G-3858-A Genetic Polymorphism in the 5'-Flanking Region of the *CYP1A2* Gene in a Randomly Selected Population

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Abstract

As a member of the cytochrome P450 superfamily, CYP1A2 plays an important role in the activation of carcinogens and in the metabolism of many drugs, including selective serotonergic reuptake inhibitors, a class of antidepressants. A mutation in CYP1A2, -3858G A (allele *CYP1A2*C*), affects the inducibility of this enzyme. The aim of this study was to determine the allelic frequency of -3858G A in Caucasians and compare it with the allelic frequency in other ethnic groups. To improve drug effectiveness or reduce toxicity, increasing importance is attributed to genotyping of drug metabolizing enzymes. For the -3858G A mutation, the allele frequency was not measurable in Caucasians, compared to the ~25% reported in Asians.

Introduction

Depression, or major depressive disorder, is defined as a despairing mood and the loss of interest or pleasure in nearly all activities previously considered pleasurable. The individual must also experience at least four other symptoms, including changes in appetite or weight, sleep, and psychomotor activity; feelings of worthlessness or guilt; difficulty thinking, concentrating, or making decisions; or recurrent thoughts of death or suicide. These symptoms must persist for most of the day, nearly every day, for at least two consecutive weeks. The depressive episode must be accompanied by distress or impairment in social, occupational, or other important areas of functioning[1]. There are also many physical symptoms associated with depression, such as tiredness and fatigue, sleep disturbances, headaches, psychomotor changes, gastrointestinal disturbances, appetite changes, and body aches and pains[2].

Up until recently, the prevailing hypothesis of depression has been that a deficit in monoamine neurotransmitters, primarily norepinephrine and serotonin (5-HT), underlies depression. Although current theories recognize that depleted monoamine neurotransmitters may be involved in the pathogenesis of depression, they also acknowledge that other factors may be involved. Although existing antidepressant medications increase levels of monoamines in the brain, there is a delay between measured increases in monoamines and corrections in mood. The monoamines, serotonin and norepinephrine, also influence and are influenced by other processes in the brain. Hence, the neurochemical basis of depression now is regarded as being more complex and not the result of any one specific deficit[3].

There are a variety of treatments available for depression, including tricyclic antidepressants (TCAs), selective serotonergic reuptake inhibitors (SSRIs), and

monamine oxidase inhibitors (MAOIs). Current antidepressants act by affecting three distinct processes: (1) neurotransmitter degradation, (2) neurotransmitter reuptake (reabsorption into the releasing cell), and (3) neurotransmitter binding.

Inhibiting neurotransmitter degradation or neurotransmitter reuptake results in an increase in neurotransmitters in the synapse. This increase in monoamine neurotransmitter concentrations results in increased binding at postsynaptic receptors. Monoamine oxidase is an enzyme that degrades monoamine neurotransmitters after their reabsorption into the presynaptic neuron. Monoamine oxidase inhibitors inhibit this enzyme, resulting in an elevation in the concentration of neurotransmitters in the synaptic cleft[4].

After release into the synapse, neurotransmitters are taken back into the axon in a process called reuptake that prevents over-stimulation of postsynaptic receptors. Neurotransmission can be enhanced acutely by blocking this reuptake with a reuptake inhibitor drug that binds to and blocks the reuptake protein. In contrast with MAOIs, tricyclic antidepressants inhibit the reuptake of norepinephrine and serotonin neurotransmitters, thus increasing levels of these neurotransmitters in the brain. Unfortunately, TCAs are not very selective and interact with several other types of receptors inside and outside the brain.

The ultimate effect of most antidepressants available today is to increase neurotransmitter binding at postsynaptic receptors by increasing levels of available neurotransmitters. Unfortunately, antidepressants also may bind directly to postsynaptic receptors, including histaminergic H1, muscarinic, H1-adrenergic, and dopaminergic D2 receptors, resulting in undesirable side effects. In general, antidepressants that bind to

these receptors do so with greater affinity for these postsynaptic receptors than neurotransmitters for these receptors[5].

