CORRELATION BETWEEN ADVERSE DRUG EFFECTS IN PSYCHIATRIC PATIENTS TREATED WITH ANTIDEPRESSANTS AND POLYMORPHISM OF *CYP2D6*

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Dedication

To my parents

To my brothers and sisters

With all my love and appreciation

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It has been my good fortune to have the advice and guidance of many talented people whose knowledge and skills have enhanced this work in many ways. For their valuable help I thank:

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CYP450	Cytochrome P450
CYP2D6	Cytochrome P 450 2D6
SNP	Single nucleotide polymorphism
ADR	Adverse Drug Reaction
TCA	Tricyclic antidepressant
SSRI	Selective serotonin reuptake inhibitor
UKU	Udvalg for Kliniske Undersøgelser

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CORRELATION BETWEEN ADVERSE DRUG EFFECTS IN PSYCHIATRIC PATIENTS TREATED WITH ANTIDEPRESSANTS AND POLYMORPHISM OF *CYP2D6*

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ABSTRACT

The polymorphic enzyme CYP2D6 has a major role in the oxidative metabolism of many xenobiotics, the genetic polymorphism of this enzyme leads to a wide intersubject variation in the metabolic activity that ranges from ultrafast to poor metabolizing capacity. Its three null alleles (*CYP2D6*3,*4* and**6*) encode for inactive enzyme. A carrier of two mutant alleles is considered as a poor metabolizer, who is more susceptible to suffer from adverse drug reactions of the substrates of this enzyme.

In the present study, the frequency of the three alleles in a group of 44 psychiatric patients treated with antidepressants that are metabolized by CYP2D6 was found to be (0) for the *CYP2D6*3* and **6* alleles, and (17%) for the *CYP2D6*4* allele, (6.8%)were found to be poor metabolizers, while the frequency of the three alleles in a group of unrelated Jordanian subjects were found to be (0) for the *CYP2D6*3* allele, (0.53%) for the *CYP2D6 *6* allele, and (9.5%) for the *CYP2D6*4* allele, with (3%) poor metabolizers.

The presence of defective alleles and its relation to the appearance of side effects was investigated between the three groups of patients (homozygous for the wild-type allele,

heterozygous for a defective allele, and homozygous for a defective allele). Every poor metabolizer did have adverse drug reactions when using the antidepressant, but the difference between the three groups did not reach statistical significance.

Further studies are needed in order to investigate more of the mutated alleles of this enzyme in a larger number of patients, so that the effect of the genetic polymorphism on the patients treated by substrates of this enzyme becomes more obvious.

1. Introduction:

It is well known that the response to drugs often shows wide inter-individual variability. A drug that shows less activity in some patients may be pharmacologically active or even toxic in others (Meyer, 1996; Özdemir *et al,* 2001; Poolsup *et al.*, 2000). The risk for drug inefficacy or toxicity is a product of the interaction of physiological (as age and gender), pathological (i.e. diabetes, hepatic or renal diseases), environmental (diet, cigarette smoke, alcohol and drugs consumption) and genetic factor (Testai *et al.*, 2001).

Genetic variations in genes coding for drug metabolizing enzymes, drug receptors and drug transporters have been associated with inter-individual variability in the efficacy and toxicity of drugs (Kalow, 2001,a). Many drug-metabolizing enzymes of both phase I and phase II are expressed polymorphically within the population (Gonzalez & Idle, 1994).

The term genetic polymorphism defines a monogenic trait that exists in the population in at least two phenotypes (and presumably at least two genotypes) with frequency of at least 1% in the population (Meyer, 2000), for a gene to be polymorphic, it must encode a product that is not required for development, reproductive or a crucial physiological function, otherwise such a high frequency of occurrence wouldn't be expected (Gonzalez & Idle, 1994).

The study of such polymorphisms associated with an individual's ability to respond to pharmacotherapy has given rise to the field of pharmacogenetics (Linder *et al.*, 1997).

The major route of phase I drug metabolism is by cytochrome P450 (CYP450).

CYP450 is a superfamily of isoenzymes that oxidate numerous lipophilic xenobiotics into more easily excreted metabolites (Rodrigues & Rushmore, 2002).

Sequencing of the human genome revealed 58 different human cytochrome P450 genes (Ingelman-sundberg, 2001,a).

Seventeen CYP gene families have been described in humans; the enzymes belonging to the families CYP1, CYP2 and CYP3 catalyze the oxidative biotransformation of exogenous compounds including many drugs (Brøsen, 1995; Hemeryck& Belpaire, 2002; Linder *et al.,* 1997). A number of CYP enzymes are known to be genetically polymorphic, thus the metabolic capacity of the CYP enzyme system is not equal in all members of a population.

Of all CYP enzymes, CYP2D6 is the best-characterized P450 enzyme that demonstrates polymorphic expression in humans. CYP2D6 is involved in the oxidative metabolism of more than 40 widely prescribed drugs such as selective serotonin reuptake inhibitors (Caccia, 1998), antipsychotics (Scordo & Spina, 2002), tricyclic antidepressants (Furlanut, 1993), and morphine derivatives (van der Weide & Steijns, 1999).

The gene that codes for CYP2D6 enzyme has been localized in chromosome 22 (Heim & Meyer, 1990). In addition to the wild-type gene, 53 different alleles of CYP2D6 have been identified in the Caucasian population (Marez *et al.*, 1997), these alleles are different from the normal or "wild-type" gene by one or multiple mutations, or gene deletion, gene duplication or multiduplications. The mutations may have no effect on enzyme activity, or lead to enzyme with decreased or absent activity, duplications lead to increased enzyme activity.

The consequent categories of phenotypes are called extensive metabolizers (EM) for individuals homozygous or heterozygous for the wild type or normal activity enzymes, intermediate metabolizers (IM) or poor metabolizers (PM) for carriers of two decreased activity or loss-of-function alleles and ultrarapid metabolizers (UM) for carriers of duplicated or multiduplicated active gene (Kalow, 2001, a).

Among extensive metabolizers, CYP2D6 activity varies more than a hundred fold (Shulman and Özdemir, 1997).

Poor metabolizers of CYP2D6-dependent drugs may be at increased risk of adverse effects arising from accumulation of the unmetabolized parent compound. In addition, drug efficacy in these subjects may be compromised if the parent compound is a prodrug, reliant upon metabolic conversion to active metabolites (Ereshefsky *et al.*, 1995).

Ultraextensive metabolizers, on the other hand, will not reach therapeutic plasma levels upon treatment with standard doses, leading to therapeutic failure (Steijns & Weide, 1998), these patients may be successfully treated with higher concentrations of these drugs.

Besides the inter-individual expression of CYP2D6, this enzyme displays also a significant interethnic variability (Gaedigk, 2000).

Lack of enzyme activity occurs with a high frequency from 5-10% in Caucasians, and a frequency usually less than 1% in Orientals.

In ~ 75% of the Caucasian poor metabolizers, the enzyme lack represents the mutation of CYP2D6*4, this mutation is absent in Orientals who have a high frequency of CYP2D6*10 mutation which leads to reduced enzyme activity with a frequency of 51% in China (Kalow,2001, b).

So, since different populations are characterized by their racial backgrounds and their exposure to different environments, it is not surprising to find interethnic differences in drug metabolism.

In the Jordanian population several studies have been done to estimate the percentage of poor metabolizers, either by phenotyping using different probe drugs as dextromethorphan (Irshaid *et al.*, 1993; Irshaid *et al.*, 1996), metoprolol (Hadidi *et al.*,

1994,a) and debrisoquine (Hadidi *et al.*,1994,b), or by genotyping for *CYP2D6*4* (Hadidi *et al.* 1994, b).

The frequency of the poor metabolizers varied in these studies, it was found to be 1.5% using metoprolol as a probe drug (Hadidi *et al.,* 1994), 2.9% (Irshaid *et al.,* 1993) and 6.8% (Irshaid *et al.,* 1996) using dextromethorphan, 7.7% (Hadidi *et al.,* 1994) using debrisoquine, whereas genotyping for *CYP2D6*4* alone yielded a percentage of 8.8 (Hadidi *et al.,* 1994).

Many studies have explored whether the CYP2D6 genotype / phenotype is related to clinical outcome and the occurrence of adverse effects in patients treated with drugs that are metabolized by this enzyme, especially in the field of psychiatry where the metabolism of many antidepressants and antipsychotics used in this field is associated with CYP2D6 to variant degrees (Scordo & Spina, 2002; Chen *et al.*, 1996; Ingelman-Sundberg, 2001,a).

The metabolism of tricyclic antidepressants (TCAs) such as amitriptyline, nortriptyline, imipramine, desipramine and clomipramine is associated with CYP2D6 (Linder & Keck, 1998). Impaired metabolism may result in higher plasma levels, and for many of the TCAs clear relations between blood levels and side effects have been shown (Tamminga *et al.*, 2003).

Novel antidepressants like selective serotonin reuptake inhibitors such as fluoxetine, fluovoxamie and paroxetine are also metabolized by CYP2D6 to various degrees, these drugs have a wider therapeutic index than TCAs, making them safer than the TCAs (Harten, 1993). However, some of these drugs are potent inhibitors of CYP2D6 mediated metabolism, thus administration of these drugs may inhibit the activity of this enzyme and convert the extensive metabolizing activity of an individual to a poor metabolizing activity (Ereshefsky *et al.*, 1995).

In the present study the prevalence of the null alleles (*CYP2D6*3, CYP2D6*4* and *CYP2D6*6*) in a group of normal Jordanian individuals and a group of psychiatric patients who use an antidepressant that is metabolized by CYP2D6 is studied, then the relationship between *CYP2D6* polymorphism and the presence of side effects at the psychiatric patients was assessed.

No previous studies had surveyed the prevalence of the *CYP2D6*3* and **6* alleles in the Jordanian population. In addition, no previous studies had investigated the prevalence of the *CYP2D6* defective alleles (*CYP2D6*3,*4* and **6*) in a population of Jordanian psychiatric patients and studied the effect of the presence of these defective alleles on the appearance of adverse drug reactions of antidepressants.

2. Literature Review:

The recommended therapeutic doses of psychiatric drugs affect individuals differently. Treatment response levels show a continuous spectrum ranging from miraculous recovery to no response or even deterioration, with the added complication of side effects present even in those individuals showing good improvement (Arranz *et al.*, 2001).

Pharmacogenetics research has helped to provide biological evidence and information that explain this variation in the response to drugs.

Pharmacogenetics is the science of the influence of heredity on pharmacological response (Steimer & Potter, 2002).

Pharmacogenetic studies have focused in two main areas: The drug metabolism and the drug site of action.

The first genes that have shown to affect outcome of therapies coded for enzymes that are involved in the metabolism of drugs.

Cytochrome P450 enzymes are considered a very important elimination pathway for drugs. A number of enzymes in this family are known to be genetically polymorphic, and thus interindividual variability in response is suspected in substrates of these enzymes.

2.1. Cytochrome P450:

Cytochrome P450 (CYP450) describes a class of heme-containing proteins that is involved in the vast majority of phase I metabolism (Ellenhorn, 1997).

The original term " cytochrome P450" represents a holdover from the time when the protein was given its provisional name, these proteins are not, in fact, cytochromes in the true sense of the word, this term is derived from the fact that these enzymes were initially believed to be similar to mitochondrial cytochromes which are red in color

(Ellenhorn, 1997), and when reduced cytochrome P450 (Fe^{+2}) forms a ligand with carbon monoxide, the maximal absorbance of light occurs at 450 nm (Parkinson, 1996). This system is also called cytochrome P450 monooxygenase system or mixed function oxidases (Testai, 2001).

This system consists of two protein components: a hemoprotein called cytochrome P450 and a flavoprotein called NADPH-cytochrome P450 reductase.

The general reaction catalyzed by P450 is an oxidation, in which an atom of molecular oxygen is incorporated into the substrate and the other one is reduced to water. The electrons involved in the reaction are derived from NADPH through the NADPH-cytochrome reductase (Meyer, 1996). So the overall reaction (figure 1) can be written as follows:

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

In vertebrates, two classes of P450 exist (Testai, 2001):

- The mitochondrial P450 forms which are localized in the inner membrane of the mitochondria and catalyze bile acids and steroid biosynthesis.

- The P450 forms that are located in the microsomal fraction of most tissues, these forms are involved in the xenobiotic metabolism.

The liver is the richest source of P450, however, significant levels have also been found in skin, lung, kidney, gastrointestinal tract and olfactory epithelia (Testai, 2001).

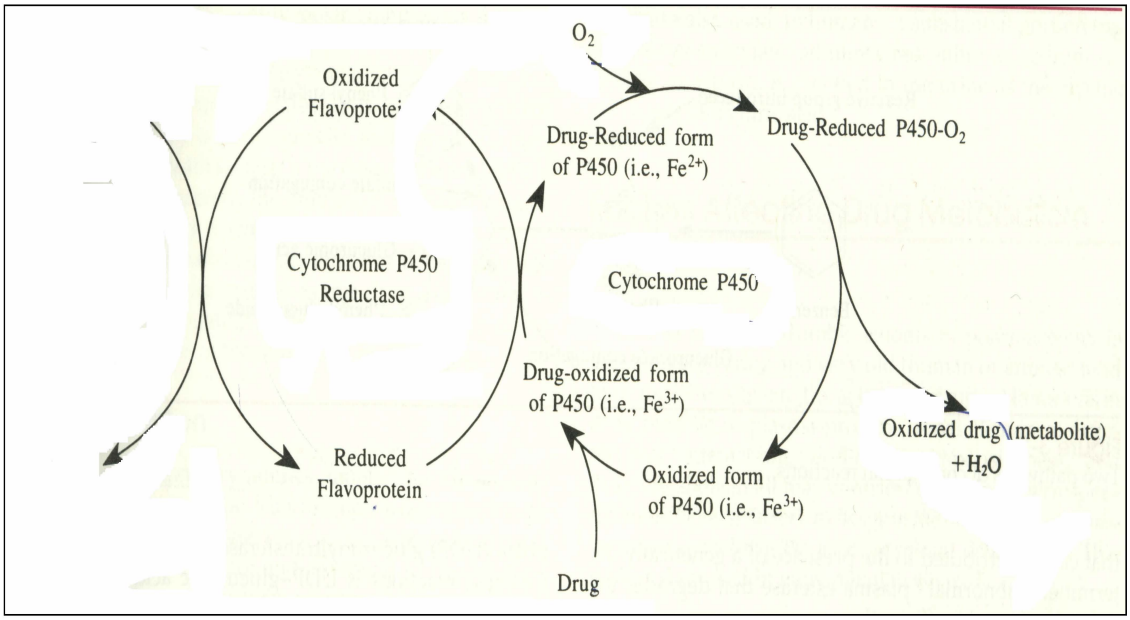

Figure 1:The working mechanism of CYP450 (Gram, 1997).

