathways involved in the metabolism of LEXAPRO were completely inactive. Subsequent to her genotyping, she was successfully treated with electroconvulsive therapy (ECT) and concurrently successfully treated with REMERON (mirtazapine), trazodone, and KILONOPIN (clonazepam), which have alternative pathways of metabolism to CYP2D6 and CYP2C19.



The fourth case is a 50-year-old Caucasian woman who had been diagnosed as having dysthymia and chronic anxiety. She had been placed on multiple psychotropic drugs,

including two CYP2D6 medications that she could not tolerate. Her genotype indicated that she was a poor CYP2D6 metabolizer (\*4/\*4) and an intermediate CYP2C19 metabolizer (\*1/\*2). She was heterozygous for the long and short form of the serotonin transporter gene. Based on her genotype of HR2A C/C, 1/s form of 5HTTR, extensive metabolizer for 1 A2 and 3A4, and poor metabolizer for 2C1 9 and 2D6, the associated medication profile indicated that fluoxetine and bupropion were acceptable for use, sertraline and mirtazapine should be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and monitoring. This patient had multiple hospitalizations at considerable cost. She also found it difficult to tolerate a range of nonpsychiatric medications, including being able to take only one dose of PROZAC (fluoxetine) and ZOLOFT (sertraline) before finding that she was acutely uncomfortable. Of particular interest, she ultimately responded to a subclinical dose of paroxetine, which represents an example of using the algorithm to find a method to administer an effective medication that in normal doses could not have been tolerated.

TABLE 9

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Based on patient no. 5's genotype of HR2A C/C, 1/1 form of 5HTTR, extensive metabolizer for 1 A2 and 3A4, and poor metabolizer for 2C1 9 and 2D6, the associated medication profile indicated that fluoxetine and bupropion were acceptable for use, sertraline and mirtazapine should be used with caution, and citalopram, escitalopram, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and monitoring.

The medication profile associated with patient no. 6's genotype (HTR2A CC; 5HTTR 1/s; 1 A2, 3A4, and 2D6 extensive; and 2C1 9 poor) indicates that sertraline, venlafaxine, bupropion, and fluoxetine are acceptable for use, and mirtazapine, fluoxetine, nortriptyline, citalopram, escitalopram, imipramine, amitriptyline, and paroxetine can be used with caution.

The medication profile associated with patient no. 7's genotype (HTR2A TT/TC; 5HTTR 1/s; 1A2, 3A4, and 2C19 extensive; and 2D6 ultra rapid) indicates that citalopram, fluoxetine, bupropion, and escitalopram are acceptable for use; sertraline and mirtazapine can be used with caution, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

The medication profile associated with patient no. 8's genotype (HTR2A TT/TC; 5HTTR 1/1; 1A2, 3A4, and 2C1 9 extensive; and 2D6 intermediate) indicates that citalopram, fluoxetine, bupropion, escitalopram, sertraline, and mirtazapine are acceptable for use; and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

The medication profile associated with patient no. 9's genotype (HTR2A TT/TC; 5HTTR 1/s; and 1A2, 3A4, 2C19, and 2D6 extensive) indicates that citalopram, fluoxetine, bupropion, escitalopram, sertraline, mirtazapine, paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline are acceptable for use.

The medication profile associated with patient no. 10's genotype (HTR2A TT/TC; 5HTTR 1/s; 1 A2, 3A4, and 2C1 9 extensive; and 2D6 poor) indicates that citalopram, fluoxetine, bupropion, and escitalopram are acceptable for use, sertraline and mirtazapine

should be used with caution, and paroxetine, fluoxetine, venlafaxine, amitriptyline, imipramine, and nortriptyline should be avoided or used with caution and close monitoring.

Example 5 The genotype of the CYP2D6, CYP2C19, CYP1A2, and HTR2A genes is determined in patients using the methods described above. Based on the CYP2D6 genotype, patients are classified as being poor, intermediate, extensive, or ultra rapid metabolizers. For the CYP2C19 genotype, patients are classified as being either extensive, intermediate, or poor metabolizers. For the 1A2 genotype, patients are classified as being poor, intermediate, extensive, or ultra rapid metabolizers. For the 5HTTR genotype, patients are classified as having the short/short (s/s) form of the gene, the short/long (s/1) form of the gene, or the long/long (1/1) form of the gene. For the HR2A genotype, patients are classified as having the C/C allele, the T/T allele, or T/C allele.

The following five rules can be used to sort the genotypes into treatment recommendations. The first rule relates to the CYP2D6 genotype. For poor metabolizers and ultra rapid metabolizers, CYP2D6 substrates can be placed in the "use very cautiously with close monitoring" category. For intermediate metabolizers, exclusive CYP2D6 substrates can be placed in the "avoid or use very cautiously with close monitoring" category. For intermediate metabolizers, substrates that are

partially metabolized by CYP2D6 can be placed in the "use cautiously" category. For extensive metabolizers, CYP2D6 substrates can be placed in the "acceptable for use" category.