The selective serotonin reuptake inhibitors have become an important component in the treatment of depression. Owing to their efficacy, good tolerability and relative safety, the SSRIs have become the most frequently prescribed antidepressant drugs. The SSRIs inhibit the reuptake of neurotransmitters by the presynaptic cell. However, their activity is specific to the serotonin reuptake transporter protein, resulting in more available serotonin in the synapse. Hence, their specificity results in fewer side effects than tricyclics and MAOIs, as well as reduced toxicity.

The efficacy of antidepressant drugs is partly under genetic control. Genetic factors contribute to the phenotype of drug response. The gene encoding the neuronal serotonin transporter protein (5-HTT) has been shown to have a functional polymorphism in the upstream regulatory region (5-HTTLPR) and recent studies have reported an association between this polymorphism and the response to certain SSRIs, such as fluvoxamine and paroxetine (see Figure 1)[6].

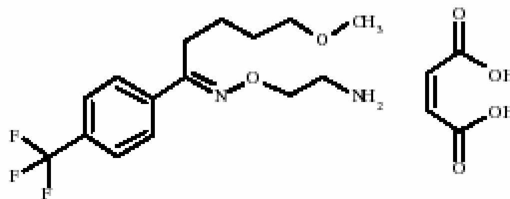

Figure 1: Structure of fluvoxamine maleate

Various components of the 5-HT system have been studied in the central nervous system and peripheral tissues. According to one study, the 5-HTTLPR polymorphism is not a major susceptibility factor in the etiology of major depression[6]. It has been

previously reported that a functional polymorphism in the upstream regulatory region of the serotonin transporter gene (5-HTTLPR) was associated with response to SSRIs[7]. The effect was more marked in subjects taking SSRIs alone. However despite the strength of the association, the explained variance was only 5%, suggesting the involvement of other genes in antidepressant response[7]. Approximately 30% of patients fail to respond to SSRIs. This is in accordance with current views on polygenic inheritance, where minor effect genes contribute only 1-10% of the total phenotypic variance, and the existence of other genes that contribute with additive multiplicative or epistatic effect is very likely[8].

While SSRIs are remarkably similar in their antidepressant activity and side effect profile, they differ substantially in their chemical structure, metabolism, pharmacokinetics, and their inhibitory effect on the cytochrome P450-system (CYP). Because many patients require long-term maintenance treatment with antidepressants, the SSRIs are frequently co-prescribed with other medications. Such polypharmacy may, however, lead to clinically important interactions with co-administered drugs. These interactions can be pharmacodynamic or pharmacokinetic.

Pharmacokinetic interactions caused by metabolic inhibition of CYP-activity represent the majority of the reported interactions with the SSRIs. Differences in their interaction potential are related to differences in their inhibitory potency toward several important CYP-isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) involved in human drug metabolism

Cytochrome P450 (CYP450) describes a class of heme-containing enzymes responsible for the oxidation and reduction of numerous endogenous substrates and drugs, including drugs, toxins and chemical carcinogens[9]. The CYP450 isoforms are divided into two major groups depending on their substrates: CYP450s that are involved in the oxidative

metabolism of endogenous substances such as steroids, fatty acids, and prostaglandins and those that are responsible for the metabolism of exogenous substances such as drugs, environmental pollutants and carcinogens. In contrast to the latter group that exhibit wide substrate specificity and a relatively poor species structural conservation, CYP450s that are involved in the oxidative metabolism of endogenous substances have very high affinities for their substrates and are well conserved within species.

The P450 enzymes are classified based on their amino acid homology: enzymes with 40% greater sequence identity are included in the same family (designated by Arabic numerals, e.g. CYP1), and within a family, enzymes with greater than 55% sequence identity are included in the same subfamily (designated by upper case letters, e.g. CYP1A). Individual isoenzymes are designated by a second Arabic numeral (e.g. CYP1A2). To date, 18 CYP families have been identified in humans. Whereas families CYP1, CYP2, and CYP3 are implicated in the metabolism of xenobiotics, the other families are involved in the synthesis and metabolism of steroids, bile, and fatty acids.