Approximately, 1200 enzymes are known in the CYP450 system which are present in species from all five biological kingdoms (Lewis, 2003).

The CYP enzymes have been classified in a systematic way on the basis of their amino acid sequence. A standard nomenclature system, categorizing enzymes in gene families and subfamilies have been developed (Nelson *et al.*, 1996):

Families are indicated by the abbreviation for Cytochrome P450 (CYP).

Enzymes with 40% or greater sequence identity are included in the same family (designated by an Arabic numerals, e.g. CYP2), and within this family, enzymes with greater than 55% sequence identity are included in the same subfamily (designated by uppercase letters, e.g. CYP2D).

Individual isoenzymes are designated by a second Arabic numeral (e.g. 2D6).

The same numbers and letters are recommended for the corresponding gene product (mRNA, cDNA, enzyme) in the non-italicized and all capital letters, italicized for the gene (e.g. *CYP1A2* or *CYP24* for the gene; CYP1A2 or CYP24 for the mRNA, cDNA and protein).

In the text of publication, one might designate the protein as P450 1A1, P450 2G1 or simply 1A1 or 2G1, but one should stay with all capital letters.

To date, 17 families have been identified in humans (Lewis, 2003).

Seven isoenzymes are involved in the hepatic metabolism of most drugs, these enzymes belong to the families 1-3, and these are: 1A2, 2A6, 2C9, 2C19, 2D6, 2E1 and 3A4 (Ingelman-Sundberg, 2001a). The majority of the genes of these enzymes are polymorphic.

Table (1) shows some of the human cytochromes P450 in gene families 1-3 that are involved in the metabolism of xenobiotics.

Table1: Some of the human cytochromes P450 in gene families 1-3 that are involved in the metabolism of xenobiotics (Meyer, 2001).

Enzyme	Substrate
CYP1A2	Caffeine, clozapine, imipramine, theophylline, R-warfarine,.
CYP2C9	Diclofinac, phenytoin, sertraline, tolbutamide, fluvoxamine, fluoxetine, S-warfarin.
CYP2C19	Citalopram, diazepam, imipramine, omeprazole.
CYP2D6	Amitriptyline, clomipramine, codeine, desipramine, dextromethorphan, fluvoxamine, fluoxetine, haloperidol, metoprolol and other β-blockers, nortriptyline, paroxetine, perphenazine, risperidone, tramadol.
CYP3A4	Alprazolam, carbamazepine, diazepam, bupropion, cyclosporine, erythromycin, lidocaine, methadone, midazolam, nefazodone, triazolam, verapamil, R-warfarin

Numerous factors influence the concentration and activity of the drug metabolizing enzymes, some of these factors are: age, liver diseases, environmental chemicals, drugs, and genetic factors (Meyer, 1996).

Genetic variation in drug-metabolizing enzymes can markedly affect drug kinetics.

A major source of inter-individual differences in drug metabolism are genetic polymorphisms. When specific gene mutations or deletions are maintained within the population, the gene is said to be polymorphic or having multiple forms (Linder *et al.,* 1997).

A number of CYP enzymes are known to be genetically polymorphic, such as 2C19 and 2D6.

2.2. Genetic Polymorphism:

2.2.1. Definition of genetic polymorphism:

The term genetic polymorphism defines a monogenic trait that exists in the population in at leas two phenotypes (and presumably at least two genotypes), neither which is rare. Indeed, the rarest phenotype is considered due to a polymorphic gene and not to a rare mutation, when occurring at a frequency of more than 1% (Meyer, 2000).

For a gene to be polymorphic, it must encode a product that is not required for development, reproductive or a crucial physiological function, otherwise, such a high frequency wouldn't be expected (Gonzalez & Idle, 1994).

The mere existence of polymorphism in enzymes responsible for xenobiotic metabolism implies that these enzymes are not required for survival under normal conditions.

However, polymorphism in drug-metabolizing enzymes can be associated with marked differences in response to drug therapy (Özdemir *et al.*, 2001).

2.2.2. Types of genetic polymorphisms:

Genetic polymorphisms occur in the form of gross structural changes including complete gene deletion, gene duplication and genetic translocation where portions of similar genes are combined creating a new gene hybrid.

By far, the most common forms of genetic polymorphism is single nucleotide polymorphisms (SNPs) where the nucleotide sequence at one specific location is changed, inserted or deleted (Wilkins, 2002)

- Genetic polymorphism resulting in gene deletion leads to loss of function and no production of the gene product.

- Gene duplication and multi-duplication most commonly leads to increased expression of the gene product and a hyperactivity phenotype. An exception to this is duplication of an allele which includes additional structural variation leading to loss of function.

- Genetic translocation typically yields a non-functional gene (Rioux, 2000).

- SNPs can result in a variety of changes in the expressed protein function depending upon where the polymorphism occurs in the overall gene structure (IngelmanSundberg, 2001,a):

- SNPs in the 5` regulatory domain may influence gene regulation.

- SNPs in the coding exons only influence function if there is a resulting amino acid change that alters the protein function (Linder & Valdes, 2001).

- SNPs within the introns regions are typically silent unless the SNP alters a nucleotide critical for splicing of the RNA during maturation which typically leads to loss or decrease in protein function (Campbell *et al.*, 2000).

Most of the genes of CYP450 belonging to families 1-3 which are associated with xenobiotic metabolism are polymorphic (Ingelman-Sundberg, 2001,b).

The field of polymorphic CYPs is quite complex, so in order to help scientists in this field, a web page has been created which contains continuously updated information regarding the polymorphic CYPs forms (http:// www.imm.ki.se/CYP alleles/). The aim of this page is to provide scientists with a useful nomenclature for all enzyme variants with links to relevant literature describing the properties of the polymorphic enzymes. An important factor is also to bring scientists up to date with the most recent knowledge allowing them to check whether allelic variants they have found have been described before.

2.3. Genetic polymorphism and population phenotypes:

Genetic polymorphism in drug metabolizing enzymes has been linked to four phenotypes ranging from extremely slow (PM) to ultrafast metabolism (UM) (Meyer, 2000):

Extensive metabolism (EM) of a drug is a characteristic of the normal population, individuals belonging to this category are homozygous or heterozygous for the wild-type or normal activity enzymes, this group constitute 75-85% of the Caucasian population.

Intermediate metabolizers (IM) (10-15%) or poor metabolizers (PM) (5-10%) are carriers of two decreased-activity alleles or loss of function alleles.

Ultrarapid metabolizers (UM), (1-10%) are carriers of duplicated or multiduplicated active genes.

In general, poor metabolizers will develop higher serum drug concentrations in comparison with EMs, causing an increased risk of suffering from side effects when subjected to recommended doses, ultrarapid metabolizers, on the other hand , will not

reach therapeutic serum concentrations upon treatment with standard doses (Kalow, 1997,b).

In addition to the inter-subject variability in the activity of drug metabolizing enzymes, inter-ethnic variations between populations have also been described (Gaedigk, 2000; Kalow, 2001).

These differences between human populations are known for some of the P450 enzymes, there are well documented data for CYP2D6 and CYP2C19 (Leathart *et al.*, 1998;Tateishi *et al.*, 1999; Wedlund *et al.*, 1984).

Of all CYP enzymes, the highly genetically polymorphic enzyme 2D6 which is also known as sparteine /debrisoquine hydroxylase has been the most extensively studied.

2.4. Determination of metabolic activity: phenotyping and genotyping

The identification of the four classes of metabolizers (PM, IM, EM and UM) is determined by either phenotyping or genotyping.

Phenotyping has been a valuable research tool, and for a long time it was the only possibility of assessing the genetic basis of a patient's metabolic capacity.

Phenotyping is accomplished by administration of a probe drug (the metabolism of which is known to be solely dependent on the function of a specific drug-metabolizing enzyme) followed by measurement of the metabolic ratio (MR, defined as the ratio of drug dosage or unchanged drug to metabolite measured in urine or serum). Because of a possible interaction of the probe drug with many other clinically used drugs, a washout period of several days is necessary to obtain meaningful results. This implies an inherent ethical problem, as patients seeking medical help for their disease would have to stay untreated for several days (Steimer *et al.*, 2001).

Genotyping involves identification of defined genetic mutations that give rise to the specific drug metabolism phenotype (Linder *et al.*,1997).

In pharmacokinetic studies, phenotyping has the advantage over genotyping in revealing drug-drug interactions or defects in the overall process of drug metabolism, but it also has several drawbacks in that it is hampered by complicated, time-consuming, expensive protocols of testing, risks of adverse drug reactions caused by the probe drug, problems with incorrect phenotype assignment due to coadministration of drugs, and the need of several samples of blood or urine (Ensom et *al.*,2001).

Genotyping tests have the advantages of having to be done only once in a life time, providing unequivocal genetic information, can be performed from only one blood sample or even more easily obtained material as hair roots or saliva, not affected by coadministered medications. However, genotyping tests only identify a group (e.g., PM, UM) and do not predict the exact individual metabolic capacity or receptor interaction, because there is still considerable variation between individuals of the same genotype (Meyer, 2001).

2.5. Cytochrome 2D6:

CYP2D6 which comprises 2% to 6% of the total hepatic CYP450 content, is the best characterized P450 enzyme that demonstrates polymorphic expression in humans (Meyer, 1996).

In 1977, physicians at London made the serendipitous observation that a volunteer's hypotensive response to debrisoquine, an antihypertensive drug, was markedly increased because of impaired metabolism (Mahgoub *et al.,* 1977). At about the same time, a group of physicians in Bonn, independently observed increased side effects associated with decreased oxidative metabolism of sparteine, an alkaloid drug with antiarrhythmic actions (Eichelbaum *et al.,* 1979). Family studies revealed that both oxidative metabolic reactions are under the same monogenic control which is now known as the *CYP2D6* gene, and that poor metabolizers are homozygous for a recessive allele.

2.5.1. *CYP2D* Genes:

The *CYP2D* wild-type locus in humans is comprised of the three highly homologous genes *CYP2D8P*, *CYP2D7P* and *CYP2D6*, which are located in this orientation (5` to 3`) on a contiguous region of about 45 Kb on the long arm of chromosme 22 (Gaedigk *et al.*, 1991).

Like other genes of the CYP2 family, *CYP2D* genes consist of nine exons and eight introns.

CYP2D8P is a pseudogene that contains multiple deletions and insertions and causes a highly disrupted open reading frame. The *CYP2D7P* gene is more similar to *CYP2D6* than to *CYP28P*, no specific mRNA product was detected in RNA from human livers, which indicates that it is also a pseudogene (Meyer & Zanger, 1997).

So, 2D6 is the only CYP2D protein product in humans.

2.5.2. *CYP2D6* Polymophism:

The *CYP2D6* gene locus is highly polymorphic, exhibiting point mutations, nucleotide deletions or insertions, gene rearrangements, duplications, multi-duplications and deletions of the entire *CYP2D6* gene (Geadigk *et al.*, 1991; Agùndez *et al.*, 1995; Marez *et al.*, 1995; Steen *et al.*, 1995; Stüven *et al.*, 1996; Yokoi *et al.*,1996; Gaedigk *et al.*, 1999).

Consequently, large variations of enzyme activities are observed in the general population ranging from ultrarapid (UM) to poor metabolizer (PM) phenotypes.

The poor metabolism phenotype is inherited as an autosomal recessive trait (Mahgoub *et al.*, 1977). Five to 10% of individuals in most studied Caucasian populations are PMs (reviewed by Bertilsson, 1995), (i.e. carrying two-non functional alleles of the *CYP2D6* gene).

The frequency of the different mutant PM alleles in Caucasian populations varies among different studies, the *CYP2D6*4* allele is the most common with a frequency of 0.10-0.20, accounting for over of 70% of all null alleles (Gonzale& Idle, 1994; Bradford, 2002).

The *CYP2D6*4* variant results from the G to A change (1846 G <A) at the last nucleotide of intron 3 leading to splicing defect (Kagimoto *et al.*, 1990).

The *2D6*5* allele is the second most common PM-associated mutation, representing about 15% of the null alleles (Gonzalez & Idle, 1994; Kalow,2001). This variant represents a complete deletion of the *CYP2D6* gene which leads to absence of enzyme production (Hiem & Meyer, 1990; Gaedigk *et al.*, 1991), this allele has been reported to occur in Caucasians with a frequency of approximately 0.05 (Steen *et al.*, 1995).

*CYP2D6*3* is another null allele, with a frequency of 5% in the Caucasian populations (Hansen *et al.*, 1995), it results from a single mutation in exon 5, the deletion of one

nucleotide (A 2549) causes a reading frame disruption in the translation of mRNA leading to a premature termination of protein synthesis (Kagimoto *et al.,* 1990).

Another null allele is *CYP2D6*6,* this variant results from a single base deletion (T 1795) in exon 3 with a subsequent frame shift and generation of a stop codon (Saxena *et al.,* 1994). The allele frequency of this allele in Caucasian populations was found to be ~ 1% (Bradford, 2002).

Several PM alleles have been described that lead to inactive CYP2D6, however evaluation of the four alleles (*CYP2D6*3,*4,*5* and **6*) can predict 93-97.5% of the PM phenotypes in the white Caucasian populations (Hersberger *et al.,* 2000).