After applying the first rule, the second rule relating to the 2C19 genotype can be applied. For poor metabolizers based on their CYP2C19 genotype, exclusive CYP2C19 substrates can be placed in the "avoid or use very cautiously with close monitoring" category. For extensive or intermediate metabolizers based on their CYP2C19 genotype, CYP2C19 substrates can be placed in the "acceptable for use" category. Substrates that are primarily metabolized by both CYP2D6 and CYP2C19 can be placed in the "avoid or use very cautiously with close monitoring" category if CYP2C19 was poor and CYP2D6 was

either intermediate or poor, or can be placed in the "use cautiously" category if CYP2C19 was poor and CYP2D6 was extensive.

A third rule relating to the 1A2 genotype can be applied after the first and second rules were applied. For poor and ultra-rapid metabolizers based on their CYP1A2 genotype, exclusive CYP1A2 substrates can be placed in the "avoid or use very cautiously with close monitoring" category (include fluvoxamine as a primary CYP1A2 substrate). For poor metabolizers based on the CYP1A2 genotype, substrates having the CYP1A2 pathway as one of their primary pathways can be placed in the "use with caution" category. For extensive or intermediate metabolizers based on their CYP1A2 genotype, CYP1A2 substrates can be placed in the "acceptable for use" category. In other embodiments, intermediate metabolizers can be placed in the "use with caution" category.

After applying the first three rules, the fourth rule relating to the 5HTT genotype can be applied. For the homozygous short genotype (s/s) of the serotonin transporter gene, SSRIs that are categorized as "acceptable for use" can be shifted to the "use with caution" category. For the homozygous short genotype (s/s) of the serotonin transporter gene, SSRIs that are categorized as "use with caution" can be shifted to the "avoid or use very cautiously with close monitoring" category. Antidepressants, outside the SSRI class, do not shift category based on 5HTT genotype.

Rule five, which relates to the 5HT2A genotype, can be applied after applying the first four rules. For the "mutant form" of the 5-hydroxytryptamine serotonin receptor 2A gene, if paroxetine is in the "acceptable for use" category, it can be shifted to the "use with caution" category. If paroxetine is in the "use with caution" category, it can be shifted to the "avoid or use very cautiously with close monitoring."

Based on the genotype of the patients and associated medication profile, medications appropriate for the patient can be selected.

#### Example 6

This example describes an electronic chart review conducted of patients who had been enrolled in a pharmacogenomic study. Thirty-eight (38) patients had been treated with

venlafaxine and genotyped for the 2D6 gene. Participants who were slow (also called "poor") metabolizers, as defined by having either two inactive 2D6 alleles or one inactive and one reduced 2D6 allele, had not been maintained on a dose of greater than 75 mg of venlafaxine. In contrast, 26 of the remaining 33 subjects (79%) who had at least one fully functioning 2D6 allele tolerated a dose of 150 mg or more of venlafaxine. ( $p < .002$ ).

Clinical guidelines recommend a typical starting dose for venlafaxine to be 75 mg/day. However, an initial dose of 37.5 mg/day is regularly prescribed for geriatric patients. For patients who do not respond to an initial dose of 75 mg/day, increasing the daily dose by increments of 75 mg is recommended up to a maximum dose of 225 mg/day. Patients with no fully active 2D6 gene are predicted to have higher serum levels of both venlafaxine and N-desmethylvenlafaxine (i.e., an inactive metabolite) for any given dose of venlafaxine when compared to patients with one or two functional copies of the 2D6 gene. Consequently, compromised metabolizers are less able to tolerate higher dosages of 2D6 substrate medications. Some patients with limited 2D6 metabolic capability can be expected to achieve a therapeutic clinical response at a relatively low dose.

#### Method

A sample of 199 patients, with a documented history of atypical response to psychotropic medications, were genotyped for the 2D6 gene as part of a clinical research protocol. Atypical response was defined as the development of adverse side effects or a non-response to the medication. Fourteen variant alleles were examined using a TM Bioscience platform. Test-retest reliability for determination of the 2D6 genotype was greater than 99% accurate. An electronic medical record review revealed that 38 of the participants in this protocol had previously been treated with venlafaxine by clinicians who had no knowledge of their 2D6 genotype before or during the course of their treatment. Table 10 provides the 2D6 alleles

and the corresponding activity level.

Table 10

Five "slow metabolizers" in this group of 38 patients were identified. Each of these patients had one completely inactive allele (e.g., \*3 or \*4). Additionally each patient had a second allele with reduced activity (e.g., \*9, \*10, \*17 or \*41). All of the other 33 patients treated with venlafaxine had at least one fully active allele.