The liver is a crucial site for CYP isoenzymes, although considerable metabolic activity has been found in the gut, kidney, and in the brain. Whereas liver and gut isoenzymes are mostly microsomal, brain isoenzymes are mostly mitochondrial. P450-mediated metabolism is associated with a limited degree of nonspecificity, i.e., one P450 isoenzyme can metabolize multiple substrates and most substrates can be metabolized by different P450 isoenzymes. However, many substrates have a high affinity for one particular P450 isoenzyme, which then becomes a major factor regulating its elimination.

CYP1A, CYP2A6, CYP2B6, CYP2C, CYP2D6, CYP2E1, and CYP3A enzymes account for approximately 70% of human liver CYP[10,11]. CYP3A participates in the metabolism of approximately 50% of all drugs, with CYP2D6 contributing to

approximately 25%, CYP2C9 to approximately 15%, and CYP1A2 to approximately 5%. Therefore, these four isoforms participate in the metabolism of 95% of all drugs[12]. They are responsible for the biotransformation, detoxification and excretion of foreign chemicals, such as drugs, from the body after the desired effect has been reached in humans by converting them into more soluble products, which makes them easier to be excreted via urine or the bile.

Through sequence comparisons, it has been shown that there are extensive similarities between CYP450s identified in man and those identified in bacteria. It has therefore been suggested that CYP450 superfamily originates from a common ancestral gene some three billion years ago through gene duplication, conversion, amplification and SNPs as a defense mechanism to protect organisms from environmental toxicants[9]. The completion of the draft sequence of the human genome revealed the presence of 57 active human CYP genes, which are divided into 18 families and 43 subfamilies and 58 pseudogenes

CYP1A2 belongs to the CYP1 family, which contains 3 subfamilies, 3 genes and 16 pseudogenes. The P450 1 family enzymes (1A1, 1A2, and 1B1) are important catalysts of carcinogen bioactivation reactions, such as polycyclic aromatic hydrocarbon epoxidation and aromatic/heterocyclic amine N-hydroxylation[13]. P450 1A2 is the only member of the P450 1 family expressed constitutively at significant levels in human liver[14], and is involved in metabolism of several clinically important drugs (see Table 1) such as clozapine[15]. Among the procarcinogenic substances that CYP1A2 metabolizes are heterocyclic amines, arylamines[16], which are derived from tobacco smoke, charcoal broiled and fried food and aflatoxin B1[17]. When these are metabolized by CYP1A2, they become reactive and potentially mutagenic substances[17].

Substrates	Amitryptyline Acetaminophen Caffeine Clomipramine Clozapine Cyclobenzaprine Fluvoxamine Imipramine N-DeMe Donepezil	Estradiol Phenactin Mexiletine Naproxen Ondansetron Melatonin Olanzapine Paracetamol Propranolol	R-warfarin Riluzole Ropivacaine Tacrine Tamoxifen Theopylline Verapmil Zileuton Zolmitriptan
Inducers	Tobacco smoking Omeprazole Rifampicine TCDD		
Inhibitors	Fluvoxamine Oral contraceptives		
Probe drug	Caffeine Melatonin Phenactin (*in vitro*) Ethoxyresorufin (*in vitro*)		

Table 1. List of inhibitors, inducers and drugs metabolized by CYP1A2 modified from (Christensen, 2003[18] and http://medicine.iupui.edu/flockhart/p450.pdf)

Endogenous substrates of CYP1A2 include estradiol and melatonin[19]. CYP1A2 is inducible by a number of xenobiotics such as polycyclic aromatic hydrocarbons (PAH) like benzo[a]pyrene from cigarette smoke, 3-methylcholanthrene and TCDD, industrial pollutants or food derived heterocyclic and aromatic mutagens[20]. This induction is through an aryl hydrocarbon receptor (AhR), which is a transcription factor that is activated by binding of polycyclic aromatic hydrocarbons[21]. Inhibition of CYP1A2 may occur by oral contraceptives and fluoroquinolones.

The *CYP1A2* gene is located on chromosome 15 and spans 7.8 kb, comprising 7 exons and 6 introns[22] (See Figure 2). Including the first noncoding exon of 55 bp, the entire mRNA is 3,121 bp long. The open reading frame starts with nucleotide 10 of exon 2. Between CYP1A2 and CYP1A1, exons 2, 4, 6, and especially 5 are strikingly conserved in both nucleotides and total number of base. Exons 2-6 are highly conserved in the

orthologous human, mouse, rat, and rabbit[22] *CYP1A2* genes, which could mean that these exons have a crucial role in the catalytic activity in these enzymes[17].

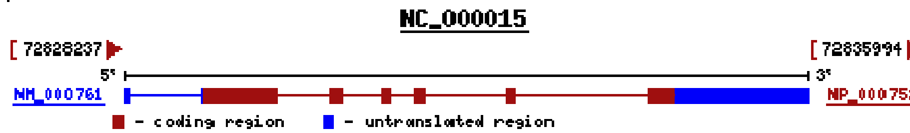

Figure 2: CYP1A2 Gene Structure (From Entrez Gene, CYP1A2 cytochrome P450, family 1, subfamily A, polypeptide 2)

CYP1A2 is induced by various xenobiotic compounds through binding to the aryl hydrocarbon receptor complex, which then binds to the xenobiotic responsive element located 3403 to 3385 bases upstream of the translational initiation site[23]. Furthermore, several other transcription factor-binding sites have been identified in the enhancer region: -3109 to –3093 for AP-1 and E-box proteins, -3020 to –2996 for HNF-1, and –2929 to –2923 for AP-1[24,25,26,27]. In addition GC and CCAAT boxes are known to be present just upstream of transcriptional start sites[24].

Approximately 15- and 40-fold interindividual variations in CYP1A2 mRNA and protein expression levels have been observed in the human liver[22,28]. In addition, there is up to a 60-fold variation in the CYP1A2 activity. These interindividual differences are likely to be associated with efficacy of drugs and cancer susceptibility caused by procarcinogens. Some of the interindividual differences are caused by environmental factors. Cigarette smoking and intake of oral contraceptive steroids are well-established modifiers for CYP1A2 activity[29]. On the other hand, it has been suggested that approximately 35-75% of the interindividual variability in CYP1A2 activity is due to genetic factors. Thus, research has focused on the identification of CYP1A2 genetic variations.

Several studies have indicated the presence of wide interindividual and ethnic differences in CYP1A2 activity. Several polymorphisms in the P450 1A2 upstream sequence and intron 1 have been discovered and some of these may affect P450 1A2 protein expression[30,31,32] and, hence, therapeutic response to drugs. Genetic polymorphisms in the CYP1A2 gene influence the magnitude of CYP1A2 induction. Indeed, three phenotypes have been identified: normal induction, diminished induction and hyperinduction.

34 *CYP1A2* alleles (haplotypes), including 9 subtypes, have been observed (see Table 2). Since *CYP1A2* is inducible by environmental factors, research has focused on the identification of single nucleotide polymorphisms (SNPs) in the transcriptional regulatory regions: the distal enhancer region, the promoter region, non-coding exon 1, and intron 1.

Table 2: CYP1A2 allele nomenclature

Allele	***Protein***	***Nucleotide changes, gene***	***Trivial name***	***Effect***	***Enzyme activity***		***References***
					In vivo	***In vitro***	
*CYP1A2*1A*	CYP1A2.1	None	Wild-type		Normal	Normal	Ikeya *et al*, 1989 Quattrochi and Tukey, 1989
*CYP1A2*1B*	CYP1A2.1	5347T>C					Nakajima *et al*, 1994 Welfare *et al*, 1999
*CYP1A2*1C*	CYP1A2.1	**-3860G>A**			Decreased		Nakajima *et al*, 1999
*CYP1A2*1D*	CYP1A2.1	-2467delT					Chida *et al*, 1999
*CYP1A2*1E*	CYP1A2.1	-739T>G					Chida *et al*, 1999
*CYP1A2*1F*	CYP1A2.1	**-163C>A**			Higher inducibility		Sachse *et al*, 1999 Chida *et al*, 1999
*CYP1A2*1G*	CYP1A2.1	-739T>G; 5347T>C					Chevalier *et al*, 2001

Allele	*Protein*	*Nucleotide changes, gene*	*Trivial name*	*Effect*	*Enzyme activity*		*References*
					In vivo	*In vitro*	
*CYP1A2*1H*	CYP1A2.1	2025A>C; 5347T>C					Chevalier *et al*, 2001
*CYP1A2*1J*	CYP1A2.1	-739T>G ; -163C>A					Aklillu *et al*, 2003
*CYP1A2*1K*	CYP1A2.1	-739T>G; **-729C>T**; -163C>A			Decreased		Aklillu *et al*, 2003
*CYP1A2*1L Predicted haplotype*	CYP1A2.1	-3860G>A; -2467delT; -163C>A					Soyama *et al.*, 2005
*CYP1A2*1M Predicted haplotype*	CYP1A2.1	-163C>A; 2159G>A; 5347C>T					Soyama *et al.*, 2005
*CYP1A2*1N Predicted haplotype*	CYP1A2.1	-3594T>G; -2467delT; -163C>A; 2321G>C; 5521A>G					Soyama *et al.*, 2005
*CYP1A2*1P Predicted haplotype*	CYP1A2.1	-3594T>G; -2467delT; -733G>C; -163C>A; 2321G>C; 5521A>G					Soyama *et al.*, 2005
*CYP1A2*1Q Predicted haplotype*	CYP1A2.1	-2808A>C; -163C>A; 2159G>A; 5347C>T					Soyama *et al.*, 2005
*CYP1A2*1R Predicted haplotype*	CYP1A2.1	-3594T>G; -2467delT; -367C>T; -163C>A; 2321G>C; 5521A>G					Soyama *et al.*, 2005
*CYP1A2*1S Predicted haplotype*	CYP1A2.1	-3053A>G					Soyama *et al.*, 2005
*CYP1A2*1T Predicted haplotype*	CYP1A2.1	-2667T>G					Soyama *et al.*, 2005
*CYP1A2*1U Predicted haplotype*	CYP1A2.1	678C>T					Soyama *et al.*, 2005
*CYP1A2*2*	CYP1A2.2	63C>G		F21L			Huang *et al*, 1999
*CYP1A2*3*	CYP1A2.3	2385G>A; 5347T>C		D348N			Chevalier *et al*, 2001

Allele	***Protein***	***Nucleotide changes, gene***	***Trivial name***	***Effect***	***Enzyme activity***		***References***
					In vivo	***In vitro***	
*CYP1A2*4*	CYP1A2.4	2499A>T		I386F			Chevalier *et al*, 2001
*CYP1A2*5*	CYP1A2.5	3497G>A		C406Y			Chevalier *et al*, 2001
*CYP1A2*6*	CYP1A2.6	5090C>T		R431W			Chevalier *et al*, 2001
*CYP1A2*7*		**3534G>A**		**Splicing defect**	Decreased		Allorge *et al*, 2003
*CYP1A2*8*	CYP1A2.8	5166G>A		R456H			Soyama *et al.*, 2005
*CYP1A2*9*	CYP1A2.9	248C>T		T83M			Murayama *et al*, 2004
*CYP1A2*10*	CYP1A2.10	502G>C		E168Q			Murayama *et al*, 2004
*CYP1A2*11*	CYP1A2.11	**558C>A**		**F186L**		Decreased	Murayama *et al*, 2004
*CYP1A2*12*	CYP1A2.12	634A>T		S212C			Murayama *et al*, 2004
*CYP1A2*13*	CYP1A2.13	1514G>A		G299S			Murayama *et al*, 2004
*CYP1A2*14*	CYP1A2.14	5112C>T		T438I			Murayama *et al*, 2004
*CYP1A2*15*	CYP1A2.15	125C>G		P42R			Soyama *et al.*, 2005
*CYP1A2*16*	CYP1A2.16	2473G>A		R377Q			Soyama *et al.*, 2005

Nucleotide variations in bold are the major SNPs/alterations responsible for the phenotype of the corresponding allele.
Nucleotide numbering is based on Genbank accession number AC091230.23 for the reference sequence.

Three important polymorphisms that cause functional changes in enzymatic activity have been identified in the *CYP1A2*1* wild-type allele. The *CYP1A2*1C* allele is the result of a single point mutation (-3858 G>A), located in the enhancer region, and is

associated with decreased CYP1A2 metabolic activity compared to the normal wild-type *CYP1A2*1A* allele[31]. The *CYP1A2*1F* allele is the result of a single point mutation (-163 C>A), located in intron 1, and is associated with increased induction, particularly in smokers, compared to the wild-type *CYP1A2*1A* allele[30,32,33].

*CYP1A2*1D* (-2467delT), located in the regulatory region, and *CYP1A2*1F* have both been reported to be common polymorphisms and occur in high frequency in Asians and Caucasians[34]. The frequencies of *CYP1A2*1C* and *CYP1A2*1F* in Caucasians are estimated to be around 2% (a much lower value than the 23% reported in Asians ($P<0.001$)) and 68% (comparable with data published for Asians and Caucasians), respectively[35]. A study in Japanese smokers has shown that there is a significant decrease of *CYP1A2*1C* activity and that the frequency is higher (23%) than in Caucasians[31]**.** Unlike other polymorphic P450s such as CYP2D6 and CYP2C19, the frequency of coding region SNPs in CYP1A2 that cause amino acid substitutions are very rare.

As for coding exons, F21L (*CYP1A2*2*) was first reported by Huang *et al*.[36]. Four non-synonymous SNPs (*CYP1A2*3*, *CYP1A2*4*, *CYP1A2*5*, *CYP1A2*6*) were reported with altered protein expression levels and/or enzymatic activities[37]. Allorge *et al*. reported an SNP at the splice donor site of intron 6, which most likely results in a splicing defect (*CYP1A2*7*)[38]. Six additional non-synonymous nucleotide alterations (*CYP1A2*9*, *CYP1A2*10*, *CYP1A2*11*, *CYP1A2*12*, *CYP1A2*13*, *CYP1A2*14*) were reported in a Japanese population, one of which (F186L) showed a markedly reduced enzymatic activity *in vitro*[39]. Three novel SNPs were reported by Soyama *et al*., 125C>G (P42R *CYP1A2*15* allele), 1130>A (R377Q, *CYP1A2*16* allele), and 1367G>A (R456H, *CYP1A2*8*)[40].

Association studies have shown that haplotypes, linked combinations of SNPs , have the advantage of enabling more precise detection of phenotype-genotype links than individual SNPs[41]. Several haplotypes for the 5'-flanking region of *CYP1A2* have been reported using SNPs found in small numbers of Africans, Asians, Europeans, and Indians[42]. *CYP1A2*1K*, which contains a combination of -739T>G, -729C>T and -163C>A, causes lower activity and inducibility of CYP1A2, whereas *CYP1A2*1J* (-739T>G; -163C>A) does not have any effect on enzyme activity[30]. Using 33 detected polymorphisms of *CYP1A2*, Soyama *et al.* identified 14 haplotypes unambiguously and inferred 17 additional haplotypes by aid of an expectation-maximization-based program (see Table 2)[40].

Objectives of Research

The objectives of my research include the determination of the allelic frequencies of *CYP1A2* G-3858-A genetic polymorphism in Caucasians and comparing these frequencies to those reported in Japanese populations. The skills learned will be used to further study the relationship between specific *CYP1A2* polymorphisms and treatment-resistant depression.

Materials and Methods

Patients and Preparation of Genomic DNA. A total of 150 patients diagnosed with unipolar, and major depressive disorder were recruited by collaborating psychiatrists from the following outpatient clinics operated by Lookout Mountain Community Services of Georgia Department of Human Resources, Division of Mental Health, Mental Retardation, and Substance Abuse; Summerville Outpatient Clinic, Summerville, GA;

Fort Oglethorpe Outpatient Clinic. All patients were Caucasians of Western European origin. A registered nurse at Lookout Mountain Community Services drew 5 ml of blood from each patient. The Genomic DNA was extracted using a Blood and Cell Culture DNA Midi Kit (Qiagen) by following the manufacturer's protocol (5).

PCR and Sequence Analysis of Amplified DNA Fragments. PCR based methods were utilized to genotype each polymorphic region. Genomic DNA samples (1 mg) were added to the PCR mixtures consisting of 5 ml Mg Buffer (10X) (Roche), 4 ml of 25 mM MgCl2 (Roche), 1 ml of 10 mM each DNTP (Promega), 1 ml of 20 mM Primer R2 (Genosys), 1 ml of 20 mM Primer R3 (Genosys), 0.25 ml Taq Polymerase (Roche), and 36.75 ml ddH2O. The total volume of the reaction was 50 ml. Thirty cycles of amplification were performed using a programmable heat block (Perkin Elmer DNA Thermal Cycler 400) under the following conditions: denaturation at 94°C for 1.5 min., annealing at 56°C for 2 min., and extension at 72°C for 2 min.

Detection of Genetic Polymorphism in the 5'-flanking Region. The allelic frequencies of the genetic polymorphism in the *CYP1A2* gene were identified as follows. The amplified DNA fragment from a genomic DNA sample was digested with Ddel. The PCR products were electrophoresed adjacent to 100 bp ladders (Promega) on a 2% agarose gel. The gel was stained with 70 ml ethidium bromide.

Results and Discussion

The PCR product size was 596 bp in each case. Therefore, all six tested samples possessed the homozygous wild type genotype (G/G) of the G-3858-A genetic polymorphism in the 5'-flanking region of the *CYP1A2* gene. None of the PCR products were cut; hence the mutant allele was not present. (See Figure 3). The fragment sizes for

heterozygous wild type genotype (A/G) would be 596 bp, 464 bp, and 132 bp. The fragment sizes for homozygous mutant type genotype (G/G) would be 464 bp and 132 bp.

[INSERT Figure 3]

Figure 3: PCR products of 6 5-HTT coding exons on a 2% agarose gel. The exon numbers (2-7) are labeled.

The six samples review indicated that the G/G genotype dominated in the Caucasian population tested. The G/A and A/A genotype appear to very rare in Caucasians of Western European origin. This is compatible with what has been previously reported by Todesco *et al.* in which the allele frequency was only 2% in Caucasians. In the Japanese population tested, the allelic frequencies are 0.77 (wild type) and 0.23 (mutant)[31,40], while in the Caucasian population tested they are 1.0 (wild type) and 0 (mutant). Hence, there is a significant difference in the frequency of the mutant allele, 3858G A (_CYP1A2*C_), based on race and ethnic origin.

Conclusions

Haplotype analysis has revealed that –3858G>A (_*1C_ allele) is highly linked with –163C>A (_*1F_ allele) with a 0.99 probability[40]. However, -163C>A is linked with –3858G>A with only a 0.37 probability[40]. Hence, further study is needed to assess the combinatorial effects of the SNPs in the transcriptional regulatory regions on transcription and induction of *CYP1A2*. It has been suggested that several non-synonymous SNPs are closely associated with the SNPs in the transcriptional regulatory regions. The combined effects of the non-synonymous SNPs and the SNPs in the transcriptional regulatory region should also be investigated. It has been reported that

function-related SNPs are closely linked with each other, suggesting that the haplotypes instead of the SNPs of CYP1A2 should be used for association studies on pharmacokinetic or clinical data.

Detecting genetic variations in drug-metabolizing enzymes is useful for identifying individuals who may experience adverse drug reactions with conventional doses of certain medications. Individuals who possess *CYP1A2*1F* and/or *CYP1A2*1C* variants, or indeed different haplotypes, may exhibit different pharmacokinetics (drug levels) than normal individuals. As a result, such individuals may require non-conventional doses of medications that require CYP1A2 for biotransformation. Conversely, medications that do not require CYP1A2 biotransformation may be preferentially selected for patients with potentially impaired CYP1A2 metabolic capacity to avoid adverse drug reactions. My future work will include extending genotyping studies of *CYP1A2* to address the problem of antidepressant drug resistance.

Acknowledgments

I thank Dr. Kathryn B. Grant and Beth Wilson for their help in this project.

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