The other extreme of the CYP2D6 activity is the ultrarapid metabolism, which is often due to inheritance of alleles with duplicated or amplified functional *CYP2D6* genes. The molecular genetic explanation for ultrarapid metabolism was found when a new variant of *CYP2D6* locus with duplicated or multiduplicated functional *CYP2D6*2* genes was characterized (Agúndez *et al.,* 1995).

It was reported that up to 13 copies of *CYP2D6*2* were found, persons who carry duplicated or multiduplicated *CYP2D6*2* genes would be expected to need much higher doses than normal of drugs metabolized by 2D6 to reach therapeutic plasma concentrations.

Extensive efforts have been undertaken to identify polymorphic *CYP2D6* alleles, characterize their gene products and assess the phenotypic consequences of an each newly discovered allele .

Daly *et al.* (1996) introduced a CYP2D6 nomenclature which is now widely accepted describing 25 different alleles termed *2D6*1* to **17*, some of them with subtypes. In this field also, Marez and coworkers (1997) revealed 36 new allelic variants increasing the total number of alleles reported for the *CYP2D6* locus to over 50.

To date, > 70 variant alleles of the *CYP2D6* locus have been described (regular update on www.imm.ki.se/CYPalleles/cyp2d6.htm), of which at least 18 encode non-functional gene products.

Table (2) demonstrates some of the polymorphisms of *CYP2D6* described by Daly *et al.* (1996).

Figure 2 shows the positions of the polymorphisms associated with *CYP2D6*3,*4,*5,*6* alleles.

Table 2: Some of the *CYP2D6* alleles, nucleotide changes and the resultant enzyme activity:

Allele	Nucleotide change	Enzyme activity
*CYP2D6*1A*	None	Normal
*CYP2D6*2*	G1749C, C2938T, G4268C	Decreased
*CYP2D6*2XN* (N=2,3,4,5,13)	G1749C, C2938T, G4268C	Increased
*CYP2D6*3*	A2637 deletion	None
*CYP2D6*4A*	C188T, C1062A, A1072G, C1085G, G1749C, **G1934A**, G4268C	None
*CYP2D6*5*	***CYP2D6* deleted**	None
*CYP2D6*6*	**T1795 deleted**	None
*CYP2D6*10A*	**C188T,** G1749C, G4268C	Decreased
*CYP2D6*17*	C1111T, G1726C, C2938T, G4268C	Decreased

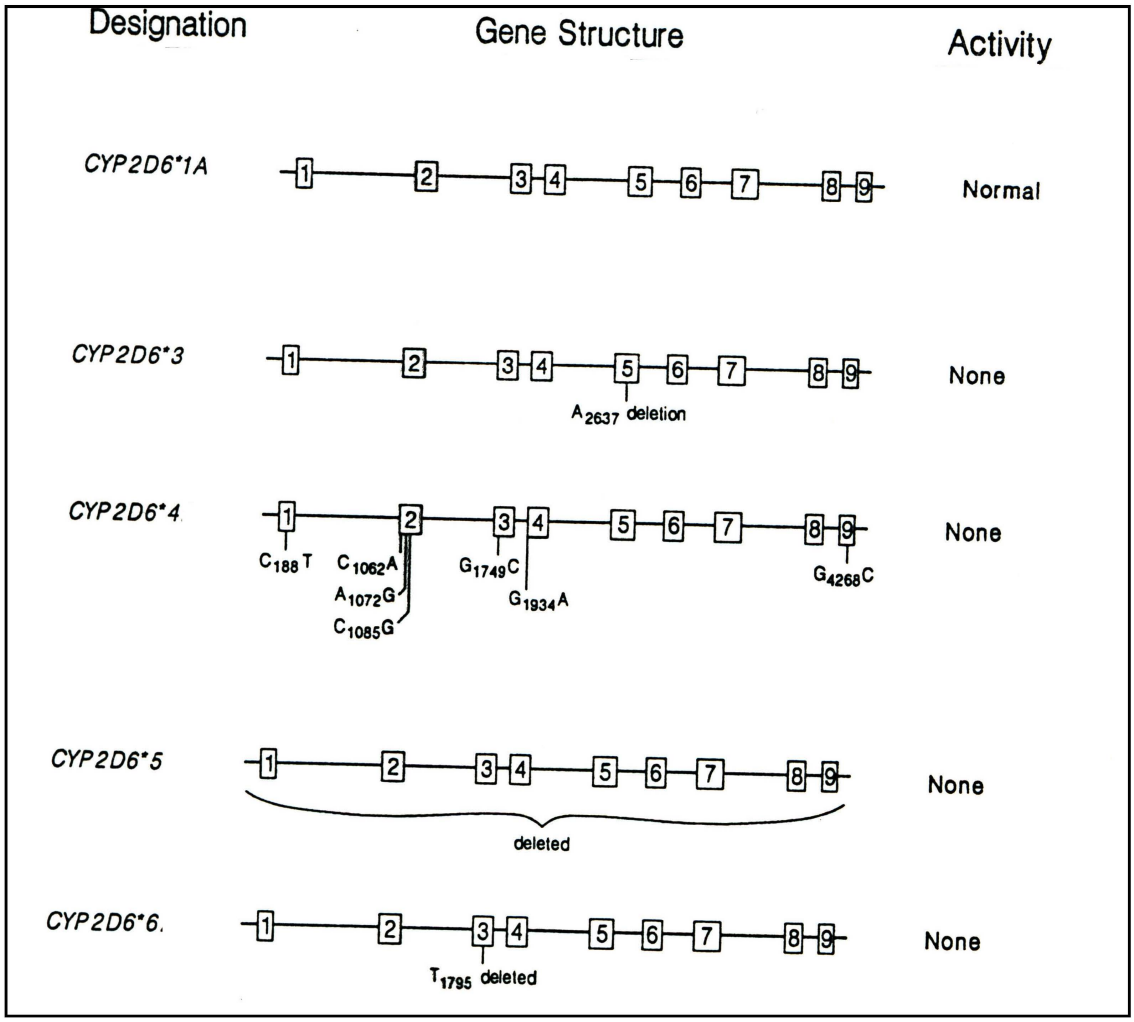

Figure 2: The positions of *CYP2D6*3, *4, *5, *6* alleles in the *CYP2D6* gene (Daly *et al.*, 1996).

2.6. Antidepressants- CYP2D6 associated metabolism:

The variability of the response of a given individual to an antidepressant is determined both by genetic and environmental factors .

Differences in metabolic rates have not yet found adequate recognition in clinical practice as an important source of interindividual variability in response (Meyer *et al.,* 1996; Arranz *et al.,* 2001). However, differences between individuals in metabolism are increasingly being demonstrated as clinically significant .

The metabolic pathways and isoenzymes involved for many drugs have not yet been completely elucidated. However, it is known that all antidepressants are extensively metabolized by different reactions, consequently, the activity of drug-metabolizing enzymes is of major importance for the kinetics and dosage of these drugs. Variations in the activity of these enzymes may be genetically determined, but also related to inhibition and induction by drugs or other substances (Alfaro *et al.,* 2000; Lam *et al.,* 2002; Caccia, 1998).

CYP2D6 is one of the important enzymes that is involved in the metabolism of many antidepressants. Polymorphisms in the gene encoding this enzyme have attracted most attention because it is responsible for the metabolism of ~ 25% of drugs, including certain antidepressants as some of the tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitiors (SSRI).

The *CYP2D6* polymorphism may pose clinical problems in two groups; the PMs and the UMs. The poor metabolizers predictably will have increased plasma concentrations of the antidepressant used when given the recommended dose.

Adverse effects occur more frequently in PMs and may be misinterpreted as symptoms of depression and lead to erroneous further increase in the dose (Chen *et al.,* 1996; Arranz *et al.,*2001).

2.6.1. Types of Adverse Drug Reactions:

An adverse drug reaction (ADR) has been defined as an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product (Edwards & Aronson,2000).

From a clinical perspective, ADRs may be classified as:

- Augmented reactions (Type A): which can be predicted from the pharmacology of either the drug or its metabolites, and represent an augmentation of its known effects. They are typically dose-dependent, and may be amenable to dose reduction (Eisenhauer, 2002).

- Type B reactions: these are unrelated to the known pharmacology of the drug and are not dose dependent. They are often termed bizarre or idiosyncratic adverse reactions, they are unpredictable and may depend heavily on host factors (Pirmohamed *et al.,* 2002).

Pharmacogenetic traits form the basis for type A adverse reactions mostly because they affect rate limiting processes in the clearance of the drug.

2.6.2. ADRs associated with antidepressants:

Most adverse effects of antidepressants can be explained by their synaptic effects. That is, the effects of these drugs on some important components of the synapse in the brain and elsewhere in the body. The most 2 important synaptic effects of antidepressants are blockade of transport of certain neurotransmitters (norepinephrine, serotonin, and dopamine) back into the nerve ending and blockade of certain receptors for some neurotransmitters. Some of these synaptic effects may be required for the therapeutic effects of antidepressants. If so, the currently available antidepressants can

never be devoid of certain adverse effects caused by interactions with neurotransmitters or their receptors (Richelson, 2001).

2.6.2.1. ADRs associated with tricyclic antidepressants:

These drugs are collectively known as tricyclic antidepressants as a result of their basic chemical structures (i.e., a three-ring core), some of the members of this group are: imipramine, clomipramine, amitriptyline, desipramine, nortriptyline.

These drugs are no longer considered first-line agents in the treatment of depression because of their prominent side effects and the need to monitor drug blood levels in order to avoid toxicity.

The mechanism of therapeutic action of the TCAs is inhibition of norepinephrine and serotonin (5-hydroxytryptamine, 5-HT) reuptake in the central nervous system (Andrews & Nemeroff, 1994). The major side effects of the TCAs are attributed to inhibition of one or more neurotransmitter receptors, including the histaminergic / muscarinic, serotonergic and dopaminergic receptors.

The anticholinergic side effects of the TCAs are the most common reasons for discontinuating antidepressant medication and include dry mouth, constipation, urinary retention, blurred vision, tachycardia, and memory dysfunction. Blockade of histamine receptors in the central nervous system (CNS) by TCAs is associated with sedation, drowsiness and weight gain. Inhibition of serotonin receptors may be related to hypontension, and dopamine receptors blockade may cause extrapyramidal movement disorders (Azzaro & Ward, 1997).

In addition, cardiac effects are observed with these drugs. The most common of the cardiovascular effects are orthostatic hypotension and / or tachycardia, these effects occur because of the α_1-adrenoceptors antagonist properties of these compounds. Cardiotoxicity associated with TCAs is of concern because of the potentially severe and

fatal nature of these side effects in patients with cardiac disease (Andrews & Nemeroff, 1994).

The CNS is also a target of TCA drug toxicity. At subtherapeutic doses these agents can produce mild sedation. However, this action is generally viewed in a positive sense by the physician and the patient, for it assists in allowing a natural sleep in the depressed patient. High serum concentrations of TCA drugs are generally associated with more severe forms of CNS toxicity. These toxicities are oven due to overtitration of the patient by the physician in an attempt to produce a therapeutic effect.

CNS toxicity presents as a delirium with affective, cognitive, motor, and psychotic symptoms that can be clinically difficult to manage.

Often the treating physician may misinterpret the delirium as a worsening of the depression and further increase of the dose of the TCA, leading to life-threatening seizures. Fortunately, TCA-induced toxicity of the CNS, like that seen for the cardiovascular system, is dose dependent and is unlikely to occur at therapeutic serum levels (Azzaro & Ward, 1997).

The TCAs have a very low therapeutic index, and careful monitoring of serum concentrations is a critical element of the therapy. TCA doses of 10-20 mg/Kg are associated with moderate to severe toxicity and doses of 30-40 mg/kg may be fatal (Andrews & Nemeroff, 1994).

2.6.2.2. ADRs associated with selective serotonin reuptake inhibitors:

The SSRIs are currently considered first-line therapy for depression because of their superior side-effect/ safety profile. These drugs are as effective as the TCAs in the treatment of depression but as a class have a much more favorable side-effect profile. This group includes fluoxetine, sertraline, paroxetine, and fluvoxamine.

In contrast to TCAs, the SSRIs are markedly safer when taken in an overdose situation (Edwards & Anderson, 1999).

The SSRIs are potent and selective inhibitors of serotonin reuptake at the presynaptic terminal. This pharmacologic characteristic results in an increase in serotonin availability at serotonergic synapses. It is believed that these agents produce their therapeutic action through an ability to modulate serotonin neurotransmission in the brain. At clinical doses, the SSRIs have little effect on the norepinephrine or dopamine transporters and a low affinity for the histaminic, muscarinic/ cholinergic, and α-adrenergic receptors (Harten, 1993).

While TCA agents commonly cause anticholinergic and cardiovascular side effects, the SSRIs are devoid of these properties. They are more likely to cause gastrointestinal effects (nausea and diarrhea), CNS stimulation(insomnia, anxiety), and sexual dysfunction. In addition a small weight loss may be seen with chronic fluoxetine treatment, as compared with the weight gain associated with the TCA agents (Azzaro & Ward, 1997).

2.6.3. 2D6 mediated metabolism of TCAs:

2.6.3.1. Imipramine:

Imipramine is a basic drug whose absorption takes place in the alkaline environment of the small intestine with no or little absorption in the stomach. A high degree of tissue binding results in an apparent volume of distribution in the range of 10-20 L/kg (Sallee & Pollock, 1990). The elimination half life of imipramine is from 6-28 hours, and it depends on the rate of hydroxylation (DeVane, 1994).

Imipramine is eliminated as shown in figure (3) by N-demethylation to the active metabolite desipramine, and by hydroxylation to 2-hydroxyimipramine and 10-hydroxyimipramine, and By N-oxidation (Sallee & Pollock, 1990).

Desipramine is largely eliminated by 2-hydroxylation (Shimoda *et al.,* 2000).

In vivo studies have shown that demethylation of imipramine and 2-hydroxylation of imipramine and desipramine are carried out by several isozymes of the cytochrome P450 (Brøsen *et al.,* 1986; Skjelbo *et al.,* 1991; Madsen *et al.,* 1997).

At least three different P450s are involved in the N-demethylation of imipramine: CYP1A2, CYP2C19 and CYPP3A4 (Skjelbo *et al.,* 1991; Madsen *et al.,* 1997), and it was found that this stage is not affected by CYP2D6 polymorphisms, the finding of high desipramine levels in 2D6 poor metabolizers suggests that imipramine demethylation is unaffected in this phenotype (Brøsen *et al.,* 1986; Brøsen *et al.,* 1991).

In a study of Skjelbo and coworkers (1991), it was shown that the demethylation of imipramine is about 50% lower in poor metabolizers of mephenytoin compared with extensive metabolizers of mephenytoin, suggesting that approximately 50% of the demethylation of imipramine to desipramine is mediated by the way of the mephenytoin oxygenase.

Madsen *et al.* (1997) found that 2C19 activity accounted for 19% of the N-demethylation of imipramine to desipramine and 29% of the N-demethylation of 2-hydroxyimipramine to 2-hydroxydesipramine.

CYP1A2 is also associated in the N-demethylation of imipramine, this was confirmed by the potent inhibition of N-demethylation of imipramine by fluvoxamine which is a potent inhibitor of 1A2 and that the N-demethylation is induced in smokers (Sallee & Pollock, 1990).

The 2-hydroxylation of imipramine and desipramine almost exclusively depends on 2D6, it was demonstrated that quinidine (a very potent inhibitor of oxidations catalyzed by 2D6) was a potent inhibitor of 2-hydroxuimipramine formation but had no effect on N-demethylation and 10-hydroxylation (Brøsen *et al.,* 1986; Brøsen *et al.,* 1988; Brøsen *et al.,* 1991).

The therapeutic efficacy of imipramine appears to be generally related to the sum of concentration of imipramine plus desipramine (Amsterdam *et al.,* 1980).

The relationship between blood level measurements and clinical outcome is clear and straightforward with imipramine, many studies have shown a linear or a sigmoid relation between blood levels of imipramine plus desipramine and the clinical outcome (Task Force on the Use of Laboratory Tests in Psychiatry, 1985).

Death of two subjects due to accumulation of imipramine and desipramine was reported, both of the patients were receiving chronic imipramine therapy, impaired metabolism due to *CYP2D6* defect was suggested as a possible mechanism for the apparent fatal accumulation of these tricyclic antidepressants (Swanson *et al.,* 1997).

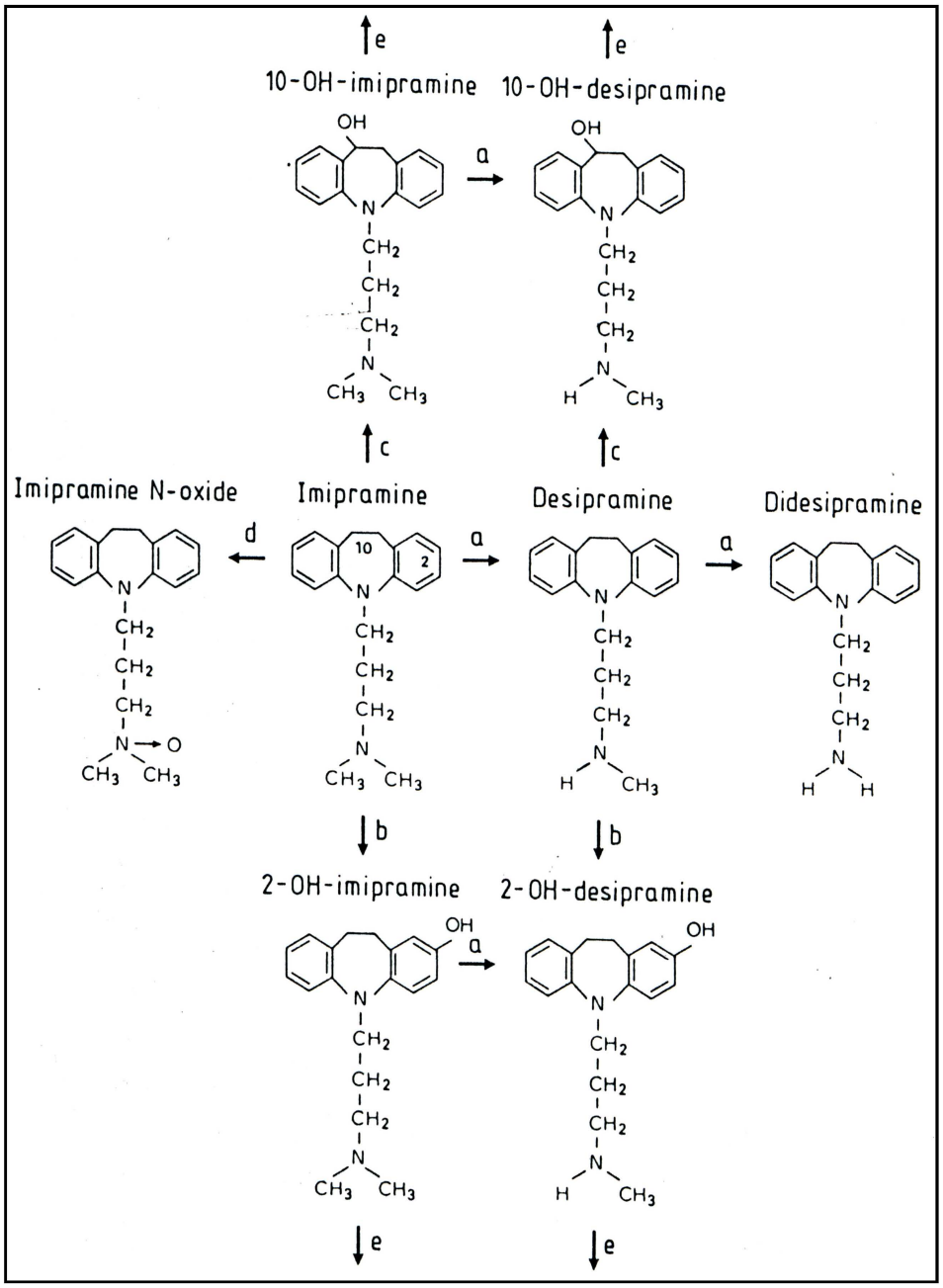

Figure 3: The metabolic pathways of imipramine (Brøsen *et al.,* 1991).

a: N-demethylation; b: 2-hydroxylation;c: 10-hydroxylation;d: N-oxidation; e:glucuronidation.

2.6.3.2. Clomipramine:

Clomipramine is another member of the TCAs family, and it is a chlorinated analogue of imipramine.

It is well absorbed from the gastrointestinal tract, hepatic first-pass metabolism to desmethylclomipramine reaches up to 50%, this represents an important source of inter-individual variability in blood concentrations of the drug and its metabolites (Balant-Gorgia *et al.,* 1991).

The mean apparent volume of distribution of clomipramine is 12 L/kg, it is 98% bound to plasma proteins and the elimination half-life is about 39 hours (Ellenhorn, 1997).

The major route of biotransformation of clomipramine is demethylation to N-desmethylclomipramine (DC), which is further hydroxylated to 8-hydroxy-N-desmethylclomipramine (HDC).It is also hydroxylated 8-hydroxyclomipramine (HC),which is subsequently demethylated to (HDC) (Balant-Gorgia *et al.,* 1992; Nielsen *et al.*, 1994), figure 4 shows the major metabolic pathways of clomipramine.

The N-demethylation of clomipramine is catalyzed by several enzymes of the CYP450 family as CYP1A2, CYP3A4 and CYP2C19 (Danish University Antidepressant Group, 1999; Terao *et al.,* 1999). The hydroxylation of the secondary amine N-desmethylclomipramine is mainly catalyzed by 2D6.

Nielsen and colleagues (1994) investigated the influence of the 2D6 and 2C19 polymorphisms on the single dose kinetics of clomipramine in healthy volunteers, they reported that poor metabolizers of 2C19 had a lower capacity to N-demethylate clomipramine than the extensive metabolizers of this enzyme, and that there was a marked interindividual variation within each genotype. They also reported that that the capacity to hydroxylate N-dismethylclomipramine was lower in PMs of 2D6 than in EMs. These findings indicate that CYP2C19 is responsible for the formation of

N-desmethylclomipramine and that 8-hydroxylation of N-dismethylclomipramine is catalyzed by CYP2D6.

The documentation of a concentration effect relationship has not been so clear for clomipramine. Comparing blood concentrations of both clomipramine and dismethylclomipramine to the clinical efficacy shows that there is a high interindividual variability which is one of the major problems related to the successful use of this drug, it was reported that there is no relationship between administered dose and concentration of clomipramine and / or desmethylclomipramine. Accordingly, daily dose is a poor predictor of treatment outcome (Amsterdam *et al.,* 1980, Terao *at al.,* 1999).

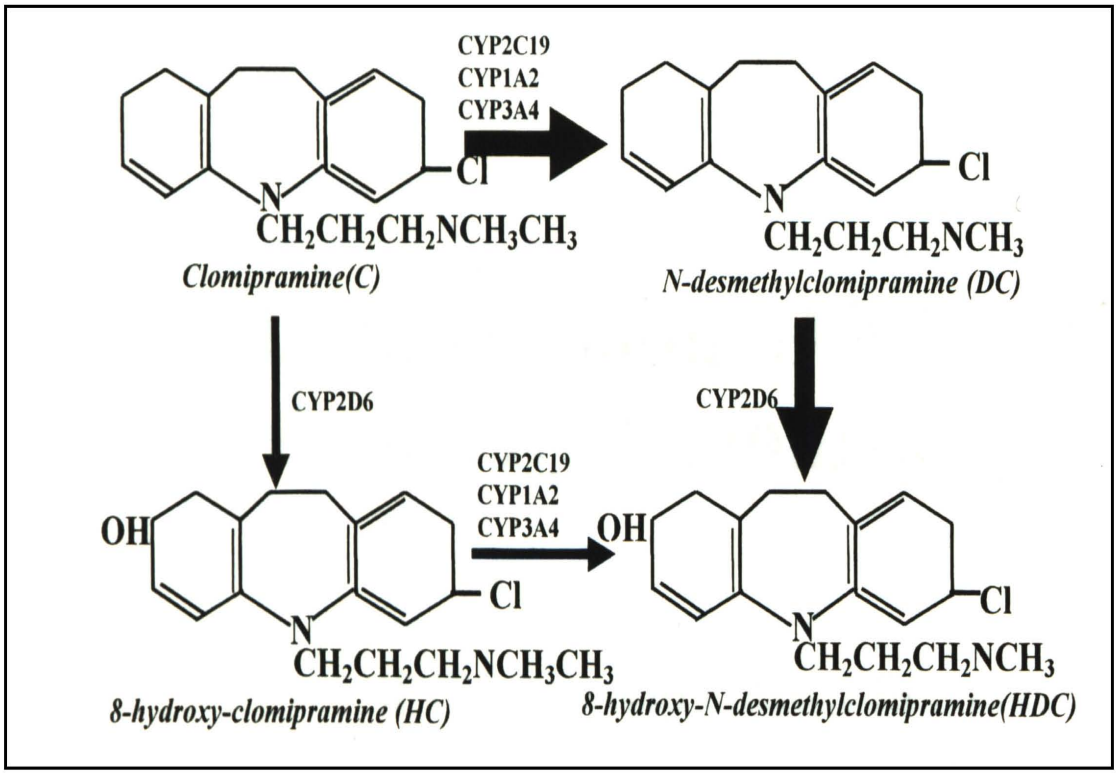

Figure 4: The metabolic pathways of clomipramine (Yokono *et al.,* 2001).

2.6.3.3. Amitriptyline:

Analogous to the metabolism of imipramine and clomipramine, the major metabolic pathway of Amitriptyline (AT) is via demethylation to nortriptyline (NT), which is further hydroxylated to isomeric metabolites; trans-10-hydroxy-nortriptyline (EHNT) and cis-10-hydroxy-nortriptyline (ZHNT). Amitriptyline is also hydroxylated to trans-10-hydroxy-amitriptyline (EHAT), and cis-10-hydroxy-amitriptyline (ZHAT), which is subsequently desmethylated to EHNT and ZHNT (Shimoda *et al.,* 2002; Breyer-Pfaff *et al.,* 1992), figure 5 shows the metabolic pathways of amitriptyline.

The desmethylation of amitriptyline has been reported to be catalyzed by several enzymes, e.g.: CYP2C19, CYP1A2, CYP3A4, CYP2D6, CYP2B6, CYP2C8 and CYP2C9 (Shimoda *et al.,* 2002, Coutts *et al.,* 1997).

The main metabolic pathway of NT, the desmethylated metabolite of AT, is by benzylic hydroxylation in the 10-position. It was reported that CYP2D6 polymorphism is the important determinant of NT metabolism (Morita *et al.,* 2000). A significant correlation was found between the metabolic ratio of debrisoquine and the total clearance of NT via trans-10-hydroxylation but not via cis-10-hydroxylation of NT (Mellström *et al.,* 1981).

The relationship between plasma concentration of AT and its clinical response has been controversial. Many studies have confirmed a linear response curve with AT and NT plasma concentrations (Amsterdam *et al.,* 1980, Task Force on the US of Laboratory Tests in Psychiatry, 1985). In contrast, other studies found no correlation between steady state plasma levels of AT or AT and NT and clinical response (Ziegler *et al.,* 1982; Kupfer *et al.,* 1985)In a study of Braithwaite and associates (1972), no significant correlation was found between plasma AT and NT levels and the total side effect scores for each of the patients, nor was there any correlation with those side effects that might be specifically associated with the drug like drowsiness and dry mouth.

The serum therapeutic concentration of amitriptyline is in the range of 100-250 ng/ml, and the elimination half-life is from 16-26 hours (Azzaro & Ward, 1997).

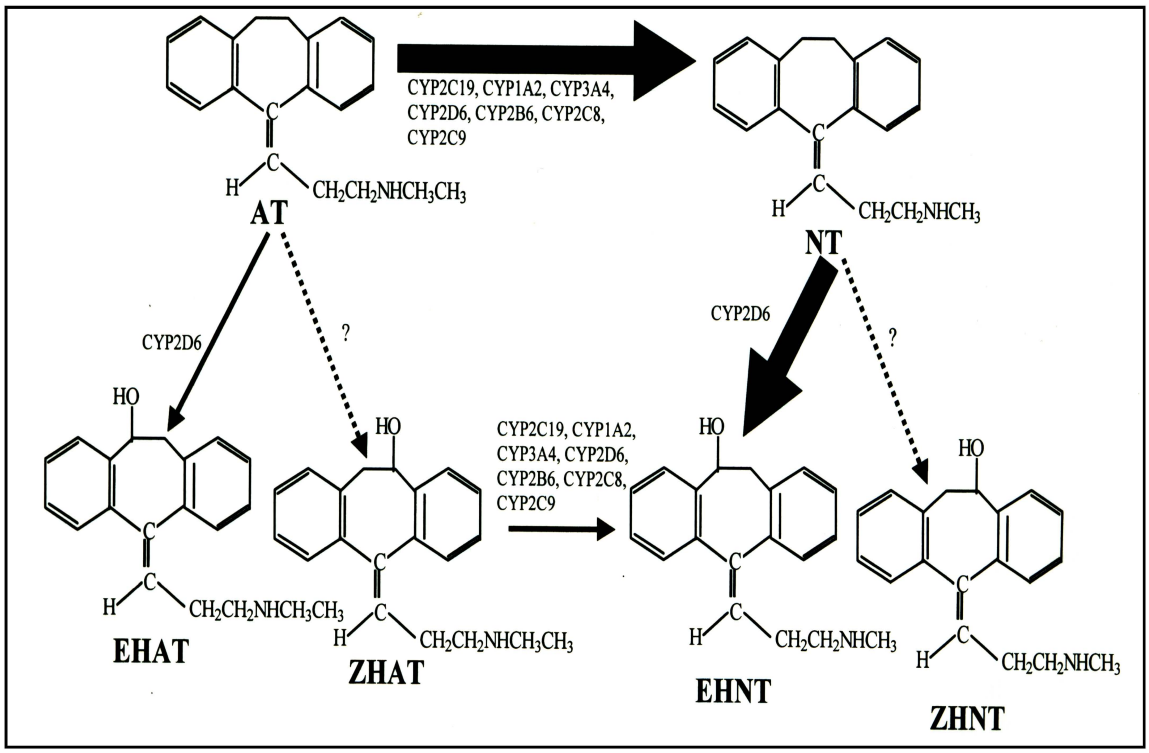

Figure 5: The metabolic pathways of amitriptyline (Shimoda *et al.,* 2002).

2.6.4. 2D6 mediated metabolism of SSRIs:

2.6.4.1. Fluoxetine:

Fluoxetine is almost completely absorbed, it is highly bound to plasma proteins (> 95%) and it has a large volume of distribution ranging from 14-100 L/kg . Fluoxetine has an elimination half-life ranging between 1-4 days, while that of its principal metabolite, norfluoxetine, ranges between 7-15 days (Hemeryck & Belpaire, 2002).

Fluoxetine which is a chiral compound, is demethylated to norfluoxetine that is also a chiral compound and a potent SSRI, other metabolites that are of no clinical relevance are also formed (Hiemke & Härtter, 2000).

From the serotonin reuptake inhibition properties of the enantiomers, it appears that S-, R-fluoxetine and S-norfluoxetine have to be considered as SSRIs (Eap *et al.,* 2001).

Fluoxetine and norfluoxetine are known to be potent inhibitors of CYP2D6 (Lam *et al.,* 2002; Otton *et al.,* 1993), fluoxetine also inhibits CYP2C19, and norfluoxetine is a potent inhibitor of CYP3A4 (Hiemke & Härtter, 2000).

The evidence is conflicting regarding the involvement of the different CYPs in the formation of norfluoxetine.

In vivo clearance of fluoxetine co-segregates with the CYP2D6 polymorphic phenotype, suggesting that fluoxetine clearance is mediated by this isoform (Hamelin *et al.,* 1996).

Recently, it has been shown that the clearance of (R)- , (S)- fluoxetine and (S)-norfluoxetine depends strongly on CYP2D6 activity (Eap *et al.,* 2001). On the other hand, the pharmacokinetics of fluoxetine and norfluoxetine are not affected by paroxetine which is a potent inhibitor of CYP2D6 (Harvey & Preskorn, 1995).

From in vitro studies, it was suggested that CYP2C9 plays a major role in the N-demethylation of fluoxetine with a possible contribution of CYP2C19 and CYP3A4,

whereas the contribution of CYP2D6 was found to be negligible (Steeven & Wrigton, 1993; von Moltke *et al.,* 1997).

It was found that substantial concentrations of norfluoxetine is found in CYP2D6-deficcient individuals, this indicates that there is a significant amount of fluoxetine is metabolized by routes other than CYP2D6 (DeVane, 1999).

As fluoxetine is a potent CYP2D6 inhibitor, under conditions of multiple dosing, the role of CYP2D6 metabolism becomes less significant and the differences between poor and extensive metabolizers diminish (Bergstom *et al.,* 1992).

In general, the clinical course of patients overdosing with fluoxetine has been benign (Borys *et al.,* 1992). However, following fluoxetine overdoses as the sole ingestant, seizures have been reported and a fluoxetine fatality documented. The alleged fluoxetine related-death of a nine-year-old boy, a CYP2D6 poor metabolizer and the subsequent murder charge of his parents demonstrates that even supposedly safe substrates of CYP2D6 can cause detrimental outcomes, the levels of fluoxetine and norfluoxetine at blood, brain, and other tissues obtained at autopsy were several-fold higher than expected based on literature reports for overdose situations (Sallee *et al.,* 2000).

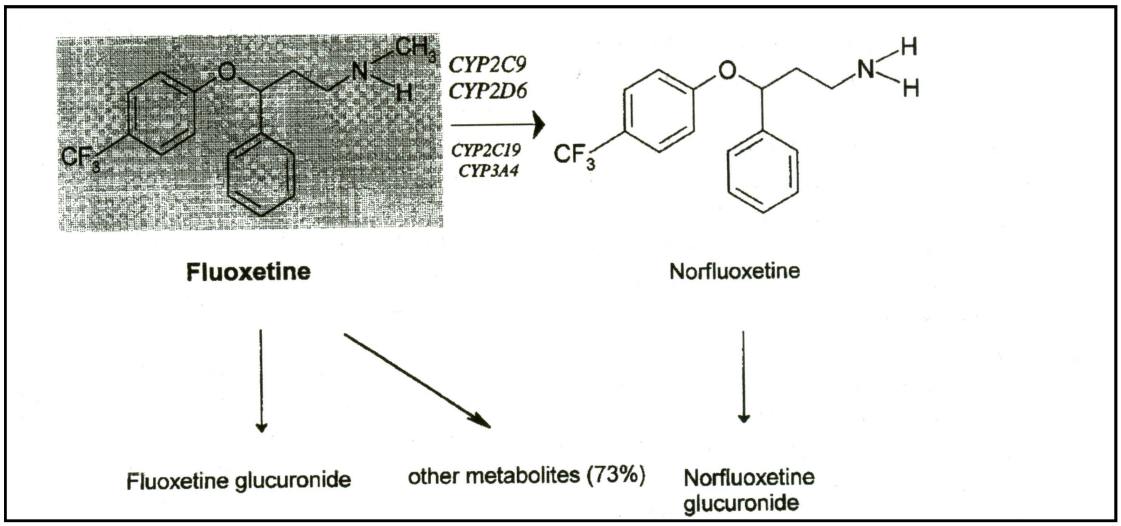

Figure 6: The metabolic pathways of fluoxetine (Hiemke & Härtter, 2000).

2.6.4.2.Fluvoxamine:

Fluvoxamine is the only achiral SSRI, more than 90% of it is absorbed. Due to extensive hepatic first-pass metabolism the oral bioavailability is only 50%.The plasma protein binding is low (77%), the volume of distribution is about 25 L/kg. The elimination half-life is ranges between 8 and 28 hours (mean 15 hours), steady state is reached within a week of treatment (Hemeryck & Belpaire, 2002).

Fluvoxamine's metabolism includes: oxidative demethylation, oxidative deamination, =N-O bond cleavage and N-acetylation (Perrucca *et al.,* 1994; Hemeryck & Belpair, 2002).

After ingestion of fluovoxamine, 11 metabolites have been found in urine, nine of them has been identified (Hiemke & Harter, 2000)

About 30-60% of the metabolites seems to be produced by oxidative demethylation of the methoxy group, whereas 20-40% seems to be produced by degradation at the amino group or by removal of the entire ethanolamino group (Overmars *et al.*,1983; Perucca *et al.,* 1994, figure 7 shows the metabolic routes of fluvoxamine.

The principal metabolites are most likely devoid of any significant pharmacological activity (Hemeryck & Belpair, 2002).

Fluvoxamine has been reported to be a substrate for CYP2D6 and CYP1A2 (Spigset *et al.*,2001), and it has been reported to have modest inhibitory action on CYP2D6 and potent inhibition of CYP1A2 (Brøsen, 1995), it is also an inhibitor of CYP2C19 and CYP2C9 (Jann *et al.,* 2002).

The major fluvoxamine metabolite was found to be produced by a process predominantly catalyzed by CYP2D6. In contrast, CYP1A2 might be more important for other metabolic pathways such as degradation at the amino / ethanolamino group (Perruca *et al.,* 1994).

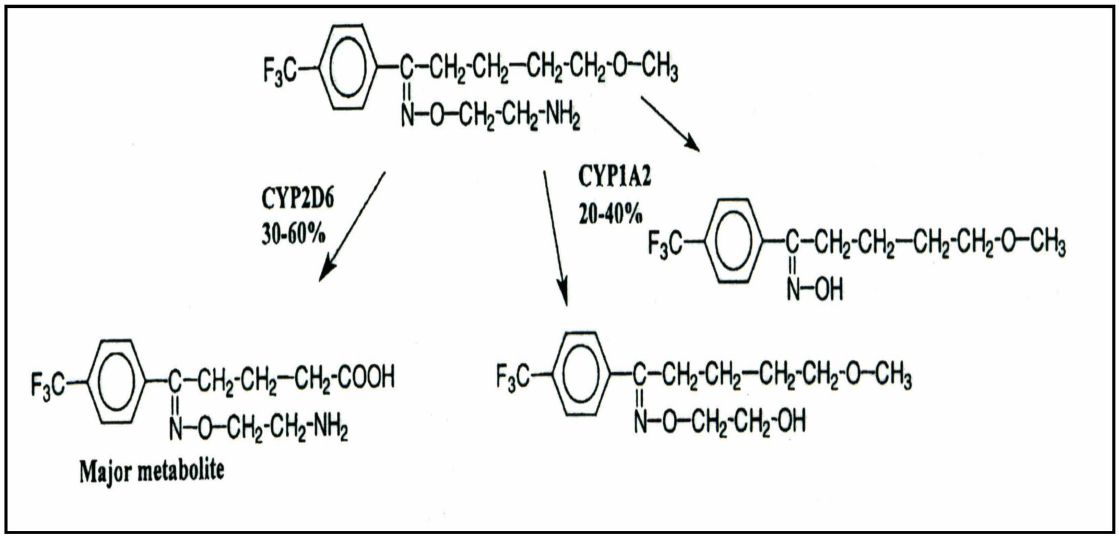

Figure 7: The metabolic pathways of fluvoxamine (Spigset *et al.,* 2001).

3. Study population, Materials and Methods:

3.1. The study population:

3.1.1. Psychiatric patients:

A group of Jordanian psychiatric patients (21 males, 23 females) who were treated with one of the following tricyclic antidepressants (Amitriptyline, Imipramine, Clomipramine) or selective serotonin reuptake inhibitor (Fluoxetine, Fluovoxamine) were included in the study.

The age of the patients ranged between 20 and 56 years, with a mean age of 37years.

All patients had been treated with one of the previously mentioned drugs for at least two months.

For inclusion in the study, patients had to meet the following criteria:

- Jordanian origin.
- No history of alcohol or other substance abuse.
- Not affected with kidney or liver diseases.

Patients were recruited from the clinics of the National Center of Psychiatric Health.

All subjects participating the study had given their written consent prior to inclusion.

3.1.2. The Jordanian population:

One hundred and ninety Jordanian, unrelated subjects were included in the study for the survey of the *CYP2D6*3* and *CYP2D6*6* alleles, and 100 from the 190 for the survey for the *CYP2D6*4* allele.

Volunteers were recruited from staff and students at University of Jordan, The Hashemite University and Applied Science University.

3.2 Materials and Methods:

3.2.1 DNA preparation:

EDTA blood samples were collected from each individual of the study population.

DNA was isolated from the whole blood using phenol / chloroform / proteinase K conventional extraction method (Davis *et al.*,1986).

Isolated DNA was dissolved in sterile distilled water, quantified by photometric measurement at 260 nm wavelength (using Pharmacia Biotech Gene Quant II, England) and diluted to concentration of 100 μg / ml.

3.2.2. Methodology:

3.2.2.1. Clinical assessment of the psychiatric patients:

All patients participating in the study had been considered to be healthy, as assessed by medical history in addition to liver and renal function tests.

The adverse drug reactions of the used antidepressant for each patient were assessed by a psychiatrist using the UKU (Udvalg for Kliniske Undersøgelser) side effect rating scale (Pech, 1995), (Appendix 1).

3.2.2.2. Data Analysis:

Patients were divided into two groups: those who had side effects and those who did not. Patients who were included in the group of having side effects were those who had two or more of the ADRs in the scale as moderate or severe (Chou *et al.*, 2000; Topić *et al.*, 2000).

The SPSS for Windows statistical package (release version 10) was used for the statistical analysis. The Fisher's exact test (two-tailed) and Student t-test were used to assess the differences between the groups. Significance was defined as $p< 0.05$.

3.2.2.3. Detection of the mutations in the study population:

The survey for *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*6* alleles was performed using nested polymerase chain reaction (PCR), where a preamplification fragment of DNA is produced in a first step, this fragment served as a template DNA in another two reactions, one for detecting the wild type and the another for detecting the mutant type.

All amplifications were run in PTC-100 Peltier Thermal Cycler (MJ Research, USA), and all PCR products were analyzed by electrophoresis in a 2% LE agarose gel (Promega, USA) in 1x Tris- Boric acid- EDT (TBE) buffer (Promega, USA).

The gels were stained with ethedium bromide (Sigma, USA),visualized under UV (254 nm),photographed with UPP-110 HD printing paper (Sony, Japan).

Blank sample generated by inclusion of all reagents except DNA, a positive quality control containing a known genotype (obtained by the courtesy of Dr. M. Hersberger, Institute of Clinical Chemistry, University Hospital Zurich, Switzerland and Dr. A. Daly, Department of Pharmacological Sciences, University of Newcastle, UK).

Negative quality control sample (which has a wild-type genotype for the mutation) and 100 bp DNA ladder (Promega, USA) were included in every run.

3.2.3. Primers:

Ten primers (Invitrogen, USA) were used to study the three mutations (Hersberger *et al.*, 2000, Heim and Meyer, 1990).

Table (3) shows the primers with their positions which were given according to the CYP2D6 gene sequence (GenBank Accession No. M33388)

The sequences are shown 5` to 3`.

Nucleotides that correspond to the target mutation are in bold.

Primer	5`position	Sequence	3`position
3-Forward	2098	GCGGAGCGAGAGACCGAGGA	2117
3-Reverse	3203	GGTCCGGCCCTGACACTCCTTCT	3181
*3-wt	2624	GCTAACTGAGCACA	2637
*3-mut	2624	GCTAACTGAGCACG	2638
1-Forward	1388	TCCCAGCTGGAATCCGGTGTCG	1409
2-Reverse	2137	GGAGCTCGCCCTGCAGAGACTCCT	2114
*4-wt	1947	CGAAAGGGGCGTCC	1934
*4-mut	1921	TCTCCCACCCCCAA	1934
*6-wt	1808	TCCTCGTCACCCA	1795
*6-mut	1782	GTCGCTGGAGCAGG	1795

3.2.4. Detection of the three mutations among the study population:

3.2.4.1. Nested PCR assay for detecting *CYP2D6*3* allele:

Three PCR reactions were performed for each DNA sample, the first reaction yielded a 1106 bp fragment using (3- forward) and (3- Reverse) primers, the amplicon produced was later used as a template DNA in another two parallel allele - specific reactions, one with a wild – type specific primer and the other with a mutation – specific primer, the second primer in both cases was the primer already used in the first PCR reaction (3- Reverse).

The PCR reaction was carried out in a final volume of 25 µL containing 2 µL (200 ng) template DNA, 2.5 µL 10x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.75 µL of 50 mM $MgCl_2$, 2.5 µL of deoxynucleotides triphosphates (dNTPs) mixture containing 2mM of each dNTP, 1 µL of 2 µM of the two primers (3-Forward) and (3-Reverse), 1.5 unit of Taq DNA polymerase and 15 µL of deionized double distilled water, (all the materials used in the PCR reaction were obtained from Invitrogen, USA).

The PCR thermal cycling program for this reaction was as follows:

First denaturation at 94°C for 10 minutes, followed by 29 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 63°C, 30 seconds extension at 72°C and a final extension at 72°C for 7 minutes.

One µL of the product of the previous PCR reaction was used as a template DNA in the other two reactions, each was carried out in a final volume of 25 µL containing 1 µL of 2 µM of each of the two primers (*3-wt) and (3-Reverse) in the wild type reaction and 1 µL of 2 µM of each of the two primers (*3-mut) and (3-Reverse) in the mutant reaction. The rest of the components of the reactions were the same as that in the first reaction.

The PCR thermal cycling conditions for the two reactions were as follows:

First denaturation at 94°C for 10 minutes, followed by 26 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 53°C, 30 seconds extension at 72°C and a final extension at 72°C for 7 minutes.

The size of the products produced for both the wild type and the mutant band was 580 bp.

3.2.4.2. Nested PCR assays for detecting *CYP2D6*4* and *CYP2D6*6* alleles:

As in the nested PCR assay for the detection of *CYP2D6*3*, three PCR reactions were performed for each sample.

The first one yielded a 750 bp fragment by carrying out a reaction of 25 µL final volume, containing 2 µL (200 ng) template DNA, 2.5 µL 10x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.75 µL of 50 mM $MgCl_2$, 2.5 µL of deoxynucleotides triphosphates (dNTPs) mixture containing 2mM of each dNTP, 1 µL of 2 µM of the two primers (1-Forward) and (2-Reverse), 1 unit of Taq DNA polymerase and 15 µL of deionized double distilled water, (all the materials used in the PCR reaction were obtained from Invitrogen, USA).

The PCR thermal cycling conditions were as follows:

First denaturation at 94°C for 10 minutes, followed by 29 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 61°C, 30 seconds extension at 72°C and a final extension at 72°C for 7 minutes.

The amplicon produced in this reaction was used as a template DNA in the detection of the *CYP2D6*4* and *CYP2D6*6* alleles.

One µL of the product of the previous PCR reaction was used as a template DNA in another two reactions (wild-type and mutant-type) for each of the two mutations.

Each was carried out in a final volume of 25 µL containing 1 µL of 2 µM of each of the two primers (1-Forward) and (*4-wt) in the wild type reaction of *CYP2D6*4* and

1 μL of 2 μM of each of the two primers (*4-mut) and (1-Forward) in the mutant reaction of *CYP2D6*4*, and 1 μL of 2 μM of each of the two primers (*6-wt) and (1-Forward) in the wild type reaction of *CYP2D6*6* and 1 μL of 2 μM of each of the two primers (*6-mut) and (1-Forward) in the mutant reaction of *CYP2D6*6* and 2.5 μL 10x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.75 μL of 50 mM $MgCl_2$, 2.5 μL of deoxynucleotides triphosphates (dNTPs) mixture containing 2mM of each dNTP, 1 unit of Taq DNA polymerase and 16 μL of deionized double distilled water. (All the materials used in the PCR reactions were obtained from Invitrogen, USA).

The cycling conditions were as follows:

First denaturation at 94°C for 10 minutes, followed by 29 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 57°C, 30 seconds extension at 72°C and a final extension at 72°C for 7 minutes.

The size of the amplicons produced for CYP2D6*4 (wild type and mutant type) is 560 bp.

The size of the amplicons produced for CYP2D6*6 (wild type and mutant type) is 421 bp.

4. Results:

4.1. Survey of *CYP2D6*3, *4* and *6 alleles in the population study:

A nested PCR assay was used to analyze each of the three mutations.

In the assay of *CYP2D6*3* allele, a preamplification step yielded a 1106 bp region of *CYP2D6* (figure 7), then the amplification of each of the wild-type and mutant allele of *CYP2D6*3* yielded a 580 bp PCR product (figure 8).

The *CYP2D6*4* allele was detected by a preamplification step that produced a 750 bp PCR product (figure 9), then the specific reactions for the wild type and mutant *CYP2D6*4* alleles yielded a 560 bp fragment (figure 10).

The 750 bp amplicon produced was also used in detecting the CYP2D6*6 allele, where each of the wilde-type and the *CYP2D6*6* produced an amplicon of 421 bp (figure 11).

If non of the three mutations were detected, homozygous *CYP2D6*1* wild type alleles were assumed.

4.1.1. Survey of *CYP2D6*3,*4* and *6* in the Jordanian population:

Analysis of the 190 genomic DNA samples of the Jordanian population for the two mutations (*CYP2D6*3* and *CYP2D6*6*) and 100 DNA samples for *CYP2D6*4* revealed the following:

- No *CYP2D6*3* alleles were found in the 190 DNA samples.
- Nineteen *CYP2D6*4* alleles were identified among the 100 DNA samples analyzed, three individuals were homozygous for *4* allele.
- Two *CYP2D6*6* alleles were found within the 190 DNA samples analyzed.

Table 4 shows the allelic frequency of the three mutations among the Jordanian population.

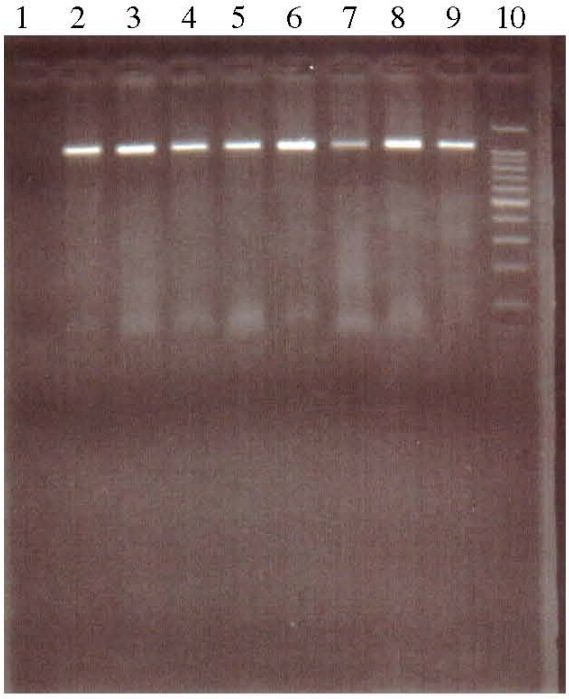

Figure 8: Analysis of the *CYP2D6*3* allele, a preamplification step of a 1106 bp of the *CYP2D6* gene. Lane 1 represents a blank reaction, lane 10 is a 100 bp DNA ladder.

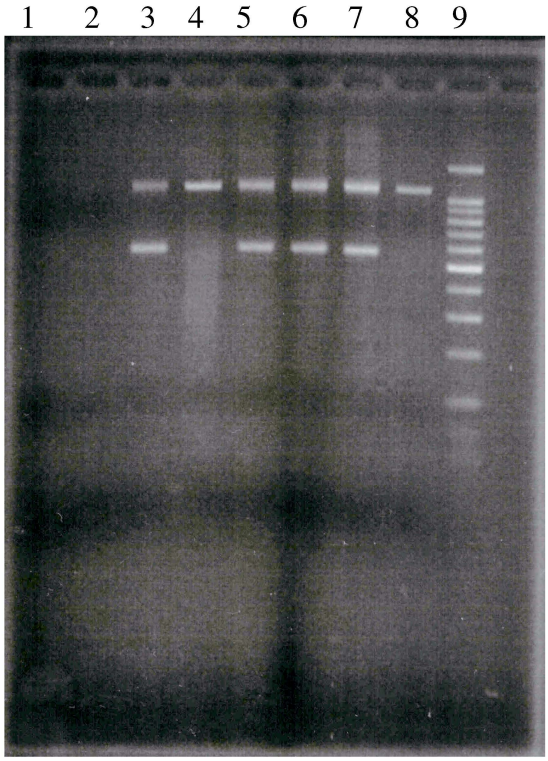

Figure 9: Analysis of *CYP2D6*3* allele.

Each DNA sample is represented by two reactions, one for the wild-type (odd lanes), and one for the mutant type (even lanes).

Lanes 1 and 2 represent blank reactions, lanes 3 and 4 represent a negative quality control (wt/wt), lanes 5 and 6 represent a positive quality control (wt/*3), lane 9 is a 100 bp DNA ladder.

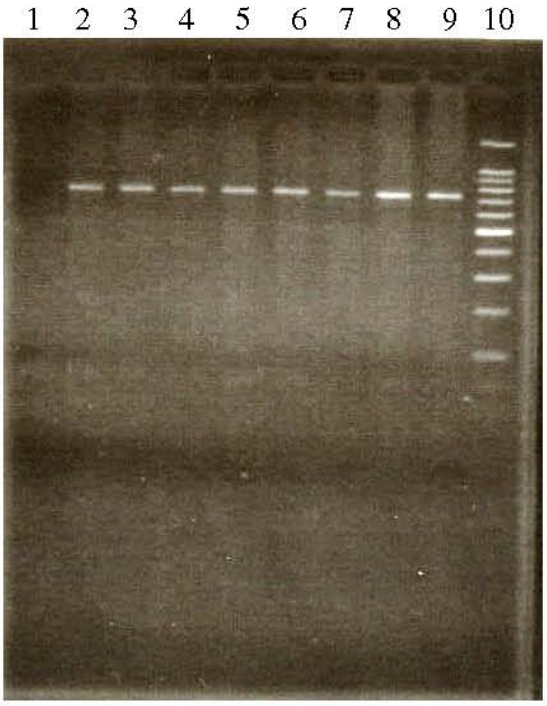

Figure 10: Analysis of the *CYP2D6*4* and**6* alleles, a preamplification step of a 750 bp of the *CYP2D6* gene.

The first lane represents a blank reaction, and the last lane represents a 100 bp DNA ladder.

Figure 11:Analysis of *CYP2D6*4* allele.

Each DNA sample is represented by two reactions, one for the wild-type (odd lanes), and one for the mutant type (even lanes).

Lanes 1 and 2 represent blank reactions, lanes 3 and 4 represent a negative quality control (wt/wt), lanes 5 and 6 represent a positive quality control (wt/*4), lane 9 is a 100 bp DNA ladder.

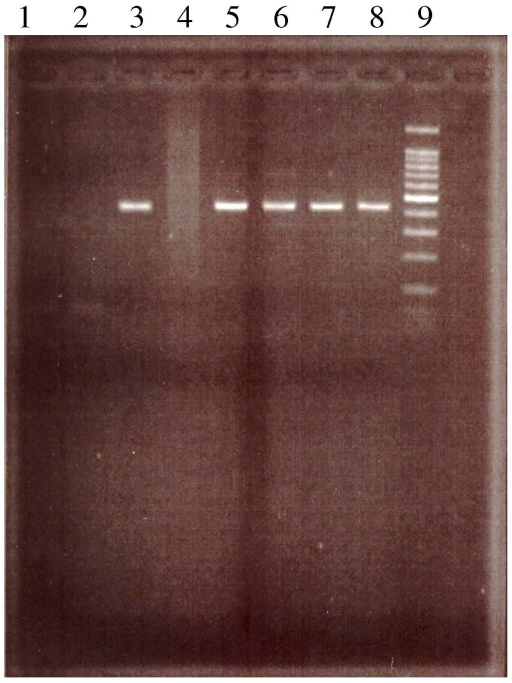

Figure 12: Analysis of *CYP2D6*6* allele.

Each DNA sample is represented by two reactions, one for the wild-type (odd lanes), and one for the mutant type (even lanes).

Lanes 1 and 2 represent blank reactions, lanes 3 and 4 represent a negative quality control (wt/wt), lanes 5 and 6 represent a positive quality control (wt/*6), lane 9 is a 100 bp DNA ladder.

Table 4: *CYP2D6*3, *4* and **6* allele frequencies in the Jordanian population:

Allele	Number of alleles	Allele frequency,%
**3*	0/380	0
**4*	19/200	9.5
**6*	2/380	0.53

4.1.2. Survey of *CYP2D6*3,*4* and **6* in the psychiatric patients:

Analyzing the 44 genomic DNA samples of the patients for the three mutations yielded, as shown in table 5:

- No *CYP2D6*3* alleles were found.

- No *CYP2D6*6* alleles were found.

- Fifteen *CYP2D6*4* alleles were identified among the 44 DNA samples, including 3 individuals homozygous for *CYP2D6*4*, comprising a percentage of 6.8 of the study population.

The frequency of the PMs (6.8%) among the patients group was more than that of the general population (3%), but this difference did not reach statistical significance (p= 0.062).

Table 5: *CYP2D6*3, *4* and **6* allele frequency in the psychiatric patients:

Allele	Number of alleles (n = 88)	Allele frequency,%
**3*	0/88	0
**4*	15/88	17
**6*	0/88	0

4.2. Assessment of the ADRs for the psychiatric patients:

The ADRs experienced by the patients were assessed by a psychiatrist using the UKU side effect rating scale.

Each patient was categorized as either having side effects (if the score of two or more of the ADRs in the scale was moderate or severe) or not having side effects.

Of the 44 patients, 21 were having side effects.

Eight of the 21 patients who had side effects had a defective allele: five intermediate metabolizers (wt/*4), and three poor metabolizers (*4/*4) who were using either clomipramine or fluoxetine, the rest were genotyped as extensive metabolizers (wt/wt).

Four IMs of the patients did not have side effects, and 13 of the patients who were genotyped as homozygous for the wild-type allele had side effects.

Comparing the group of patients who had no defective allele with those who did ((wt/*4) and (*4/*4)), revealed no significant difference between the two groups ($p = 0.135$).

The difference between patients who were genotyped as EMs and those who were PMs was highly significant ($p= 0.001$).

There was no significant difference between the patients who were genotyped as EMs and those who were IMs ($p= 0.438$).

The preliminary data suggest that patients who had two non-functional *CYP2D6* alleles and treated with antidepressants that are metabolized by CYP2D6 suffer from ADRs.

Unfortunately, the population size examined was too small to confirm the significance in the differences observed between patients who are homozygous for the wild-type allele and those who are heterozygous for the defected *CYP2D6* allele.

5. Discussion:

The cytochrome P450 2D6 is involved in the oxidative metabolism of numerous commonly prescribed psychoactive drugs, including many antidepressants and antipsychotics (Scordo & Spina, 20002; Furlanut, 1993; Caccia; 1998).

This enzyme is genetically polymorphic. Several mutant alleles are known, associated with enzyme activities ranging from ultrafast to a complete absence. Therefore, metabolic capacity varies from one person to another, leading to intersubject differences in the final plasma drug concentrations (Kalow, 2001,a).

This polymorphism is one of multiple factors that affect the response to a drug.

In addition to the genetic polymorphism, pharmacokinetic differences may be caused by drug interactions, in addition to factors like age, gender, renal or hepatic diseases (Testai *et al.,* 2001, Murray, 1992).

Besides the polymorphisms in the genes encoding drug metabolizing enzymes, genetic variations in receptors and transporters can produce variations in drug response (Arranz *et al.,* 2001; Poolsup *et al.,* 2000).

All or many of these and additional factors may work together to cause the ever present person to person differences in drug response.

Several studies have found an increased rate of adverse effects in poor metabolizers treated with substrates of CYP2D6 (Topić *et al.,* 2000; Chen *et al.,* 1996; de Leon *et al.,*1998).

Currently, the most popular method for identifying a patient's metabolic status is by genotyping using PCR analysis (Weide & Steijns, 1999, Linder *et al.,* 1997).

The objective of this preliminary study was to investigate whether psychiatric patients had different frequency of defective *CYP2D6* alleles compared to a control group of Jordanian individuals, and whether psychiatric patients with defective *CYP2D6* alleles

were more likely to develop side effects when treated with antidepressants metabolized by CYP2D6.

In the genotyping analysis used in this study, a nested PCR assays were used. Determining the mutations by a single round allele-specific PCR is not possible as the *CYP2D6* gene sequence is highly homologous to the neighboring *CYP2D7* pseudogene. As some of the mutations in the *CYP2D6* gene are also contained in the *CYP2D7*(Meyer & Zanger, 1997), a single allele-specific PCR cannot be designed without the possibility of *CYP2D7* interfering with the interpretation. So, a larger PCR fragment is amplified initially using primers complementary to the *CYP2D6* unique sequences on either side of the mutations studied.

Of the three mutant alleles investigated in this study, only *CYP2D6*4* and *CYP2D6*6* alleles were found in the Jordanian population, while the *CYP2D6*3* allele was not found.

The frequency of *CYP2D6*6* allele, which is a null allele contributing to the poor metabolism phenotype when occurs in a homozygous form, was found to be 0.53% in the Jordanian population, this result is lower than that reported in other Caucasian populations, where the frequency of this allele was reported to be around 1% (Gaedigk, 2000, Bradford, 2002).

The frequency of *CYP2D6*3* in this study was 0, this result is lower than that observed in some of the Caucasian populations, where the frequency of this allele was found to be 1-2%, but some Caucasian populations reported a less frequency of this allele, for example the frequency of this allele in Turkish population was 0 (Bradford, 2002).

The allele frequency of *CYP2D6*4* was the highest in this study, it was found to be 9.5%. This result is lower than that obtained by Hadidi and coworkers (1994), where genotyping of this allele for 91 individuals, revealed a frequency of 20%, and 8.8% of

the 91 subjects analyzed for this allele alone were found to be poor metabolizers, in this study the poor metabolizers were found to be 3% (homozygous for the *CYP2D6*4* allele).

The percentage of poor metabolizers obtained in this study (by genotyping analysis for the three alleles) was lower than that reported by Hadidi and coworkers (1994,a) where it was found to be 8.8% by genotyping of 91 subjects for the *CYP2D6*4* allele alone, 7% by phenotyping using debrisoquine as a probe drug for 191 individuals, other studies by Irshaid and associates (1993;1996) reported the frequency of poor metabolizers using dextromethorphan as a probe drug in 241 Jordanian subjects to be 2.9%, and 6.8% in 266 subjects using the same probe drug. Another study by Hadidi and associates (1994,b) using metoprolol as a probe drug in 65 Jordanian individuals yielded a percentage of 1.5 of poor metabolizers.

The differences between the percentage of poor metabolizers obtained by genotyping and phenotyping is possible, it was reported that 100% accurate prediction of phenotype by genotyping may not be possible, especially when not all the allelic variants are investigated (Chen *et al.,* 1996, Rodrigues & Rushmore, 2002).

In the patients population, the *CYP2D6*4* allele was the only allele found in a frequency of 17%, which was higher than that found in the normal population, and the frequency of poor metabolizers was 6.8% which is also higher than that in the normal population, but this difference did not reach statistical significance.

It has been reported that higher frequency of poor metabolizers is observed in the psychiatric patients (Topić *et al.,* 2000; Wolf & Smith, 1999).

Data on the association of non-functional *CYP2D6* alleles with psychiatric diseases, are controversial, it has been suggested that CYP2D6 is involved in the metabolism of tryptamine which is suggested to play a role in the determining of personality and

normal character. Some studies suggested that CYP2D6 poor metabolizers are more anxiety prone and have less well developed social skills than extensive metabolizers .

The second objective of this study was to investigate whether psychiatric patients with defective *CYP2D6* alleles are more likely to develop side effects when treated with antidepressants that are metabolized by CYP2D6 (Am *et al.,* 2003, Miksys *et al.,* 2002, Wolf & Smith, 1999).

The patients were divided in three groups; homozygous for the wild-type allele, heterozygous for *CYP2D6*4* allele, and homozygous for the *CYP2D6*4* allele, and the presence of the side effects in these groups was compared. Although every poor metabolizer in this study did have side effects, the differences between the three groups did not reach statistical significance, where 4 IM and 13 EM developed side effects.

This could be to various factors:

- The small number of patients, which makes it hard to derive any solid conclusions, consequently, a larger study population may provide a better opportunity to detect differences between the groups.
- It is also possible that subtle differences between EM and IM groups are overwhelmed by the many factors that contribute to the final clinical outcome.
- If genotyping is used as method replacing phenotyping in order to determine metabolic capacity, all relevant mutations must be known and tested for. In this study, only three allelic variants are investigated, the presence of the *CYP2D6*5* allele which is the second most common mutation, was not screened for.

In the PCR assay used in this study, individuals who are heterozygous for this allele or any other defected allele that is not investigated, will be genotyped as homozygous for the wild type allele, for example a person who has a wt/*5 genotype will be genotyped

as wt/wt, so changing him from an intermediate metabolizer to an extensive metabolizer.

So, it is possible that patients who were genotyped as normal, are actually heterozygous for the *CYP2D6*5* allele, or for other defective alleles that are not investigated in this study. The presence of duplicated or multiduplicated alleles was also not detected in the present study, which may affect the net result of the occurrence of side effects according to the genotype.

Although reliable procedures have been reported and recommendations for routine testing have been made, there is no clear consensus as to how many allele-specific assays must be performed to provide a high degree of statistical probability that the correct phenotype will be assigned from genotyping data (Rodrigues & Rushmore, 2002). In addition, the predictive power of the genotype for the in-vivo catalytic function is limited, it only subdivides the population to the four phenotypes.

It was suggested that even if the CYP2D6 is lower in IM patients, this may not have any clinical relevance (Meyer *et al.,* 1996), and that there may be overlap in the CYP2D6 activity between IM and EM groups, so the existence of the IM group demonstrates the limitations of genotyping in studies like this study, which link the PM genotype to drug-concentration related side effects by assuming a clear cut difference between the EM and PM groups.

In addition, it was shown that within the same group of EM, the metabolic capacity differs in hundreds folds (Dahl *et al.,*1992; Shulman & Özdemir, 1997).

- Another factor contributing to the insignificance of differences between the groups, is the involvement of other CYP enzymes which possibly compensating for the lack of CYP2D6 activity in the intermediate netabolizers. For example CYP2C19 and CYP3A4 are also involved in the metabolism of these drugs and also show polymorphisms in

their activities among the population, so, for example, an EM for CYP2D6 may be a PM for CYP2C19, and it was found that the percentage of CYP2C19 poor metabolizers in the Jordanian population is 4.9 (Hadidi *et al.,* 1995), or the patient may have an induced activity of CYP1A2 due to environmental factors as smoking.

- Drug metabolism is not the only requisite variable, in addition to the polymorphisms in drug-metabolizing enzymes, genetic variations in receptors and transporters can produce variations in drug response, although these variables have been characterized less extensively than drug metabolism, they are emerging as novel and important predictors of safety and efficacy of drug therapy (Poolsup *et al.,* 2000; Arranz *et al.,* 2001).

6. Conclusion:

At present, pharmacogenetic testing is restricted to a limited number of patients or volunteers in academic institutions and clinical drug trials, although the evidence is accumulating that prospective testing could be of major benefit to many patients.

Obviously, prospective trials are needed to prove the value of genotyping in patients with depression in selecting the proper starting dose to increase therapeutic efficacy and prevent toxicity.

In conclusion, additional resources are needed for an extension of this study to:

- Evaluate more of the *CYP2D6* alleles in order to predict more precisely the metabolic capacity.
- Expanding the number of patients recruited in such studies.
- Genotyping another enzymes of the P450 family that are highly associated with the metabolism of the antidepressants and other antipsychotic drugs.
- Following the cost of treating the patients for the ADRs and compare it with the cost of genotyping test.

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Appendices:

Appendix (1): UKU Side Effect Rating Scale:

Each item is defined by a four-point-scale (0-3). In general, degree (0) means not or doubtfully present, 1,2 and 3 indicate that a symptom is present to a mild, moderate or severe degree, respectively.

1. Concentration difficulties:

Difficulties in ability to concentrate, to collect one's thoughts, or to sustain one's attention.

0. No or doubtful difficulties in concentrating.
1. The patient must try harder than usual to collect his thoughts, but not to the degree that it hampers him in his everyday life.
2. The difficulties in concentrating are pronounced enough to hamper the patient in his everyday life.
3. The patient's difficulties in concentrating are obvious to the interviewer during the interview.

2. Sedation:

Diminished ability to stay awake during the day. The assessment is based on clinical signs during the interview.

0. No or doubtful sleepiness.
1. Slightly sleepy/ drowsy as regards facial expression and speech.
2. More markedly sleepy/ drowsy. The patient yawns and tends to fall asleep when there is a pause in the conversation.
3. Difficult to keep the patient awake and to wake the patient, respectively.

3. Failing memory:

Impaired memory, assessment should be independent of any concentration difficulties.

0. No or doubtful disturbances in memory.
1. Slight, subjective feeling of reduced memory compared with the patient's usual condition; however, not interfering with functioning.
2. The failing memory hampers the patient or slight signs of this are observed during the interview.
3. The patient shows clear signs of failing memory during the interview.

4. Tension/Inner unrest:

Inability to relax, nervous restlessness. This item is to be assessed on the basis of the patient's experience and must be distinguished from motor akathisia.

0. No or doubtful tension/ nervous restlessness.
1. The patient states that he is slightly tense and restless; however, this does not interfere with his functioning.
2. Considerable tension and inner unrest; however, without this being so intense or constant that the patient's daily life is influenced to any marked degree.
3. The patient feels tension or restlessness that is so marked that his daily life is clearly effected.

5. Increased duration of sleep:

This should be assessed on the basis of the average of sleep over the preceding nights. The assessment is to be made in relation to the patient's usual pre-illness state.

0. No or doubtful increase of the duration of sleep.
1. Sleeps up to 2 h longer than usual.
2. Sleeps 2-3 h longer than usual.
3. Sleeps more than 3 h longer than usual.

6. Reduced duration of sleep:

This should be assessed on the basis of the average of sleep over the preceding 3 nights. The assessment is to be made in relation to the patient's usual pre-illness state.

0. No or doubtful reduction of the duration of sleep.
1. Sleeps up to 2 h less than usual.
2. Sleeps 2-3 h less than usual.
3. Sleeps more than 3 h less than usual.

7. Increase dream activity:

This should be assessed independently of dream content and based on the average of sleep over the preceding 3 nights in relation to the usual pre-illness dream activity.

0. No or doubtful change in the dream activity.
1. Slightly increased dream activity but does not disturb the night's sleep.
2. More pronounced increase in dream activity.
3. Very pronounced increase in dream activity.

8. Emotional Indifference:

A diminution of the patient's empathy, leading to apathy.

0. No or doubtful emotional indifference.
1. Slight subduing of the patient's empathy.
2. Obvious emotional indifference.
3. Pronounced indifference so that the patient behaves apathetically in relation to his surroundings.

9.Confusion:

This deals with the degree of clouded ness of consciousness. This may be accompanied by loss of orientation, including time, place and personal data in different degrees and may be accompanied by psychotic symptoms.

0. The patient is fully orientated in time and place and personal data. The patient shows clearness of consciousness, answers relevantly and is fully orientated in time and place and personal data.
1. The patient may occasionally lack clearness of consciousness and full orientation, he may occasionally answer irrelevantly, but he is capable of correcting errors.
2. The patient frequently shows clouded consciousness, answers irrelevantly and shows signs of lack of orientation, he may for example correctly state month or year, but not the day of the week, or he may show problems naming the place, even if he without help can find his way around the ward.
3. The patient answers in a noticeably irrelevant manner. He is not orientated, neither in time, place, nor in personal data.

10.Tremor:

This item comprises all forms of tremor.

0. No or doubtful tremor.
1. Very slight tremor that does not hamper the patient.
2. Clear tremor hampering the patient, the amplitude of finger tremor being less than 3 cm.
3. Clear tremor with an amplitude of more than 3 cm and which cannot be controlled by the patient.

11. Accommodation disturbances:

Difficulty in seeing clearly or distinctly at close quarters (with or without glasses), although the patient sees clearly at a long distance. If the patient uses bifocal glasses, the condition must be assessed on the basis of the use of the distance glasses.

0. No difficulty in reading any ordinary newspaper text.
1. Newspaper text can be read, but the patient's eyes tire rapidly or he must hold the paper further away.
2. The patient cannot read an ordinary newspaper text, but still manages to read texts printed in larger types.
3. The patient can read large type, such as headline, only with aid, such as a magnifying glass.

12. Increase salivation:

Increased, non-stimulated salivation:

0. No or doubtful increase of salivation.
1. Salivation clearly increased but not bothersome.
2. Disturbing increase of salivation, need for spitting or frequent swallowing of saliva, only exceptional dribbling.
3. Frequent or constant dribbling, perhaps concomitant speech disturbances.

13. Reduced salivation (Dryness of mouth):

Dryness of mouth because of diminished salivation. This may result in increased consumption of liquids, but it must be distinguished from thirst.

0. No or doubtful dryness of mouth.
1. Slight dryness of mouth, not disturbing the patient.
2. Moderate and slightly disturbing dryness of mouth.
3. Marked dryness of mouth which clearly disturbs the patient's daily life.

14. Nausea/ Vomiting:

To be recorded on the basis of the past 3 days.

0. No or doubtful nausea.
1. Slight nausea.

2. Disturbing nausea, but without vomiting.

3. Nausea with vomiting.

15. Diarrhea:

Increase frequency and / or thinner consistency of feces.

0. No or doubtful diarrhea.

1. Clearly present, but does not disturb work or other performance.

2. Disturbing, with need for several daily, inconvenient stools.

3. Marked, imperative need for defecation, threatening or actual incontinence, results in frequent interruptions of work.

16. Constipation:

Reduced frequency of defecation and / or thicker consistency of feces.

0. No or doubtful constipation.

1. Slight constipation, but bearable.

2. More marked constipation which hamper the patient.

3. Very pronounced constipation.

17. Micturition disturbances:

Feeling of difficulty in starting and of resistance to micturition, weaker stream or increased time of micturition. Should be assessed on the basis of the past 3 days.

0. No or doubtful micturition disturbances.

1. Clearly present but bearable.

2. Poor stream, considerably increased time of micturition, feeling of incomplete emptying bladder.

3. Retention of urine with high volume residual urine and/ or threatened or actual acute retention.

18. Polyuria/ Polydipsia:

Increased urine production resulting in increased frequency of micturtion and discharge of an abundant quantity of urine at each micturition, secondarily increased consumption of fluid.

0. No or doubtful.
1. Clearly present, but not hampering, nocturia at most once a night (in young people).
2. Moderately hampering because of frequent thirst, nocturia two or three times a night, or micturition more frequent than every 2h.
3. Very hampering, almost constant thirst, nocturia at least four times a night, or micturition at least every hour.

19. Orthostatic dizziness:

Feeling of weakness, everything going black, buzzing in the ears, increasing tendency of faint when changing from supine or sitting position.

0. No or doubtful.
1. Clearly present, but requires no special countermeasures.
2. Hampering, but can be neutralized by slow and/ or stagewise change to upright position.
3. Threatening fainting or real episodes of fainting despite careful change of position, with a tendency to this type of dizziness as long as the patient is in an upright position.

20. Palpitations/ Tachycardia:

Palpitation, feeling of rapid, strong and/ or irregular heartbeats.

0. No or doubtful.
1. clearly present, but not hampering, only short occasional attacks or more constant, but not marked palpitation.

2. Hampering frequent or constant palpitation that worries the patient or disturbs his night's sleep, however, without concomitant symptoms.
3. Suspicion of real tachycardia, for instance because of concomitant feeling of weakness and need to lie down, dyspnea, tendency to fainting, or precordial pain.

21. Increased tendency of sweating:

Localized to the whole body, not only palms and soles of the foot.

0. No or doubtful.
1. Clearly present, but mild, for example a profuse outburst of sweat only after considerable effort.
2. Hampering, requires frequent change of clothes, profuse sweating after moderate activity, for instance walking upstairs.
3. Profuse outbursts of sweat after slight activity or when resting, the patient is constantly wet, must change clothes several times a day and must also change night clothes or bedclothes.

22. Weight gain:

Rating is to be made on the basis of the preceding month.

0. No or doubtful weight gain during the preceding month.
1. Weight gain of 1-2 kg during the preceding month.
2. Weight gain of 3-4 kg during the preceding month.
3. Weight gain of more than 4 kg during the preceding month.

23. Weight loss:

0. No or doubtful weight loss.
1. Weight loss of 1-2 kg during the preceding month.
2. Weight loss of 3-4 kg during the preceding month.
3. Weight loss of more than 4 kg during the preceding month.

24. Diminished sexual desire:

Reduced desire of sexual activity.

0. No or doubtful.
1. The desire of sexual activity is slightly diminished, but without hampering the patient.
2. A distinct reduction of the patient's desire and interest in sexual activities so that it becomes a problem for the patient.
3. Desire and interest have diminished to such an extent that sexual intercourse occurs extremely seldom or has stopped.

25. Orgasmic dysfunction:

0. No or doubtful.
1. It is more difficult for the patient than usual to obtain orgasm or the experience of orgasm is slightly influenced.
2. The patient states that there is a clear change in the ability to obtain orgasm. This change has reached a degree that troubles the patient.
3. When the patient rarely or never obtain orgasm or the experience of orgasm is markedly reduced.

26. Headache:

On the scoring sheet, headache is classified as: (a) tension headache (b) migraine (c) or other forms of headache.

0. No or doubtful headache.
1. Slight headache.
2. Moderate, hampering headache which does not interfere with the patient's daily life.
3. Pronounced headache interfering with the patient's daily life.

Appendix 2:

Descriptions of the patients recruited in the study:

Patient number	Drug	Dose (mg/day)	Age	Sex	Genotyepe (*CYP2D6*4*)
1	Clomipramine	75	37	Female	wt/wt
2	Clomipramine	50	27	Male	wt/wt
3	Clomipramine	175	42	Male	wt/wt
4	Clomipramine	150	37	Male	wt/wt
5	Clomipramine	150	47	Male	wt/wt
6	Clomipramine	75	21	Female	wt/wt
7	Clomipramine	225	29	Female	wt/wt
8	Clomipramine	25	42	Female	wt/wt
9	Clomipramine	200	30	Male	wt/wt
10	Clomipramine	75	44	Female	wt/*4
11	Clomipramine	100	43	Female	*4/*4
12	Clomipramine	150	45	Female	wt/wt
13	Clomipramine	150	34	Male	wt/wt
14	Clomipramine	25	52	Male	wt/wt
15	Clomipramine	225	43	Male	wt/wt
16	Clomipramine	75	42	Female	wt/wt
17	Clomipramine	75	39	Female	wt/wt
18	Amitriptyline	125	29	Male	wt/wt
19	Amitriptyline	150	39	Female	wt/wt
20	Amitriptyline	150	41	Female	wt/wt
21	Amitriptyline	100	39	Male	wt/*4
22	Amitriptyline	25	33	Female	wt/wt
23	Amitriptyline	150	45	Female	wt/wt
24	Imipramine	75	30	Female	wt/wt
25	Imipramine	100	33	Male	wt/wt
26	Imipramine	75	36	Female	wt/*4
27	Imipramine	75	50	Female	wt/wt
28	Imipramine	75	45	Male	wt/*4
29	Fluoxetine	40	56	Female	*4/*4
30	Fluoxetine	40	34	Male	wt/wt
31	Fluoxetine	60	38	Male	wt/wt
32	Fluoxetine	40	24	Male	wt/wt
33	Fluoxetine	40	41	Female	wt/wt
34	Fluoxetine	40	35	Male	*4/*4
35	Fluoxetine	40	20	Female	wt/*4
36	Fluoxetine	40	24	Male	wt/wt
37	Fluoxetine	20	22	Male	wt/*4
38	Fluoxetine	40	35	Female	wt/*4
39	Fluvoxamine	100	25	Female	wt/wt
40	Fluvoxamine	100	42	Male	wt/wt
41	Fluvoxamine	200	37	Male	wt/*4
42	Fluvoxamine	100	49	Female	wt/wt
43	Fluvoxamine	150	46	Female	wt/wt
44	Fluvoxamine	200	29	Female	wt/wt

العلاقة بين احتمالية ظهور الأعراض الضارة لمضادات الاكتئاب والتنوع الشكلي ل *CYP2D6* لدى المرضى النفسيين

إعداد

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المشرف

الأستاذ الدكتور محمد الخطيب

يعتبر الأنزيم (CYP2D6) أحد الأنزيمات المهمة في عملية أيض أدوية مضادات الاكتئاب،ويتميز هذا الأنزيم بالتغير الشكلي الناتج عن طفرات في الجين المسؤول عن هذا الأنزيم،مما يؤدي إلى فروقات كبيرة بين الأشخاص في فاعلية هذا الأنزيم والتي تتراوح من قدرة فائقة على الأيض إلى قدرة معدومة.من الطفرات العديدة التي تؤدي إلى انعدام فاعلية هذا الأنزيم هي: (*CYP2D6*3, *4,*6*)،حيث يكون وجود إحدى هذه الطفرات في كلا الجينين عاملا مهما في العرضة للإصابة بالأعراض الضارة للأدوية التي يقوم هذا الأنزيم بأيضها.

في هذا البحث تم دراسة التكرار الجيني للطفرات الثلاث في مجموعة من المرضى النفسيين وعددهم 44 مريض، حيث وجد أن التكرار الجيني للطفرتين (*CYP2D6*3,*6*)هو

صفر وللطفرة*CYP2D6*4* هو 17%، ونسبة الأشخاص الذين لديهم قدرة هذا الأنزيم معدومة هي 6.8%. كما تم تحديد نسبة وجود هذه الطفرات في عدد من الأشخاص الأردنيين (لا تربطهم أي صلة قرابة)، ووجد أن التكرار الجيني للطفرة (*CYP2D6*3*) هو صفر، وللطفرة (*CYP2D6*6*) 0.53%،وللطفرة (*CYP2D6*4*) 9.5%، ونسبة الأشخاص الذين لديهم قدرة الأنزيم معدومة 3%.

ثم تم دراسة العلاقة بين وجود هذه الطفرات وظهور الأعراض الضارة لمضادات الاكتئاب عند المرضى الذين ليس لديهم الطفرات والذين لديهم إحداها،حيث وجد أن كل مريض لديه إحدى هذه الطفرات في كلا المورثين ظهرت لديه الأعراض الضارة للدواء المستخدم، إلا أن هذا الاختلاف لم يصل بالنتائج إلى فروقات ذات دلالة إحصائية.

هذا البحث يحتاج إلى توسع اكثر في دراسة الطفرات المؤدية الى انعدام فاعلية هذا الأنزيم في عدد اكبر من المرضى حتى يظهر تأثير هذه الطفرات بشكل أوضح.