None of the five patients with a slow 2D6 metabolism had been maintained on a dose of venlafaxine that was greater than 75 mg. In contrast, 26 of the remaining 33 subjects (79%) were maintained on dosage levels of 150 mg or 225 mg of venlafaxine. This difference between dosage levels was significant using a Fisher's exact test ( $p < .002$ ). Clinical descriptions of the five subjects who lacked a functional 2D6 gene are shown below.

Case 1 : A 44-year-old Caucasian woman was diagnosed with dysthymic disorder and was prescribed 75 mg of venlafaxine. She experienced dry mouth and an increase in appetite, but reported a decrease in depressive symptoms. Her dose was increased to 112.5

mg, at which time, her depression became worse and she complained of fatigue. Her dose was subsequently decreased to 75 mg and she did well for four months on this dose. She had a \*3 2D6 allele and a \*9 2D6 allele.

Case 2: A 16-year-old Caucasian teenager was diagnosed as having attention deficit hyperactivity disorder at 6 years of age. She subsequently became depressed at age 13, developed psychotic symptoms at age 15 and was diagnosed with bipolar disorder at age 16. She was prescribed 75 mg of venlafaxine for one month after which the medication was discontinued due to excessive somnolence and no improvement in her mood. She had a \*3 2D6 allele and a \*9 2D6 allele.

Case 3: A 54-year-old Caucasian woman who was diagnosed with major depressive disorder was initially prescribed 37.5 mg of venlafaxine. She discontinued the medication after four days due to intolerable nausea, insomnia, and decreased appetite. She had a \*4 2D6 allele and a \*17 2D6 allele.

Case 4: A 46-year-old Caucasian man was diagnosed with generalized anxiety disorder. He was prescribed 37.5 mg of venlafaxine a day and became more anxious over the next four days. As his complaints of anxiety increased, he also developed physiological symptoms that included palpitations of his heart. When his venlafaxine was discontinued, his anxiety symptoms improved. He had a \*4 2D6 allele and a \*41 2D6 allele.

Case 5: A 15-year-old Asian- American teenager was diagnosed as having major depressive disorder and was documented to have a past psychiatric history of three suicide attempts by overdose and five hospitalizations. Although there was concern that she had reported some mood fluctuations, she was prescribed 75 mg of venlafaxine. The venlafaxine was subsequently discontinued after five days because of intolerable side effects. She had previously experienced side effects when she had been placed on low doses of other 2D6 substrate medications (e.g., paroxetine and fluoxetine). She had a \*4 2D6 allele and a \*10 2D6 allele.

The analyses of this clinical sample of psychiatric patients indicates that individuals who lacked a full functioning copy of the 2D6 gene did not tolerate a maintenance dose of venlafaxine above 75 mg. In one case, a subject had briefly been able to take 112.5 mg before the treating clinician decreased the dose back to 75 mg (Case 5). This was the only patient with an inactive and partially inactive 2D6 allele that tolerated venlafaxine at any dose. In contrast, 79% of patients who had at least one fully functional copy of the 2D6 gene tolerated doses of venlafaxine of 150 mg or greater.

In reviewing the five patients who were identified as "slow metabolizers," two patterns were demonstrated. Four patients did not tolerate venlafaxine because of a range of side effects that included nausea, insomnia, anorexia, palpitation and somnolence. One subject who was a "slow metabolizer" had a therapeutic response at 75 mg, but she did not tolerate a dose increase to 112.5 mg.

One clinical implication of these analyses is that for patients who lack a functional copy of the 2D6 allele, venlafaxine should be initiated at a dose of 37.5 mg and the dosage should be increased cautiously. An alternative strategy would be to select an antidepressant with a primary metabolic pathway other than 2D6, such as fluvoxamine or an antidepressant with alternative pathways of metabolism, such as escitalopram or sertraline.

A strength of this clinical report is that none of the treating psychiatrists who cared for these patients were aware of their 2D6 genotypes. Consequently, dosing decisions were based on the clinical responses of the patients rather than predictions of phenotype based on genotypic information.

One limitation of this report is that the clinical data was retrieved from the electronic medical record. While venlafaxine dosage was always documented precisely, the descriptions of the effects and the therapeutic responses of patients were not reported in a standardized manner. The inclusion criteria for participating in the study was demonstrating an atypical

response to antidepressant medication, defined as the development of adverse side effects or a non-response to the medication. Consequently, the decision to maintain, raise or lower medication dosage was based on the tolerance and response of the patient to the medication and was independent of diagnosis and comorbidity.

In summary, patients who did not have at least one fully functional allele of the 2D6 gene were not maintained on doses of venlafaxine greater than 75 mg. This association of compromised genotypes and a clinical history of intolerance to higher doses of venlafaxine, indicates that genetic variations of cytochrome P450 2D6 may contribute to the long observed patient specific variation in response to antidepressant medications.

#### OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims