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CYTOCHROME P450 2D6: GENETIC POLYMORPHISM IN SUBJECTS ABUSING COCAINE

by

SAVITA CHAUDHARI

A thesis submitted in conformity with the requirements for the Degree of Master of Science
Graduate Department of Pharmacology
University of Toronto

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ABSTRACT

Cytochrome P450 2D6 - Genetic Polymorphism in Subjects Abusing Cocaine

Savita Chaudhari

Master of Science, Department of Pharmacology, University of Toronto, 1998

The debrisoquine-4-hydroxylase polymorphism is a genetic variation in oxidative drug metabolism characterized by two phenotypes, extensive metabolizers (EM) and the poor metabolizers (PM). Five to 10% of the Caucasian populations of Europe and North America are of the PM phenotype and are unable to metabolize debrisoquine, dextromethorphan and numerous other drugs. The impaired drug metabolism in PMs is due to the absence of the cytochrome P450 2D6 (CYP2D6) protein. The dextromethorphan metabolizer phenotype in 59 current and former (within the past two years) regular cocaine users (meeting DSM-IV criteria for psychoactive substance abuse or dependence for cocaine) was determined after oral administration of a 30 mg dose. In eight hours-post dose urine samples the log O-demethylation ratio was determined by HPLC and all 59 subjects were identified as EMs. Genotyping results by PCR were consistent with the EM phenotype for all 59 subjects. Hair samples taken from the subjects were analysed for a metabolite of cocaine to confirm self-reported drug use history. The phenotyping results of the cocaine dependent population were compared to a non-cocaine dependent control population (n=210). The EM frequency in the control group was found to be 93% and the PM frequency was 7%. Using Chi square analysis, a significant difference was found between the EM and PM genotypes of the cocaine dependent population and the control groups (p<0.05). The homogeneity of the EM phenotype among a sample of cocaine users, and the complete absence of PMs, suggests that individuals with the PM phenotype may be protected against the risk of development of cocaine use/dependence.
I wish to express my thanks to my supervisor Dr. Edward M. Sellers for providing me with the opportunity to pursue research in this field. I am grateful for his support and intellectual guidance throughout this program, and especially for his patience.

I would like to thank Dr. S. Victoria Otton for her advice and guidance in this work, as well as Dr. Khanna and Dr. Sen for their support. Special thanks to Linda Sunahara and Ewa Hoffman for analysing the control samples, to Kurt Droll and Howard Zhong for the genotyping analysis and to Franca Ursitti for analysing the hair samples. I would also like to thank Siu Cheung for her assistance during my laboratory work, as well as all the members of the Pharmacogenetic group for their help and support. Special thanks to Cathy Van Der Giessen for her patience and help throughout this program.

I am very grateful to Anindya Sen for assisting me during the data analysis stage of this project. His assistance was essential for completion of this thesis.

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<tr>
<td>3HM</td>
<td>3-Hydroxymorphinan</td>
</tr>
<tr>
<td>3MM</td>
<td>3-Methoxymorphinan</td>
</tr>
<tr>
<td>BZ</td>
<td>Benzoylecgonine</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Cytochrome P450 2D6</td>
</tr>
<tr>
<td>DEX</td>
<td>Dextromethorphan</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DOR</td>
<td>Dextrophan</td>
</tr>
<tr>
<td>DRD2</td>
<td>Dopamine Receptor D2</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual</td>
</tr>
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<td>EM (s)</td>
<td>Extensive Metabolizer (s)</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
</tr>
<tr>
<td>IS</td>
<td>Internal Standard</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobase</td>
</tr>
<tr>
<td>Ki</td>
<td>Inhibition Constant</td>
</tr>
<tr>
<td>MR</td>
<td>Metabolic Ratio</td>
</tr>
<tr>
<td>ODMR</td>
<td>O-Demethylated Metabolic Ratio</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PM (s)</td>
<td>Poor Metabolizer (s)</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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1. INTRODUCTION

1.1 Cytochrome P450s and Genetic Polymorphisms

Cytochrome P450s are enzymes important in the oxidative metabolism of a vast variety of endogenous compounds, such as cholesterol, fatty acids, steroids; and exogenous compounds, such as drugs, environmental chemicals and natural plant toxins (Schuster, 1989). Multiple isozymes of P450 have been shown to exist, each displaying broad and overlapping substrate specificity. Cytochrome P450s are believed to be among the most nonspecific enzymes known. Drug biotransformation by hepatic microsomal cytochrome P450 isozymes is a major determinant of therapeutic and toxic responses to a broad variety of clinically important drugs. Many different P450 isozymes have been purified from human liver, their corresponding cDNAs cloned (Gonzalez, 1990), and they have been classified into families and subfamilies based on their DNA and amino acid sequence similarities (Nebert et al., 1991). Several forms have been found to exhibit polymorphic activity under genetic control (Jacqz, 1986).

Genetic variation is an important cause of the large differences in drug metabolism, and thus drug response, between individuals. It is well known that drugs may be activated and/or eliminated in the body by metabolic routes that are under genetic control. Distinct genetic variants of certain drug metabolizing enzymes have been identified for a number of drugs, some of which were discovered because of unusual pharmacological responses in individual subjects. These individuals were shown to have a reduced ability to metabolize the drug when compared with other members of the population. Genetic polymorphism consists of a monogenic trait that appears in a population in two or more phenotypes. It is the result of the action of different alleles at a single gene locus (Voget et al., 1979). When pharmacokinetic parameters are expressed as a frequency distribution
histogram, a genetic polymorphism is visible by a clear separation into at least two modes. Genetic polymorphisms associated with the metabolism of drugs have previously been reported for a number of compounds. These drugs include debrisoquine (Mahgoub et al., 1977; Eichelbaum, 1982; Evans et al., 1983; Roy et al., 1984; Woosley et al., 1986), sparteine (Eichelbaum, 1982; Evans et al., 1983), isoniazid (Drayer et al., 1977), hydralazine (Drayer et al., 1977), mephenytoin (Wedlund, 1984; Kupfer, 1984), methoxyphenamine (Roy et al., 1984) and encainide (Woosley et al., 1986). In several cases the occurrence of intolerance and adverse effects of drug treatment have been attributed to pharmacogenetic differences (Dengler et al., 1977).

For the majority of drugs oxidative metabolic degradation is important. Several monooxygenase isoenzymes of the cytochrome P450 system that catalyse drug oxidations have been isolated and characterized pharmacogenetically. Great emphasis has been placed on the discoveries of genetic polymorphisms related to some of these P450s. The best known is the debrisoquine-sparteine type related to the cytochrome P450 2D6 (CYP2D6) isoenzyme (also previously called P450db1, P450IID1, and P450Bufl) (Mahgoub, 1977). Although the P450 enzymes exhibit a broad and overlapping substrate specificity, many drugs which are substrates for P450 2D6 are only marginally metabolized in individuals affected by this polymorphism (poor metabolizers).

1.2 Cytochrome 450 2D6

1.2.1 CYP2D6 (Debrisoquine / Sparteine) Polymorphism

The 4-hydroxylation of debrisoquine, a sympatholytic antihypertensive drug, is catalysed by a specific isozyme of cytochrome P450 (CYP2D6) in humans and exhibits a genetic polymorphism first reported in 1977 by Mahgoub et al.(1977). The detection of this polymorphism of debrisoquine oxidation was based originally upon observations of the effects of the drug. Certain
individuals were found to have a severe hypotensive response after a small dose and this was related to the subjects' failure to oxidize the drug to its major 4-hydroxy debrisoquine metabolite. The drug was excreted mainly unchanged in urine (Angelo et al., 1975; Silas et al., 1977). Genetic polymorphism of the oxidation of sparteine was discovered similarly, but independently, from that of debrisoquine (Eichelbaum, 1975). Increased side effects were associated with decreased oxidative metabolism of sparteine, an antiarrhythmic and oxytocic drug (Smith, 1986). It was subsequently shown that debrisoquine and sparteine are both oxidized by a cytochrome P450 isozyme called cytochrome P450 2D6, and that multiple mutations exist in the gene directing the synthesis of CYP2D6 (Broly et al., 1991).

Since the detection of the genetic polymorphism of debrisoquine/sparteine metabolism there has been a growing interest in the pharmacogenetics of oxidative drug metabolism. Two debrisoquine metabolic phenotypes, extensive and poor metabolizers, have been identified in large-scale epidemiological studies (Schmid et al., 1985; Steiner et al., 1988). The urinary concentration ratio of debrisoquine and 4-hydroxydebrisoquine was initially used to classify individuals as extensive metabolizers or poor metabolizers. A defect in the hydroxylation of debrisoquine is present in 5% to 10% of white Caucasians and is inherited as an autosomal recessive trait (Evans et al., 1980; Steiner et al., 1985). The poor metabolizer phenotype (5-10% of white Caucasians) is associated with the absence of CYP2D6, which results from mutant CYP2D6 genes. Debrisoquine oxidative phenotype is a determinant of pharmacological response for many drugs.

1.2.2 Genetic Basis of CYP2D6

Family pedigree analyses and population studies in adults have indicated that CYP2D6 is under monogenic control and that phenotypes are inherited as an autosomal recessive trait (Schmid
et al., 1985; Brosen et al., 1989). The human CYP2D6 complementary DNA has been cloned and expressed in mammalian cell culture, and has provided the first molecular insights into the genetic basis of this polymorphism (Gonzalez et al., 1988). The gene responsible for this has been located on human chromosome 22 and consists of 9 exons. Three genes of the human CYP2D subfamily have been identified: CYP2D6, CYP2D7, and CYP2D8 (Nebert and Gonzalez, 1990). However, only CYP2D6 is functional, CYP2D7 is not functional and CYP2D8 is a pseudogene.

The PM trait is characterized clinically by a deficiency in forming the relevant metabolite(s) of affected substrates, which can result in either drug toxicity or inefficacy (Mahgoub et al., 1977; Sindrup et al., 1991). Biochemically, there is a relative absence of CYP2D6 activity and lack of immunodetectable CYP2D6 in microsomes prepared from livers of PMs (Zanger et al., 1988). Family studies and DNA analysis of poor and extensive metabolizer subjects have established that PMs are homozygous for a recessive allele, whereas the EM phenotype can be either homozygous (wt/wt) or a heterozygous combination of a wt allele with a defective allele (Evans et al., 1983; Steiner et al., 1985; Brosen et al., 1986). The PM trait in several populations has been shown to be the homozygous occurrence of one of several defective alleles of the CYP2D6 gene. These alleles give rise to variant messenger RNA, resulting in lack of functional hemoprotein. Several CYP2D6 alleles have been isolated which contain point mutations or codon deletions (Skoda et al., 1988; Heim et al., 1990; Gonzalez et al., 1988b; Gough et al., 1990; Kagimoto et al., 1990; Broly et al., 1991; Johansson et al., 1991; Masimirembwa et al., 1993; Saxena et al., 1994) (summarized in Table 1). Figure 1 illustrates the wildtype (CYP2D6*I) allele and the location of the allelic variants in the CYP2D6 gene. In Caucasians, the CYP2D6*3, CYP2D6*4 and CYP2D6*5 are the most frequently occurring defective alleles (Broly et al., 1991).
## Table 1: Summary of CYP2D6 Alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Protein</th>
<th>Nucleotide Changes</th>
<th>Xba I haplotype</th>
<th>Trivial name</th>
<th>Effect</th>
<th>Enzyme activity</th>
<th>In vivo</th>
<th>In vitro</th>
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<tr>
<td>CYP2D6*1A</td>
<td>CYP2D6 1</td>
<td>None</td>
<td>29</td>
<td>Wild-type</td>
<td>Normal</td>
<td>Normal</td>
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<td></td>
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<tr>
<td>CYP2D6*1B</td>
<td>CYP2D6 2</td>
<td>G&lt;sub&gt;191&lt;/sub&gt;A</td>
<td>29</td>
<td></td>
<td>Normal (s)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Decreased (dx. d)&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>CYP2D6*2</td>
<td>CYP2D6 2</td>
<td>G&lt;sub&gt;174&lt;/sub&gt;C: C&lt;sub&gt;213&lt;/sub&gt;T: G&lt;sub&gt;172&lt;/sub&gt;C</td>
<td>29</td>
<td>CYP2D6L</td>
<td>R&lt;sub&gt;196&lt;/sub&gt;C: S&lt;sub&gt;468&lt;/sub&gt;T</td>
<td>Inactive genes</td>
<td></td>
<td></td>
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<tr>
<td>CYP2D6*2XN</td>
<td>CYP2D6 6</td>
<td>G&lt;sub&gt;174&lt;/sub&gt;C: C&lt;sub&gt;213&lt;/sub&gt;T: G&lt;sub&gt;172&lt;/sub&gt;C</td>
<td>42-175&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>Increased (d)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CYP2D6*1</td>
<td>A&lt;sub&gt;1847&lt;/sub&gt; deletion</td>
<td>29</td>
<td>CYP2D6A</td>
<td>Frameshift</td>
<td>None (d. s)</td>
<td>None (b)&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>CYP2D6*4A</td>
<td>C&lt;sub&gt;182&lt;/sub&gt;T: C&lt;sub&gt;194&lt;/sub&gt;A: A&lt;sub&gt;167&lt;/sub&gt;G; C&lt;sub&gt;193&lt;/sub&gt;G: G&lt;sub&gt;174&lt;/sub&gt;C: G&lt;sub&gt;191&lt;/sub&gt;A; G&lt;sub&gt;162&lt;/sub&gt;C</td>
<td>44/29/16 + 9</td>
<td>CYP2D6B</td>
<td>Splicing defect</td>
<td>None (d. s)</td>
<td>None (b)</td>
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<td>CYP2D6*5</td>
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<td>11.5 or 13&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>CYP2D6T</td>
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<td>None (b, s, d)</td>
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<td>CYP2D6E</td>
<td>H&lt;sub&gt;123&lt;/sub&gt;P</td>
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<td>CYP2D6*8</td>
<td>CYP2D6 8</td>
<td>G&lt;sub&gt;174&lt;/sub&gt;C: G&lt;sub&gt;184&lt;/sub&gt;T: C&lt;sub&gt;213&lt;/sub&gt;T: G&lt;sub&gt;172&lt;/sub&gt;C</td>
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<td>CYP2D6G</td>
<td>Stop codon</td>
<td>None (d. s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*9</td>
<td>CYP2D6 9</td>
<td>A&lt;sub&gt;162&lt;/sub&gt;-A&lt;sub&gt;170&lt;/sub&gt; or G&lt;sub&gt;162&lt;/sub&gt;-A&lt;sub&gt;170&lt;/sub&gt; deleted</td>
<td>29</td>
<td>CYP2D6C</td>
<td>K&lt;sub&gt;161&lt;/sub&gt; deleted</td>
<td>Decreased (b. s. d)</td>
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<td></td>
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<tr>
<td>CYP2D6*10A</td>
<td>CYP2D6 10A</td>
<td>G&lt;sub&gt;182&lt;/sub&gt;T: G&lt;sub&gt;194&lt;/sub&gt;C: G&lt;sub&gt;172&lt;/sub&gt;C</td>
<td>44.29</td>
<td>CYP2D6f</td>
<td>P&lt;sub&gt;146&lt;/sub&gt;S: S&lt;sub&gt;468&lt;/sub&gt;T</td>
<td>Decreased (s)</td>
<td></td>
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<tr>
<td>CYP2D6*10B</td>
<td>CYP2D6 10B</td>
<td>G&lt;sub&gt;182&lt;/sub&gt;T: G&lt;sub&gt;194&lt;/sub&gt;C: G&lt;sub&gt;172&lt;/sub&gt;C</td>
<td>44.29</td>
<td>CYP2D6Ch1</td>
<td>P&lt;sub&gt;146&lt;/sub&gt;S: S&lt;sub&gt;468&lt;/sub&gt;T</td>
<td>Decreased (d)</td>
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From: Daly et al. (1996).
Table 1: Continued

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<th>Allele</th>
<th>Protein</th>
<th>Nucleotide changes</th>
<th>Xba I haplotype</th>
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<th>Enzyme activity</th>
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<tr>
<td>CYP2D6*10c</td>
<td>CYP2D6 10K</td>
<td>C_{i48}T; C_{i117}T; C_{i24a}C; G_{i14b}C and gene conversion to CYP2D7 in exon 9</td>
<td>44/29</td>
<td>CYP2D6Ch2</td>
<td>P_{14S}: S_{46a}T</td>
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<td>Decreased (b)</td>
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<td>CYP2D6 12</td>
<td>G_{i77}C; G_{i117}C; C_{i24a}T; G_{i14b}C</td>
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<td>Splicing defect</td>
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<td>CYP2D6 12</td>
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<td>None</td>
<td>None (s)</td>
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<tr>
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<td>CYP2D6 12</td>
<td>CYP2D7p/CYP2D6 hybrid Exon 1 CYP2D7, exons 2–9 CYP2D6</td>
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<td>CYP2D6 14</td>
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<tr>
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<td>T_{11a} Insertion</td>
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<td>CYP2D7p/CYP2D6 hybrid Exons 1–7 CYP2D7p-related, Exons 8–9 CYP2D6</td>
<td>11</td>
<td>CYP2D6D2</td>
<td>Frameshift</td>
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<td>C_{i111}T; G_{i117}C; C_{i24a}T; G_{i14b}C</td>
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<td>CYP2D6Z</td>
<td>T_{1101}: R_{126S}: S_{46a}T</td>
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1. sparteine 2. dextromethorphan; 3. debrisoquine; 4. size of fragment is dependent on the number of copies of the gene; 5. bufaralol; 6. two different size estimates have been reported for this fragment.
<table>
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<tr>
<th>Designation</th>
<th>Gene Structure</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1A</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>Normal</td>
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<tr>
<td>CYP2D6*1B</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>Normal</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*2xN</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>Increased</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*4A</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*4B</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*4C</td>
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<td>None</td>
</tr>
<tr>
<td>CYP2D6*4D</td>
<td>1 2 3 4 5 6 7 G G</td>
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</tr>
<tr>
<td>CYP2D6*5</td>
<td>1 2 3 4 5 6 7 G G</td>
<td>None</td>
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<tr>
<td>CYP2D6*6A</td>
<td>1 2 3 4 5 6 7 G G</td>
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<tr>
<td>CYP2D6*6B</td>
<td>1 2 3 4 5 6 7 G G</td>
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</table>

*continued overleaf*

**Figure 1:** Summary of the presently known variant alleles of the CYP2D6 gene. The positions of the various polymorphisms associated with each allele are indicated. From Daly et al., 1996.
<table>
<thead>
<tr>
<th>Designation</th>
<th>Gene Structure</th>
<th>Activity</th>
</tr>
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<tr>
<td>CYP2D6*8</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*9</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
<td>Decreased</td>
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<tr>
<td>CYP2D6*10A</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*10B</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*10C</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>(exon 9 conversion to CYP2D7)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*11</td>
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<td>CYP2D6*12</td>
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<td>CYP2D6*13</td>
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<td>CYP2D6*14</td>
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<td>CYP2D6*15</td>
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<tr>
<td>CYP2D6*16</td>
<td><img src="#" alt="Gene Structure Diagram" /></td>
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</tr>
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</table>

**Figure 1:** Continued.
1.2.3 Variability Between Ethnic Groups

The frequency of the PM phenotype of CYP2D6 is known to exhibit large interethnic differences (Kalow, 1982; Woolhouse et al., 1979) (see Table 2). Phenotyping studies using a number of different substrates have been performed in various ethnic groups, including European whites (Eichelbaum et al., 1979; Benitez et al., 1988; Schmid et al., 1985; Evans et al., 1983; Drahse et al., 1989; Jacquez et al., 1988a; Evans et al., 1980; Peart et al., 1986), American whites (Nakamura et al., 1985; Wedlund et al., 1984), Japanese (Nakamura et al., 1985), and various African black groups (Woolhouse et al., 1985; Eichelbaum et al., 1985; Iyun et al., 1986). The prevalence of PMs varies from 5-10% in European and American white Caucasian populations (Brosen et al., 1985; Drahse et al., 1989; Eichelbaum et al., 1979; Evans et al., 1980; Guttendorf et al., 1990; Henthorn et al., 1989; Schmid et al., 1985; Steiner et al., 1985; Steiner et al., 1988). There is lower reported prevalence of poor metabolizers among Chinese (Lou et al., 1987) and Japanese (Nakamura et al., 1985) subjects. 1 to 3% of Japanese and Chinese (Nakamura et al., 1985; Lou et al., 1987; Yue et al., 1989; Horai et al., 1989) are poor metabolizers. However, there is one report of the frequency of PMs being 30% in a small Hong Kong Chinese population living in Canada (Kalow et al., 1980).

It has been suggested that this unexpectedly high occurrence of PMs may be due to the small sample size studied or due to the measure of CYP2D6 status used (Kalow, 1984). There have been several phenotyping studies of black African groups. There are reports of 8.9% PMs in Nigeria (Mbandefo et al., 1980), 7.1% in Ghana (Woolhouse et al., 1985), 1% in Egypt (Steiner et al., 1988), 19% in South Africa (Sommers et al., 1988), 5% Burundi (Nsabiyumva, 1990).

Differences in the frequency of different gene types have also been demonstrated in various populations. Spaniards have a lower frequency of the CYP2D6*4 allele and a higher frequency of the wild-type allele than other Caucasian populations (Agundez et al., 1994). Chinese subjects were
Table 2: Frequency of CYP2D6 Deficiency in Different Populations

<table>
<thead>
<tr>
<th>Ethnicity (Nationality)</th>
<th>Probe Drug</th>
<th>% (PMs/Total)</th>
</tr>
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<tr>
<td><strong>AFRICA</strong></td>
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<td></td>
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<tr>
<td>Americans (USA, children)</td>
<td>DB</td>
<td>1.9 (2/106)</td>
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<tr>
<td>Ghanaians (Ghana)</td>
<td>AP</td>
<td>0 (0/154)</td>
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<td>Ghanaians (Ghana)</td>
<td>DB</td>
<td>7 (10/14)</td>
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<tr>
<td>Ghanaians (Ghana)</td>
<td>DB</td>
<td>6.3 (5/80)</td>
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<tr>
<td>Nigerians (Nigeria)</td>
<td>SP</td>
<td>4 (7/165)</td>
</tr>
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<td>Nigerians (Nigeria)</td>
<td>SP</td>
<td>8 (10/123)</td>
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<tr>
<td>Nigerians (Nigeria)</td>
<td>DB</td>
<td>0 (0/138)</td>
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<tr>
<td>San Bushmen (Soth Africa)</td>
<td>DB</td>
<td>19 (18/96)</td>
</tr>
<tr>
<td>Zambians (Zambia)</td>
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<td>2 (2/102)</td>
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<td><strong>AMERICAN INDIANS</strong></td>
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<tr>
<td>Cuan (Panama)</td>
<td>SB</td>
<td>0 (0/89)</td>
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<tr>
<td>Ngawbe Guayme (Panama)</td>
<td>SP</td>
<td>5.2 (5/121)</td>
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<tr>
<td>Chinese (China)</td>
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<td>1 (7/695)</td>
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<td>DX</td>
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<td>Japanese (Japan)</td>
<td>DB</td>
<td>0 (0/100)</td>
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<tr>
<td>Japanese (Japan)</td>
<td>SP</td>
<td>2 (2/84)</td>
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<tr>
<td>Japanese (Japan)</td>
<td>Metoprolol</td>
<td>0.5 (1/200)</td>
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<td>Maori (New Zealand)</td>
<td>DB</td>
<td>5 (5/101)</td>
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<td>Thai (Thailand)</td>
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<td>Saudis (Saudi Arabia)</td>
<td>DX</td>
<td>2 (2/102)</td>
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From: Edeki, 1996. For complete list of references see Edeki, 1996.
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<td>DB</td>
<td>3.4 (11/136)</td>
</tr>
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</table>
found to have a higher frequency of the 44 kb length allele than Caucasians (Yue et al., 1985). Also, the CYP2D6*10B alleles are very frequent in Oriental populations, whereas the CYP2D6*3 or CYP2D6*4 alleles were not identified (Dayer et al., 1982).

1.2.4 Clinical Relevance of CYP2D6

The oxidative metabolism of a large number of therapeutically important drugs is found to be controlled by the CYP2D6 polymorphism. It is now well-recognized that this phenomenon has important implications for drug efficiency as well as drug safety (Eichelbaum, 1982; Kupfer et al., 1983). Over 40 clinically important drugs, many of them derived from plant alkaloids, have been identified as substrates of CYP2D6 using various probe drugs, including debrisoquine, sparteine and dextromethorphan (Masimirembwa et al., 1996). Examples of such drugs include antidepressants, beta-blockers, antiarrhythmics, opiates, neuroleptics and amphetamine analogues (Edeki, 1996), and still new substrates are continuously being discovered (see Table 3).

Clinical implications have been investigated and these studies have shown that PMs are more at risk than EMs. PM subjects experience side effects at normal doses of drugs metabolized by CYP2D6 (Dayer et al., 1982; Eichelbaum et al., 1990; Idle, 1984). For example, it has been shown that the PM phenotype was associated with a greater incidence of hepatotoxicity (Morgan et al., 1984) and neurotoxicity (Shah et al., 1982) with perhexiline, a greater incidence of lactic acidosis with phenformin (Oates et al., 1981), or side effects with propafenone (Siddoway et al., 1987) and tricyclic antidepressants (Brosen, et al., 1989). Identification of EM and PM subjects may have important clinical implications because the response to several drugs may be predicted from the phenotype. The consequences of absent CYP2D6 isozyme activity for any drug metabolized by this enzyme depends on the relative activity of the parent drug and its various
<table>
<thead>
<tr>
<th>Table 3: Some Substrates of CYP2D6</th>
</tr>
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<tbody>
<tr>
<td><strong>ANTIDEPRESSANTS</strong></td>
</tr>
<tr>
<td><strong>BETA-BLOCKERS</strong></td>
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<td><strong>ANTIARRHYTHMICS</strong></td>
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<td><strong>AMPHETAMINE ANALOGUES</strong></td>
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</tbody>
</table>
metabolites. In PMs, serious toxic reactions may occur, related either to the accumulation of drugs whose elimination depends on the activity of CYP2D6 or to increased formation of toxic reactive metabolites formed by another metabolic pathway (Kupfer et al., 1983; Jacqz et al., 1986). Poor metabolizers may be unable to bioactivate some drugs, and thus are less likely to derive therapeutic benefit if these pathways are required for the activation of the drug (Woosley et al., 1986). For example codeine is O-demethylated to morphine by CYP2D6 (Yue et al., 1989). Even though approximately only 10% of codeine is transformed to morphine, there is evidence (Sindrup et al., 1990) that morphine formation is essential for analgesia during codeine treatment. So in comparison with EMs, PMs may not benefit as much from the analgesic effect of codeine. For PMs the analgesic effect of codeine itself makes it important to correlate the therapeutic outcome with the concentrations of codeine administered.

This polymorphism can also result in adverse reactions in EMs. The extensive metabolizer phenotype has been associated with increased susceptibility to lung cancer (Ayesh et al., 1984; Caporaso et al., 1990; Evans, 1984; Faccini et al., 1990; Gough et al., 1990; Idle et al., 1981; Ilett, 1987), and bladder cancer (Gough et al., 1990; Kaisary et al., 1987). The significance of these cancer studies is unclear since the CYP2D6 enzyme does not metabolize any known environmental carcinogens. It has been hypothesized that the enzyme may activate some unknown carcinogen or on the other hand the CYP2D6 gene may be linked to an oncogene, tumor suppressor gene or genes encoding enzymes that do metabolize carcinogens.

Thus it has been suggested that in addition to the usual parameters in the determination of an effective individual dose (i.e. age and weight of patient), to optimize drug therapy and avoid unnecessary side effects for drugs which are already known to be subject to a polymorphism, genetic factors should be taken into account. Failure to consider the metabolic phenotype could lead to
several different outcomes depending on the consequences of CYP2D6 dependent metabolism of the particular drugs being administered.

1.3 Pharmacogenetics Tests

The existence of genetic polymorphisms is of great importance, since enzymatic deficiencies could lead to differences in drug action and to an increased incidence of side-effects. Therefore, pharmacogenetic tests have been introduced to determine the ability of patients to metabolize well-characterized drugs. It has been demonstrated that PMs possess negligible amounts of the P450 isozyme, CYP2D6 (rather than an aberrant one), because variant messenger RNAs important in the isozyme's formation are products of mutant genes (Gonzalez et al., 1988). An assay for CYP2D6 would require the acquisition of liver tissue (Dayer et al., 1987), and thus such a test on a routine basis is not a realistic option.

1.3.1 Genotyping

The status of the CYP2D6 enzyme system for a given individual can be determined by two methods. The first method, called genotyping, involves relatively new molecular biological techniques developed to detect specific mutations in the CYP2D6 gene. Genotyping studies involve polymerase chain reaction (PCR) amplification, restriction fragment length polymorphisms (RFLP), eg. XbaI haplotypes or allele-specific endonuclease digestion of PCR products to genotype the CYP2D6 gene. Mutant CYP2D6 genes were first identified through complementary deoxyribonucleic acid (cDNA) studies (Gonzalez et al., 1988). Initially, the presence of variant alleles of CYP2D6 was established by RFLP linkage analysis, and this was used to genotype EM and PM subjects (Meyer et al., 1990; Kagimoto et al., 1990; Heim et al., 1990; Tyndale et al., 1991a;
Broly et al., 1991; Gaedigk et al., 1991). RFLPs, after digestion with the restriction endonuclease XbaI, identify several haplotypes of this gene cluster. The XbaI restriction enzyme produces fragments of size 29, 44, 13, 11, 16+9, or 9.4 kb (Kagimoto et al., 1990). The 13 kb and 11 kb are characteristic of a complete gene deletion (CYP2D6*5) (Stein et al., 1995). However, the other fragments could contain both wild-type or variant alleles. Thus, the presence of the two mutant alleles allows prediction of the phenotype in only 25% of PMs. PCR amplification assays have now been developed for the detection of specific mutations in the CYP2D6 gene. Due to the high homology with the CYP2D gene cluster, the genotyping assays which use the PCR technology have an initial amplification which is specific for CYP2D6 introns in order to exclude amplification of the CYP2D7 and CYP2D8 genes (Droll, 1996). XbaI RFLP coupled with allele-specific PCR amplification allows accurate genotyping of 100% of EMs and over 95% of all mutant PM alleles found in Caucasians (Broly et al., 1991).

1.3.2 Phenotyping

The second method of determining CYP2D6 status, called phenotyping, allows an indirect measure of CYP2D6 activity in humans (Brosen et al., 1989; Eichelbaum et al., 1990). Phenotyping is usually performed by the oral administration of a specific probe drug and the subsequent determination of the amount of parent drug and its metabolites excreted in the urine over several hours. The metabolic ratio of parent drug to metabolites defines subjects as extensive metabolizers (EMs) or poor metabolizers (PMs) and is usually plotted as a frequency distribution histogram. A polymorphism is indicated by a bimodal frequency distribution curve with the antimode between the two populations, separating individuals who are PMs from those that are EMs. The metabolic ratio is used in phenotyping because it accounts for the possibilities of incomplete urine collection and/or
impaired absorption.

One of the major considerations with the use of phenotyping tests in humans is that other drugs that the test subjects may be concurrently using might interfere with the results of the test. In fact, all substrates of CYP2D6 can competitively inhibit the biotransformation of a test compound by the isozyme and, thus, alter the calculation of the metabolic ratio (Fonne-Pfister and Meyer, 1988; Otton et al., 1984). Nonsubstrate inhibitors of CYP2D6, such as quinidine (Otton et al., 1988) can also inhibit CYP2D6 and transform the EM phenotype into a pseudo-PM phenotype (Broly et al., 1991; Funck-Brentano et al., 1989a,b).

Initially, debrisoquine and sparteine were commonly used as probes to phenotype patients for the CYP2D6 polymorphism. However, debrisoquine and sparteine are marketed only in a limited number of countries. Also, debrisoquine, an antihypertensive agent, is associated with a small but significant incidence of hypotensive and allergic reactions (Rosendorff et al., 1968; Orgain and Kern, 1970; Almeyda and Levantine, 1973).

Dextromethorphan, one of the active ingredients in many over the counter antitussive formulations is relatively safe, well characterized and generally available. It has been established that the polymorphic oxidative O-demethylation of dextromethorphan genetically cosegregates with that of the polymorphic debrisoquine hydroxylation. The oxidative phenotype determined by the urinary metabolic ratio using dextromethorphan has also been shown to yield metabolic phenotypes that completely cosegregate with phenotypes that were determined with the use of debrisoquine as the substrate probe (Kupfer et al., 1984; Schmid et al., 1985; Kronbach et al., 1987). Thus, dextromethorphan became widely used as a test probe to determine the metabolic phenotype of individuals. A secondary consideration for the use of dextromethorphan as a pharmacogenetic probe drug is the relative simplicity and speed of the assay used to measure its metabolites in urine.
specimens (Kupfer et al., 1984; 1985). The use of dextromethorphan as a probe substrate for the status of the CYP2D6 enzyme system has been well-documented (Schmid et al., 1985; Hildebrand et al., 1989; Vetticaden et al., 1989; Freche et al., 1990; Relling et al., 1991) with similar results by several investigators. These authors have found that the rates of hepatic dextromethorphan O-demethylation, as expressed by the urinary metabolic ratio, exhibit a bimodal frequency distribution. 5-10% of the Caucasian population were found to have a deficiency of the isozyme responsible for dextromethorphan metabolism, ie. PMs, while the remainder are EMs.

Since dextromethorphan (3-methoxy-17-methylmorphinan) was introduced in the early 1950s (Benson et al., 1953; Cass et al., 1953), it has become one of the most widely used non-opioid cough suppressants. Although it does not possess the CNS pharmacology of other opiates in humans (ie. analgesia, respiratory depression, abuse liability or psychomimetic properties), it maintains the centrally mediated antitussive activity (Goodman and Gilman, 1990). There have also been reports of anticonvulsant and neuroprotective properties (Tortella, 1989). Because of its lack of addictive and analgesic properties, dextromethorphan is available without prescription as a safe antitussive over the counter drug even in pediatric practice. The most common side effects produced by dextromethorphan have been nausea, drowsiness and dizziness. The highest recommended daily dose of dextromethorphan is 120 mg. However, toxic doses could cause vomiting, blurred vision, nystagmus, ataxia, shallow respiration, urinary retention, euphoria, hallucinations, stupor and coma (Dodds and Revai, 1967; Jasinski, 1970; Shaul et al., 1977).

The availability of several analytic methods, the safety and general availability of dextromethorphan have made this drug the preferred choice for worldwide screening of the CYP2D6 polymorphism.
1.3.2.a. Dextromethorphan Metabolic Pathway

Upon oral administration, dextromethorphan undergoes rapid and extensive hepatic first-pass metabolism, the main routes of biotransformation being O- and N-demethylation (Barnhart, 1980). The major metabolic pathway is the O-demethylation of dextromethorphan (DEX) to dextrorphan (DOR) and is catalysed by the polymorphic CYP2D6 isozyme in humans (Kupfer et al., 1984). Dextrorphan (3-hydroxy-17-methylmorphinan) is further metabolized by N-demethylation to 3-hydroxymorphinan (3HM) and both metabolites are excreted after conjugation to glucuronic or sulphuric acid (Vetticaden et al., 1989). Dextromethorphan is also N-demethylated to 3-methoxymorphinan (3MM) which is further O-demethylated to 3HM. Thus, 3HM can be formed from DEX by two separate pathways (Kerry, 1993) (see Figure 2).

1.3.2.b. Dextromethorphan Metabolic Ratio

Interindividual differences in rates of dextromethorphan metabolism are expressed by the urinary O-demethylation ratio (ODMR). The dextromethorphan ODMR was calculated from the urinary ratio of total O-demethylated metabolites to non-O-demethylated compounds. Determination of the metabolic phenotype involves having the subjects take one dose of dextromethorphan orally and then collect urine for 8 to 10 hours (Schmid et al., 1985; Henthorn et al., 1989; Larrey et al., 1989). After a single dose of dextromethorphan, the total concentrations of DEX, DOR, 3HM, and 3MM can be determined by high pressure liquid chromatography (HPLC) and the urine metabolic ratio can be used to define the extensive metabolizer (EM) and poor metabolizer (PM) phenotypes for the CYP2D6 polymorphism (Schmid et al., 1985; Henthorn et al., 1989; Larrey et al., 1989).
Figure 2: Dextromethorphan Metabolic Pathway
From Vetticaden et al., 1989; Jacqz-Aigrain et al., 1993.
The O-demethylation of DEX to DOR and 3MM to 3HM
is mediated by CYP2D6. The site of metabolism of CYP2D6
is indicated by the arrow.
1.3.2.c. Analysis of Dextromethorphan

Analytical methods that are suitable for detecting low concentrations of dextromethorphan and dextrorphan in biological samples include gas-liquid chromatography (Baumann et al., 1988; Pfaff et al., 1983; Furlanut et al., 1977; Barnhart, 1979), thin-layer chromatography (DeZeeuw et al., 1992), direct fluorescence spectrometry (Ramachander et al., 1977) and radioimmunoassay (Dixon et al., 1978).

Also, numerous HPLC assays have been published. Several have used phenyl columns with UV detection (Jacqz, 1986; Park, 1984). Sensitivity was improved with the use of fluorescence detection (Jacqz-Aigrain, 1988; East and Dye, 1985). There have been few reports of the determination of urine concentrations of dextromethorphan and its principal metabolites in a single run (Johansson et al., 1988). These assays are generally complicated and time-consuming. Although East and Dye (1985) could determine the four compounds in plasma and urine by HPLC, four separate procedures, including different solvent extraction procedures, mobile phases, and HPLC columns were used. Chen et al. (1990) developed a procedure to analyse the metabolism of dextromethorphan using a simple, sensitive and specific procedure which can simultaneously and rapidly determine concentrations of dextromethorphan, dextrorphan, 3-hydroxymorphinan and 3-methoxymorphinan in urine using HPLC with fluorescence detection.

1.4 Relevance to Drug Abuse: Metabolic Risk Factor Hypothesis

The three major characteristics of drugs of abuse are 1) they have reinforcing properties, 2) they cause harm to the individual and to society; and 3) they cause dependence. A number of drugs of abuse are already known to be substrates of CYP2D6, eg. codeine (Dayer et al., 1988; Chen et al., 1988), oxycodone [Percodan®] (Otton et al., 1993), hydrocodone (Otton et al., 1992), amphetamine
(Smith, 1986) and p-methoxyamphetamine (Kitchen et al., 1979; Wu et al., 1992) or inhibitors of CYP2D6, eg. pentazocine, d-propoxyphene, and (-)-cocaine (Wu et al., 1993).

The metabolic risk factor hypothesis refers to the role of CYP2D6 in abuse liability through its effects on biotransformation (Sellers et al., 1990). Thus PMs should experience greater risk than EMs of abuse and of toxicity to a drug which is inactivated by CYP2D6 (eg. P-methoxyamphetamine, methamphetamine). On the other hand, PMs should have decreased probability of abusing a drug converted from an inactive pro-drug to an active drug of abuse by CYP2D6 (eg. codeine to morphine). In EMs, the probability should increase. Inhibitors have been suggested to decrease a drug's reinforcing properties if these are mediated by an active metabolite, and thus reduce the risk of physical dependence (Tyndale et al., 1997). Thus, an impaired ability to metabolize an active drug to its inactive metabolite or inability to produce a more active metabolite will, depending on each specific drug, be associated with an increased or decreased abuse liability (Tyndale, 1997).

In a recent study by Tyndale et al. (1997) evidence was provided to support the metabolic risk factor hypothesis. The PM genotype was under-represented in subjects dependent on oral opiates, suggesting that the CYP2D6 defective genotype is a pharmacogenetic protection factor for oral opiate dependence. Oral opiates (eg. codeine, oxycodone and hydrocodone) are metabolized by CYP2D6 to metabolites of increased activity, and therefore increased abuse potential (eg. morphine, oxymorphine and hydromorphone). Thus, the most likely explanation for these results appears to be the metabolic risk factor hypothesis. This study was the first demonstration of differences in genetically determined variation in CYP-mediated metabolism influencing the occurrence of substance dependence.
1.5 Cocaine Abuse

During the last decade there has been a substantial increase in recreational use of cocaine in North America in all age and socioeconomic groups. Cocaine use has been associated with criminal activities and a variety of serious health problems (Abelson et al., 1985).

Cocaine is the most potent of the naturally occurring central nervous system stimulants. The compound is found in the leaves of *Erythroxylon coca*, a South American shrub, in amounts of up to 2% by weight. Cocaine is a chemical with strong reinforcement properties, addictive capability and potential for harm. Cocaine is used on the street in two forms: cocaine hydrochloride and cocaine freebase, otherwise known as crack. Cocaine hydrochloride is a white powder; usually 8 to 100 mg of this powder is spread in a 4 to 6 cm "line" and is snorted (administered intranasally) or taken by intravenous injection. Freebase cocaine is more volatile and can be administered by smoking (inhalation) (Commissaris, 1989). It is well-established that the intensity of cocaine's effect depends on its route of administration. Intranasal use results in plasma cocaine concentrations that peak at 60 minutes (Van Dyke et al., 1976). When taken by intravenous injection peak blood levels occur within 5 minutes of injection (Jarvaid et al., 1978) When cocaine is smoked as freebase or crack, faster peak blood levels are achieved with cocaine reaching the brain in about 8 seconds after inhalation compared with 16 seconds after intravenous use (Mofenson et al., 1987).

Several psychosocial theories have been suggested to account for the abuse of cocaine and other illicit drugs. However, in contrast to alcoholism where growing empirical evidence is implicating hereditary factors (Winokur and Clayton, 1968; Goodwin, 1979; Cloninger, 1987; Blum et al., 1990), very little is known about the genetics of human cocaine dependence. Recent studies have shown an association between the less prevalent A1 allele of the DRD2 gene and alcoholism (Blum et al., 1990). The few recent studies available on humans (Cadoret et al., 1986; Pickens et
al., 1991) and on animals (George, 1991; Ruth et al., 1988; Seale et al., 1991; Smolen et al., 1991) provide some evidence that genetic differences are also involved in the use and abuse of cocaine as well as other illicit drugs. Two recent reports have also implicated alleles of the D<sub>2</sub> dopamine receptor (DRD2) gene in samples of polysubstance abusers (Comings et al., 1991; Smith et al., 1992).

1.6 Verification of Cocaine Abuse

1.6.1 Urine, Blood and Self-Report

A major issue in addressing the role of CYP2D6 in cocaine abuse is verification of subject use of cocaine. Traditionally, verification of substance abuse has been accomplished through urine and blood testing, and self-reports have been relied upon for abuse histories. However, self-report has been shown to be very inaccurate, and urine and blood testing for illicit drugs are positive only during the first few days after their use (Frank et al., 1988). Cocaine is eliminated in the urine as parent drug (1% - 9%, dependent on urine pH), and its metabolites, namely benzoylecggonine (BZ) (29% - 45%), ecgonine methyl ester (32% - 49%), and ecgonine (not quantitated) in a 24-hour period (Fish et al., 1969; Inaba et al., 1978). For example, after a 1.5 mg/kg intranasal application, cocaine concentrations in urine averaged 6.7 mg/L during the first hour, and declined rapidly to undetectable levels in 12 hours; benzoylecggonine urine concentrations reached an average peak of 35 mg/L during the 4-8 hour period and diminished slowly to an average of 0.4 mg/L for the 48-72 hour collection period (Hamilton et al., 1977).

Thus, the detection of cocaine and its metabolites in blood and urine is limited by the short elimination half-life of these compounds. Consequently, individuals who have stopped consuming cocaine are likely to test negative a few days later.
1.6.2 Hair Analysis

The use of hair to calculate long-term systemic exposure to xenobiotics has been used for years by forensic scientists to measure various medicines and drugs of abuse (Baumgartner et al., 1979; Puschel et al., 1983), generally in the context of postmortem examination. Hair-based assays have been developed for morphine, methadone, opiates, cocaine, amphetamines, phencyclidine and other illicit substances in both clinical and forensic context (Marsh et al., 1992). The use of hair to prove cocaine exposure in adults has been established by Baumgartner and Berka (1989).

Drugs transfer into the growing hair shaft through the capillaries nourishing it, along with nutrients and other small molecules (Forman et al., 1992). Cocaine and its major metabolite benzoylcegonine (BE) are incorporated into hair during the growth of the shaft and stay there for the whole life of the hair (Graham et al., 1989; Valente et al., 1981; Baumgartner et al., 1982). Drugs and their metabolites are incorporated into the hair matrix in such a stable manner that analysis can be done even beyond the grave: hair analyses have been conducted on both Napoleon and Keats, showing the presence of arsenic and laudanum respectively (Smith et al., 1962; Lyon, 1986). Rates of hair growth vary with race, sex and age (Saitoh et al., 1969). Human hair on a healthy head does not grow continuously but goes through a cycle of growth, fall and replacement, in which each follicle's cycle is different. However, the rate of scalp growth is relatively constant within individuals although there may be slight between-individual variation. The posterior vertex region is less subject to variation in hair growth by age and sex and has a more constant number of hairs in the growing phase (85%) with a growth rate of approximately 1.5 cm/month (Harkey and Henderson, 1989; Harry's Cosmeticology, 1992). Because hair grows in adults at an average rate of 1.5 cm per month, hair can be examined in segments to detect drug usage at different time periods depending upon the length of the hair (Saitoh et al., 1967).
Figure 3: Cocaine Metabolic Pathway
From: Frank et al., 1988.
Cocaine is very lipophilic and therefore easily crosses biological membranes (Chow et al., 1985; Chasnoff et al., 1987). It is rapidly metabolized either spontaneously or by serum and hepatic cholinesterases to active compounds such as norcocaine and to inactive compounds (BE and ecgonine methyl ester) which are renally excreted (Stewart et al., 1979). The conversion of cocaine to BE occurs in the plasma by spontaneous nonenzymatic hydrolysis. See Figure 3 for metabolic pathway of cocaine. Cocaine is less polar and thus is accumulated in hair more rapidly than BE. However, BE has a longer elimination half-life, and therefore it is likely to eventually reach the same hair levels (Spiehler et al., 1985). Although the mechanisms involved with drug transport into hair are not well-understood, systemic exposure to the drug will determine the amount of the drug deposited in the hair.

At present, hair analysis for drugs has not become a routine test. However, there is growing appreciation of its capabilities, principally because there is the potential for examination over a large timescale, depending on hair length. Also, hair cannot easily be altered by substitution and chemical interference after it has grown.

1.7 Rationale

CYP2D6 may prove to have an important role in abuse liability through its effects on biotransformation (Metabolic Risk Factor Hypothesis). Recently, Tyndale et al. (1997) have provided evidence that the PM genotype is protective against the development of oral opiate dependence. This observation has been attributed to the metabolic risk factor hypothesis. Alternatively, however, it is possible that the PM phenotype may be a trait marker for protection against drug dependence in a more general way, eg. somehow associated with the propensity to become dependent. Testing this hypothesis can be done by testing a drug of abuse not metabolized
by CYP2D6, like cocaine (Tyndale et al., 1991b), and comparing the frequency distribution to healthy non-drug using controls. This would control for the fact that protection is not due to metabolism.

1.8 Objectives

To determine if CYP2D6 genotype protects against drug dependence only for drugs that are substrates or protects in a more general way.

1.9 Hypothesis

1. Metabolic Risk Factor Hypothesis:

There should be no difference between the EM and PM frequencies in cocaine dependent and control populations since cocaine is not a substrate of CYP2D6.

2. Trait Marker Hypothesis:

If CYP2D6 genotype is a marker for dependence risk for another reason than the PM frequency should be less in the cocaine dependent population than in the control group.

1.10 General Strategy

The frequency of CYP2D6 phenotype and genotype was determined in cocaine dependent individuals, and compared to the phenotype and genotype in non-cocaine dependent control populations.
2. MATERIALS AND METHODS

2.1 Subjects

2.1.1 Cocaine Users

The subjects were recruited from the Toronto area through newspaper advertisements in local papers (see Appendix 1), intake and other similar studies at the Addiction Research Foundation, as well as from drug rehabilitation centres, i.e. Harbourlight, Salvation Army.

A total of 60 subjects completed the study (see Table 4 for Inclusion/Exclusion criteria). They were all current or former (within the past 2 years) regular cocaine users. For all subjects cocaine was the primary substance of abuse or dependence, i.e. largest amounts, largest consequences. A brief telephone interview (see Appendix 2) determined initial eligibility. All subjects were unrelated. The study was approved by the Ethics Committee, University of Toronto and Addiction Research Foundation. All subjects gave a written informed consent (see Appendix 3) before participating in the study.

2.1.2 Control Population

The control population consisted of 210 healthy non-cocaine dependent subjects. All subjects were unrelated and all of the subjects were Caucasian (both parents Caucasians). Subjects were recruited from the Toronto area through advertisements posted at the University of Toronto and the Addiction Research Foundation. Most of the subjects were students and staff of the University of Toronto and the Addiction Research Foundation. Spot urine specimens were taken and analysed for several drugs of abuse, including cocaine, by the Toxicology Lab at the Addiction Research Foundation. This urine drug screen confirmed the drug free status of all 210 subjects. The subjects
**Table 4: Inclusion/Exclusion Criteria**

**Inclusion Criteria:**

a) Males or females.

b) 18 - 70 years.

c) Agree to the phenotyping test.

d) Sign the consent.

e) Currently or in the past two years meet DSM-III-R for psychoactive substance abuse or dependence for cocaine.

f) The index drug (cocaine) must be the primary substance of abuse or dependence, i.e. largest amounts. largest consequences.

**Exclusion Criteria:**

a) Known sensitivity to dextromethorphan.

b) Orientals, i.e. Japanese, Chinese.
completed a questionnaire to obtain information on drug use and to confirm that the subjects were non-drug users.

2.2 Data Collection

2.2.1 Study Design

The study involved two visits to the Addiction Research Foundation. Recruited subjects were given an appointment at the Addiction Research Foundation (ARF) where they were given a consent form to sign (see Appendix 3) and asked to complete the "Cocaine questionnaire" (see Appendix 4) to obtain information on demographic characteristics, history of drug use and patterns of use. At this time, subjects also provided a spot urine specimen. Each subject received an "Information to Subjects" sheet explaining the study (see Appendix 5), a 500 mL plastic bottle (Nalgene), a capsule of 30 mg dextromethorphan HBr (Contac® CoughCaps™ DM, SK&B), and an "Instructions" sheet (see Appendix 6). Subjects were instructed to empty their bladder prior to drug ingestion, and take the dextromethorphan capsule with water at bedtime that night. Subsequently for the next 8 hours (overnight) after ingestion of the probe drug, dextromethorphan, they collected all of their urine in the container provided. Subjects returned their urine containers to ARF the following day at their next appointment. On return to the laboratory, the urine volume was measured and recorded. Approximately a 15 mL aliquot was kept frozen in a scintillation vial at -20°C until analysed.

At the second appointment a 20 mL blood sample was drawn from a vein in their arm into a 3.5 mL citrate/glucose buffer. The sample was put in a plastic screw-capped bottle and stored frozen at -70°C for genotyping analysis. A hair sample was also taken from some of the subjects for drug use confirmation and questions were asked regarding their pattern of cocaine use over the
previous two years. With subject cooperation, cocaine use was documented on a time chart, "Assessment Timeline Calendar" (see Appendix 7), for a two year period up to the time of the interview. Subjects were given a consent form to sign for the hair analysis portion of this study (see Appendix 8).

2.2.2 Cocaine Questionnaire

This questionnaire was designed to obtain information on demographic characteristics, history of cocaine use and patterns of cocaine use and other drug use (see Appendix 4). Current and former cocaine and opiate abuse/dependence was diagnosed using criteria from the Diagnostic and Statistical Manual (DSM III-R) of the American Psychiatric Association (1987) (see Appendix 9). The questionnaire had a page for subjects to consent should they want to be contacted regarding participation in another research project or request information about treatment services.

2.3 Data Analysis

2.3.1 Subject Characteristics

Demographic information was obtained from the cocaine questionnaire, ie. age, race and sex. Information was also obtained on cocaine patterns of use and other significant drugs of abuse, ie. cannabis, barbiturates, anxiolytics, stimulants, opiates, as well as alcohol and tobacco.

2.3.2 DSM-III-R Classifications

DSM criteria were used to diagnose substance abuse and dependence for a wide variety of psychoactive substances. For dependence, the subject must have had at least three or more DSM-III-R symptoms present at any time in the same twelve-month period with the symptoms lasting for
at least one month.

2.4 Urine Drug Screen

A spot urine sample was taken from each subject during their first visit. These samples were analysed by the Toxicology Lab at the Addiction Research Foundation (ARF). They were analysed by TLC (thin layer chromatography) for cocaine and ecgonine methyl ester (a metabolite of cocaine). The detection limit for all compounds was 1 ug/mL.

2.5 Phenotyping Procedure By HPLC

2.5.1 Drugs and Chemicals

Contac® CoughCaps™ DM with 30 mg dextromethorphan hydrobromide were purchased from SmithKline Beecham, Weston, Ontario. The dosage used was that recommended for adults ie. 1 caplet every 8 hours as required. None of the subjects reported any side effects.

Dextromethorphan hydrobromide powder and beta-glucuronidase (type H-1, containing sulfatase) were purchased from Sigma Chemical Co., St. Louis, Missouri; dextrorphan tartrate, 3-methoxymorphinan and 3-hydroxymorphinan were obtained from Hoffmann-La Roche Ltd., Nutley, New Jersey; buspirone hydrochloride was obtained from Bristol Myers, Evansville, IN. All other chemicals were of analytical reagent grade.

2.5.2 Chromatographic Conditions

A Hewlett Packard pump and autosampler series 1050 HPLC system was used for the analysis of dextromethorphan and its metabolites, dextrorphan, 3-methoxymorphinan and 3-hydroxymorphinan. The system was attached to an Applied Biosystems 980 Programmable
Fluorescence detector with excitation at 195 nm and emission at 280 nm. A 15 x 0.46 cm, 5 micron phenyl column (Chromatographic Sciences Company, Montreal, Quebec) was used. Buspirone was used as the internal standard. Quantitative analysis, including linear regression analysis of the standard curves and calculation of the concentrations of DEX and each of its metabolites, is carried out using the multilevel calibration procedure offered by the HP 3396 Series II Integrator.

Mobile Phase: The mobile phase used was 10 mM monobasic potassium phosphate (KH$_2$PO$_4$) buffer (1.36 g/L) containing 1.0 mM heptanesulphonic acid (0.22 g/L) with the pH adjusted to 3.8 with 85% orthophosphoric acid:acetonitrile mixture (80:20, v/v). The solution was filtered through a 0.45 um membrane, degassed under vacuum, and used as the mobile phase. The flow rate of the mobile phase was 1.5 mL/min.

2.5.3 Sample Pretreatment

The urine samples were simultaneously analysed for unchanged dextromethorphan, and its metabolites, dextrorphan, 3-methoxymorphinan and 3-hydroxymorphinan according to a modification of the procedure described by Chen et al. (1990) (see Figure 4). Thawed urine (250 mL) was mixed with 250 mL of 0.2 M acetate buffer (brought to pH 5.0 by 3N HCl) containing 20 uL/mL beta-glucuronidase (107200 units/mL suspension). This mixture was incubated in a shaking water bath at 37°C overnight (18 hours) to ensure complete hydrolysis of the conjugates. After this overnight hydrolysis with beta-glucuronidase, the deconjugation reaction was stopped by adding 110 uL of 0.5 N NaOH. The urine sample was adjusted to pH 11-11.5 with the addition of this NaOH. The tubes were vortexed for 5 minutes and 2.5 ug buspirone hydrochloride (25 ug/mL in distilled water) was added as internal standard.

Hexane:ether (4:1, vol/vol) was used as the extracting solvent. 3 mL of this mixture was
added and the tube was vortexed for 5 minutes. The organic and aqueous phases were separated by centrifugation at 3000 rpm for 10 minutes, and the upper organic phase was back-extracted (transferred) to another tube containing 200 uL of 0.01 N hydrochloric acid (HCl). This mixture was vortexed for 5 minutes and the two phases were separated by centrifugation at 3000 rpm for 5 minutes. The top organic layer was removed by aspiration and discarded. The bottom aqueous layer was washed with 1.0 mL ether, vortexed for 5 minutes and centrifuged for 5 minutes at 3000 rpm. After centrifugation, the top ether layer was aspirated and discarded, and a 30 uL aliquot was used for the analysis. The standard and assay preparations were injected into the chromatographic system via the automatic injector, and each chromatogram was allowed to run for 19.5 minutes. This procedure was performed in duplicate for each sample and averages were taken.

2.5.4 Standard Curve

Stock solutions were prepared by dissolving 35.24 mg DEX, 40.76 mg DOR, 33.85 mg 3HM and 32.43 mg 3MM in 10 mL methanol/water (1:1) mixture and stored at 4°C. These solutions were diluted with deionized water and added to drug free urine in order to construct a seven point standard curve.

Final concentrations of the standards ranged from 0.02 to 10 nmoles/mL of DEX, from 0.2 to 120 nmoles/mL of DOR, from 0.2 to 80 nmoles/mL of 3HM and 0.02 to 0.7 nmoles/mL of 3MM. The resulting solutions were treated the same way as the urine samples.
Hydrolysis

0.25 mL Urine
+0.25 mL 0.2M Acetate Buffer
pH 5.0 Containing β-Glucuronidase

Incubate overnight at 37 °C

Stop reaction by adding 110 μL 0.5N NaOH

Vortex 5 minutes

Add 100 μL Buspirone as the Internal Standard

Add 3 mL extractant (hexane/ether 4:1)

Centrifuge 10 minutes at 3000 rpm

Back extract top (organic) layer into 200 μL 0.01 N HCl

Vortex 5 minutes

Centrifuge 5 minutes at 3000 rpm

Aspirate the top organic layer

Wash the bottom layer with 1 mL ether

Vortex 5 minutes

Centrifuge 5 minutes at 3000 rpm

Aspirate off top layer

Inject 30 μL of clean extract

Figure 4: Hydrolysis and Extraction Procedure
2.5.5 Calculations

For each day's analysis, standard curves for DEX, DOR, 3HM and 3MM were plotted as peak area ratio to internal standard vs. the concentration of the corresponding drug or metabolite (in nmoles). Linear regression analysis was performed to calculate the slope, intercept and correlation coefficient.

The identity of the DEX and its metabolites in urine was based on the retention times obtained from the standards. The amount of DEX and each of its metabolites in the subjects' samples was calculated by substituting the peak area ratio into the equations determined by linear regression of the standard curves.

2.5.6 Precision and Accuracy

Precision was evaluated by analysis of a set of standards which were treated as samples. Within-day coefficients of variation (CVs) were determined by running five replicates on one day and between-day CVs were done by running a set of samples on five days. A CV of less than 11% was considered acceptable.

Accuracy was determined from the difference between nominal concentrations and concentrations determined by the HPLC analysis.

2.5.7 Recovery

For each subject the percent of dose excreted as unchanged DEX and each metabolite in urine was calculated. The amount of DEX, DOR, 3HM and 3MM was adjusted for each subject's urine volume, and expressed as a percentage of the dose (mean ± SD) of the parent drug excreted in 0-8 hours.
\[
\% \text{ Metabolite Recovery} = \frac{\text{Metabolite Concentration (nmol/mL)} \times \text{Urine Volume} \times 100\%}{85154.6977 \text{ nmol of DEX administered}}
\]

Total recovery was calculated by the summation of the percent dose of DEX and each of its metabolites.

**2.5.8 Evaluation of CYP2D6 Status**

The metabolic ratio or O-demethylation ratio (ODMR) was used to express CYP2D6 activity. The ODMR value was calculated from the ratio of non-O-demethylated compounds (dextromethorphan plus 3-methoxymorphinan) to O-demethylated metabolites (dextrorphan plus 3-hydroxymorphinan). The logarithms of the ODMR were used to assess the phenotype of the subjects and were plotted as frequency distribution histograms. The frequency distribution of the logarithms of the ODMRs is bimodal with the antimode between -0.5 and 0 (Wu et al., 1992). Thus, poor metabolizers had a log ODMR of >-0.3 and extensive metabolizers had a log ODMR < -0.5.

**2.6 Genotyping Procedure**

This procedure was conducted in 1993 by Kurt Droll and Howard Zhong. Genomic DNA was isolated according to a protocol modified from Sambrook (1989). Genotyping was carried out by allele-specific polymerase chain reaction amplification (ASPCR). Preparation of oligonucleotides for ASPCR was done by a modified version of the procedure described in Sambrook (1989). The \textit{CYP2D6*3} and \textit{CYP2D6*4} mutant alleles were detected by a nested PCR method described by Heim and Meyer (1990).
2.7 Hair Analysis

This analysis was conducted in 1993-1995 by Franca Ursitti. Hair was sampled from the posterior vertex region of the scalp by cutting approximately 8 strands of hair close to the root with scissors. The entire length of the hair was measured. A portion from the root of the hair was mixed with a section from the end and this mixture of hair was analysed for benzoylecgonine (BZ), a major metabolite of cocaine using Abuscreeen radioimmunoassay (RIA) specific for this compound (Hoffman La Roche, Etobicoke, Ontario, Canada). The hair was washed and then assayed as previously described (Graham et al., 1989). For hair washing, the method of Baumgartner and Berka (1989) was followed. The analytical methods followed were from Koren et al. (1992).

2.7.1 Calculations

Results are expressed as concentration of BZ (ng) / (mg) hair. The length of hair was used to calculate the time period represented by the hair sample. An average growth rate of 1.5 cm/month (Saitoh et al., 1967) was assumed. Self-reported use was calculated for the months represented by the hair sample analysed by taking an average of the dose of cocaine (in grams/month) taken during the months represented by the root and the end.

2.8 Statistical Analysis

SAS and JMP (version 2, 1989, SAS Institute Inc.) statistical softwares were used for the statistical analyses. Average data were expressed as mean values ± standard deviation (SD). Chi-square analysis was performed to assess differences among the EMs and PMs in the cocaine dependent group and previously collected data of control groups. Significance was defined as p≤0.05.
Correlations between several characteristics assessed by the cocaine questionnaire and log ODMR were calculated using Pearson correlation coefficients. Characteristics tested included method of cocaine use (by intravenous, inhalation or intranasally), grams of cocaine used per month, frequency of cocaine use per month, gender, age, other drug use, as well as BZ concentrations from the hair analysis.

Correlation between subject-reported use of cocaine (in grams) and hair concentrations of BZ (ng/mg) was studied by least-square regression analysis. A smaller sample of 21 subjects with more accurate recall as determined by the extent of detail and recency of use (Table 14), was further analysed for correlations. These subjects were selected by their ability to remember cocaine use patterns as determined by their interviewer. Those subjects who spent time carefully calculating their use and those who linked cocaine use to certain memorable events were designated as more accurate.
3. RESULTS

3.1 Subject Characteristics

Sixty current and former (within the last 2 years) regular cocaine users were recruited for this study. The study group comprised 11 females and 49 males. The age of the subjects ranged from 19-52 years with a mean age of $32.8 \pm 7.8$ years. All subjects were unrelated, 50 of the subjects were Caucasian (both parents Caucasian), 5 had one Caucasian and one non-Caucasian parent (3 African, 2 Native American), 4 were of African descent and one was of Indo-Pakistani descent.

All sixty current and former cocaine dependent subjects met DSM-III-R criteria for psychoactive substance dependence for cocaine. In their lifetime 10 of the subjects have met DSM-III-R criteria for opiate dependence, which in 7 of 10 cases was heroin dependence.

Table 5 provides various cocaine use characteristics. Of particular interest among characteristics of cocaine use are the percentages of subjects using this drug by various administration routes. Intranasal and inhalation were the most frequent route used, however intravenous use was also noted. Tables 6-8 summarize use of other drugs of abuse by the cocaine dependent population. Table 6 shows use of cannabis, barbiturates, anxiolytics, stimulants, and opiates. Tables 7 & 8 show the use of alcohol and tobacco respectively. Substance use characteristics revealed that many of the subjects had histories of regular cannabis use, followed in descending order by anxiolytics, stimulants, opiates, and barbiturates. High alcohol consumption and tobacco use was also noted.
Table 5: Cocaine use Characteristics of Subjects (n=60)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first cocaine use</td>
<td>22.0 ± 6.6 (20.0) years *</td>
</tr>
<tr>
<td>Age at first regular cocaine use (i.e. &gt; 10 times/month)</td>
<td>25.7 ± 6.4 (25) years *</td>
</tr>
<tr>
<td>Money spent on cocaine in average month</td>
<td>1862 ± 1702 (1000) dollars *</td>
</tr>
<tr>
<td>Grams of cocaine used in average month</td>
<td>67.8 ± 174.7 (20.0) grams *</td>
</tr>
<tr>
<td>Days used in an average month</td>
<td>16.3 ± 11.3 (15) days *</td>
</tr>
<tr>
<td>% of subjects using cocaine intranasally</td>
<td>90%</td>
</tr>
<tr>
<td>% of subjects using cocaine by inhalation</td>
<td>90%</td>
</tr>
<tr>
<td>% of subjects using cocaine by intravenous</td>
<td>62%</td>
</tr>
<tr>
<td>% of subjects using cocaine intranasally and by inhalation</td>
<td>83%</td>
</tr>
<tr>
<td>% of subjects using cocaine intranasally and by intravenous</td>
<td>57%</td>
</tr>
<tr>
<td>% of subjects using cocaine by inhalation and by intravenous</td>
<td>55%</td>
</tr>
<tr>
<td>% of subjects using cocaine intranasally, intravenously &amp; by inhalation</td>
<td>53%</td>
</tr>
<tr>
<td>% of subjects using cocaine daily</td>
<td>45%</td>
</tr>
</tbody>
</table>

* Mean ± SD (median)

Note: For former users the statistics were taken from their period of regular cocaine abuse (within last 2 years).
<table>
<thead>
<tr>
<th>Times Used</th>
<th>Cannabis %</th>
<th>Barbiturate %</th>
<th>Anxiolytics %</th>
<th>Stimulants %</th>
<th>Opiates %</th>
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</thead>
<tbody>
<tr>
<td><strong>Lifetime</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
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<td>43.3</td>
<td>15.0</td>
<td>15.0</td>
<td>3.3</td>
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<td>6.7</td>
<td>6.7</td>
<td>5.0</td>
</tr>
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<td>1.7</td>
<td>8.3</td>
<td>6.7</td>
</tr>
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<td>1.7</td>
<td>6.7</td>
<td>1.7</td>
<td>6.7</td>
<td>11.7</td>
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<td>5.0</td>
<td>13.3</td>
<td>10.0</td>
<td>13.3</td>
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<tr>
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<td>6.7</td>
<td>11.7</td>
<td>11.7</td>
<td>21.7</td>
</tr>
<tr>
<td>40-99</td>
<td>8.3</td>
<td>11.7</td>
<td>15.0</td>
<td>11.7</td>
<td>15.0</td>
</tr>
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<td>35.0</td>
<td>30.0</td>
<td>23.3</td>
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<td>25.0</td>
<td>71.7</td>
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<td>53.4</td>
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<td>90.0</td>
<td>70.0</td>
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<td>8.3</td>
<td>1.7</td>
<td>3.3</td>
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<td>1.7</td>
<td>6.7</td>
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<td>1.7</td>
<td>6.7</td>
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<td>3.3</td>
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<td>3.3</td>
<td>0</td>
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<td>1.7</td>
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</table>
Table 7: Alcohol use of Subjects (n=60)

<table>
<thead>
<tr>
<th></th>
<th>Number of Drinks in a one week period</th>
<th>% of Subjects</th>
</tr>
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<tr>
<td><strong>Lifetime</strong></td>
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<tr>
<td>None</td>
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<td>0</td>
</tr>
<tr>
<td>1-14</td>
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<td>10.0</td>
</tr>
<tr>
<td>15-28</td>
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<td>13.3</td>
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<tr>
<td>29-56</td>
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<td>25.0</td>
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<tr>
<td>57-84</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>85-112</td>
<td></td>
<td>13.3</td>
</tr>
<tr>
<td>&gt;112</td>
<td></td>
<td>28.3</td>
</tr>
<tr>
<td><strong>Past Year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0</td>
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<tr>
<td>1-14</td>
<td></td>
<td>25.0</td>
</tr>
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<td>15-28</td>
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<td>29-56</td>
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<td>8.3</td>
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<td>16.7</td>
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<td>85-112</td>
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<td>5.0</td>
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<tr>
<td>&gt;112</td>
<td></td>
<td>1.7</td>
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</table>
Table 8: Tobacco use of Subjects (n=60)

<table>
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<tr>
<th></th>
<th>Cigarette Packs Smoked in a one week period</th>
<th>% of Subjects</th>
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<td>20-39</td>
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<td>1.7</td>
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<tr>
<td>40+</td>
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<tr>
<td><strong>Last 30 Days</strong></td>
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<tr>
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</table>
3.2 Urine Drug Screen

The TLC tests for cocaine and ecgonine methyl ester on the spot urine samples collected from the subjects were negative for all subjects (ie. cocaine dependent and control population).

3.3 Phenotyping

DEX, DOR, 3HM and 3MM all exhibited a linear relationship between peak area and concentration within the range described by the standard curve. The standard curve for DEX is linear between 0.02 nmol/mL and 10 nmol/mL, DOR is linear between 0.2 nmol/mL and 120 nmol/mL, 3HM is linear between 0.02 nmol/mL and 80 nmol/mL and 3MM is linear between 0.02 nmol/mL and 0.7 nmol/mL. Linear regression analysis gave correlation coefficients of \( r > 0.99 \). Sample standard curves are included for DEX, DOR, 3HM and 3MM in Figure 5.

Analysis of a set of standards which were treated as samples \((n=5)\) showed within day and between day coefficients of variations <11\% (Table 9). These values are within the ranges of variability reported by other investigators (Larrey, et al., 1987; Chen et al., 1990; Freche et al., 1990). DEX and its three metabolites (DOR, 3HM, 3MM) were separated on a phenyl column and detected by fluorescence within 20 minutes after injection of the urine sample in a single HPLC run. A sample chromatogram is included in Figure 6. The conditions resulted in retention times of 3.8 min for 3HM, 5.5 min for DOR, 7.7 min for 3MM and 11.7 min for DEX and 15.9 min for the internal standard (buspirone). One subject's urine sample was eliminated from the study since DEX and its metabolites were not measurable in his urine sample, thus it can be assumed that the DEX capsule was not taken as expected.

A summary of results of DEX, DOR, 3HM and 3MM 8 hour urinary recoveries and log ODMRs for the control and cocaine population are presented in Table 10. HPLC analysis of the
Figure 5  Sample standard curves extracted from urine: a-DEX, b-DOR, c-3HM, d-3MM
The abscissa is concentration and the ordinate is peak area ratio. Linear regression analysis gave correlation coefficients of $r>0.995$. 
Table 9: Between day and within day accuracy and precision

<table>
<thead>
<tr>
<th></th>
<th>Expected nmol/ml</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>CV%</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>CV%</th>
</tr>
</thead>
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<tr>
<td><strong>DEX</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.02</td>
<td>0.027</td>
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<td>7.5</td>
<td>0.024</td>
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<tr>
<td>2</td>
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<td>0.003</td>
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<td>5.1</td>
<td>40.00</td>
<td>2.449</td>
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<td>7</td>
<td>120</td>
<td>137.13</td>
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<td>8.0</td>
<td>146.63</td>
<td>15.89</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>3HM</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.2162</td>
<td>0.023</td>
<td>10.6</td>
<td>0.217</td>
<td>0.023</td>
<td>10.6</td>
</tr>
<tr>
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<td>2.057</td>
<td>0.1836</td>
<td>8.9</td>
<td>1.998</td>
<td>0.128</td>
<td>6.4</td>
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<td>0.3214</td>
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<td>3.772</td>
<td>0.264</td>
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</tr>
<tr>
<td>5</td>
<td>20.0</td>
<td>20.216</td>
<td>1.216</td>
<td>6.0</td>
<td>19.64</td>
<td>1.114</td>
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</tr>
<tr>
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<td>2.6526</td>
<td>5.9</td>
<td>39.03</td>
<td>1.433</td>
<td>3.7</td>
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<td>7</td>
<td>80</td>
<td>78.524</td>
<td>6.2938</td>
<td>8.0</td>
<td>78.67</td>
<td>8.249</td>
<td>10.5</td>
</tr>
</tbody>
</table>

|     | **3MM**          |       |                    |      |       |                    |      |
| 1   | 0.02             | 0.0264| 0.002              | 7.6  | 0.026 | 0.002              | 7.7  |
| 2   | 0.04             | 0.067 | 0.005              | 7.5  | 0.061 | 0.004              | 6.6  |
| 3   | 0.2              | 0.2164| 0.0193             | 8.9  | 0.221 | 0.021              | 9.5  |
| 4   | 0.3              | 0.3194| 0.0112             | 3.5  | 0.288 | 0.020              | 6.9  |
| 5   | 0.4              | 0.4338| 0.0385             | 8.9  | 0.387 | 0.027              | 7.0  |
| 6   | 0.6              | 0.6088| 0.0455             | 7.5  | 0.5944| 0.041              | 6.9  |
| 7   | 0.7              | 0.7178| 0.0288             | 4.0  | 0.6372| 0.064              | 10.0 |
Figure 6: Sample HPLC Chromatogram of an EM subject (log ODMR= -1.38). From urine sample collected 8 hours post-dose of the probe substrate DEX. For better graphic representation, the peaks were magnified by a factor of 4. Retention times in brackets (minutes).

Min nmol/ml

- Peak 1 3HM (3.842) = 48.451
- Peak 2 DOR (5.499) = 44.773
- Peak 3 3MM (7.666) = 0.184
- Peak 4 DEX (11.676) = 3.766
- Peak 5 IS (15.816)
control population was done previously in 1992 by Linda Sunahara and Ewa Hoffmann. Analysis of urine samples after ingestion of 30 mg DEX found marked differences between EMs and PMs with regard to the recovery of DEX and each of its metabolites (see Table 10). In urine samples of EMs, considerable amounts of DOR and 3HM were recovered, whereas 3MM and DEX recoveries were rather low. In PMs, DOR and 3HM recoveries were low and the predominant recoveries were 3MM and DEX. The mean total urinary recovery calculated for the EM population in the control group (26.68% ±18.05) was found to be much lower than that of the EMs in the cocaine population (54.30% ± 34.67). Using a t-test, a significant difference was found between the total recovery in the EM populations of the cocaine dependent population compared to those in the control group (p< 0.05).

The frequency distribution histograms of the log ODMR in 210 healthy control subjects and the 59 cocaine dependent subjects are shown in Figures 7 and 8. The distribution of the control group is bimodal with 15 of 210 (7%) having the PM phenotype (log ODMRs > -0.3) and 95 of 210 (93%) having the EM phenotype (log ODMRs < -0.5). The distribution of the cocaine population is unimodal with no PMs (0%) and all 59 subjects (100%) having the EM phenotype. The log ODMRs in the control population ranged between -3.62 and -0.64 (mean -2.35 ± 0.60) in EMs and between 0.1 and 0.88 (mean 0.46 ± 0.20) in PMs (see Table 10). In the cocaine dependent population, the log ODMRs for the EMs ranged between -4.34 and -0.72 with a mean of -2.46 ± 0.77. Using the chi square test, a significant difference was found between the proportions of EMs and PMs in the cocaine dependent and control groups (p<0.05). A significant difference was also found by chi-square analysis (p<0.05) when several Caucasian control groups (Table 2) were compared with the proportions of EMs and PMs in the cocaine dependent population from this study.

Pearson correlation coefficients were insignificant for all subject characteristics compared
Table 10: Summary of dextromethorphan metabolism among subjects.

The values are given are means ± SD. The recoveries are given as percent of dose.

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Control Subjects (Never cocaine dependent)</th>
<th>Cocaine Dependent Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EM</td>
<td>PM</td>
</tr>
<tr>
<td>Number of Subjects</td>
<td>195</td>
<td>15</td>
</tr>
<tr>
<td>Phenotype prevalence (%)</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>DEX recovery (%)</td>
<td>0.22±0.45</td>
<td>2.15±0.94</td>
</tr>
<tr>
<td>DOR recovery (%)</td>
<td>21.18±11.51</td>
<td>0.59±0.32</td>
</tr>
<tr>
<td>3HM recovery (%)</td>
<td>7.26±7.78</td>
<td>0.24±0.15</td>
</tr>
<tr>
<td>3MM recovery (%)</td>
<td>0.02±0.03</td>
<td>0.16±0.08</td>
</tr>
<tr>
<td>Total recovery (%)</td>
<td>26.68±18.05</td>
<td>3.13±1.24</td>
</tr>
<tr>
<td>Range of log ODMR</td>
<td>-3.62 to -0.64</td>
<td>0.1 to 0.88</td>
</tr>
<tr>
<td>Mean ± SD of log ODMR</td>
<td>-2.35 ± 0.60</td>
<td>0.46± 0.20</td>
</tr>
</tbody>
</table>
Figure 7: Frequency Distribution Histogram of Control Subjects (n=210)

Figure 8: Frequency Distribution Histogram of Cocaine Dependent Subjects (n=59)
to the log ODMR. Thus the log ODMR does not seem to be affected by the characteristics tested, including gender, age at first use, age at regular use, methods of use (inhalation, intranasally, or intravenously), days of use per month, money spent per month, grams used per month and concentration of BZ in hair samples.

3.4 Genotyping

Tables 11 and 12 give the genotypic distributions of $CYP2D6^*$1 and mutant $CYP2D6^*$3 and $CYP2D6^*$4 alleles in the cocaine dependent population. Of the 59 cocaine dependent subjects, 59.3% were homozygous $CYP2D6^*$1/$CYP2D6^*$1, 40.7% were heterozygous with one mutant allele and one nonmutant allele, i.e. 1.7% were $CYP2D6^*$1/$CYP2D6^*$3 and 39.0% were $CYP2D6^*$1/$CYP2D6^*$4. Thus 100% of the cocaine dependent group had the $CYP2D6^*$1 allele and are considered EMs.

Table 12 compares the genotype results of the cocaine dependent population to three Caucasian control groups from the literature (Agundez et al. 1994; Tefre et al., 1994; Wolf et al., 1992). While 100% of the cocaine dependent group were designated as EMs by their genotype, the average EM genotype in the three control groups was 95%, with 5% designated as the PM genotype. Using chi square analysis, a significant difference was found between the EM and PM genotypes of the cocaine dependent population and the control groups ($p<0.05$). Figure 9 shows the frequency distribution of log ODMRs by genotype in the cocaine dependent subjects. The allele frequencies in the cocaine dependent population were as follows: 79.7% for the $CYP2D6^*$1 allele, 19.5% for the $CYP2D6^*$4 allele and 0.8% for the $CYP2D6^*$3 allele (Table 13).
**Table 11:** Genotype of Cocaine Dependent subjects by PCR (n=59)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6<em>1/CYP2D6</em>1 (EM)</td>
<td>35</td>
<td>59.3</td>
</tr>
<tr>
<td>CYP2D6<em>1/CYP2D6</em>3 (EM)</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>CYP2D6<em>1/CYP2D6</em>4 (EM)</td>
<td>23</td>
<td>39.0</td>
</tr>
<tr>
<td>EM genotype</td>
<td>59</td>
<td>100</td>
</tr>
<tr>
<td>PM genotype</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 12:** Genotypic distribution of CYP2D6 alleles in Cocaine-dependent subjects and controls from literature

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotype Frequency (% total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CYP2D6<em>1/CYP2D6</em>1(EM)</td>
</tr>
<tr>
<td>Cocaine-dependent subjects</td>
<td>59</td>
<td>59.3 (35/59)</td>
</tr>
<tr>
<td>Non-drug using controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>258</td>
<td>72</td>
</tr>
<tr>
<td>b</td>
<td>118</td>
<td>59</td>
</tr>
<tr>
<td>c</td>
<td>720</td>
<td>63.8</td>
</tr>
<tr>
<td>Average of Controls</td>
<td></td>
<td>64.9</td>
</tr>
</tbody>
</table>

a Agundez et al., 1994, b Tefre et al., 1994, c Wolf et al., 1992

**Table 13:** CYP2D6 Allele Frequencies for Cocaine Dependent Subjects

<table>
<thead>
<tr>
<th>Allele Frequency (n, % total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>59</td>
</tr>
</tbody>
</table>
Figure 9: Frequency distribution of log ODMRs by Genotype in Cocaine Dependent Subjects (n=59)
3.5 Hair Analysis

A total of 37 hair samples from the cocaine dependent population who volunteered complete history of their cocaine use were analysed (Table 14). All hair samples were positive for BZ, confirming that the DSM-III-R dependence diagnosis was correct in all cases. The concentration of BZ ranged between 0.019 ng/mg hair and 7 ng/mg hair (mean 2.96 ± 4.18) (see Figure 10 for Frequency Distribution Histogram). The number of months represented by the length of the hair ranged between 2.2 to 22.7 (mean 9.07 ± 5.49). Subjects reported use of cocaine during the time which reflected the hair sample analysed ranged from 0 to 56 grams in one month (mean 12.05 ± 14.92).

A linear regression model was fit to two data sets to test the reliability of self-reported data (see Figure 11 and 12). An R-square of 0.0567 was obtained using a data set of all subjects (n=37) (Figure 11) and an R-square of 0.634 using a data set of only subjects thought to have accurate recall (Figure 12) of their cocaine use (n=21) (see * in Table 14). The improvement in the R-square from the complete to the more restricted data set indicates the potential accuracy of self-reported data. The t statistic of the estimated coefficient also confirmed the accuracy of self-report in the restricted data set. In order to be a reliable indicator of self-reported use, BZ (ng/mg) should have a significant and positive coefficient estimate. The results in the restricted data set confirm this hypothesis. BZ ng/mg hair is positive and significant at p<0.01 (t ratio = 5.73). It should be noted that the potential accuracy of self-reported data in subjects who were thought to have accurate recall may be influenced strongly by the results of a single individual (Figure 12).
Table 14: Cocaine use of subjects through hair analysis (n=37)

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Concentrations of BZ (ng/mg)</th>
<th>Time represented by hair (months)</th>
<th>Subject reported use of cocaine (g)</th>
<th>Accurate Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.641</td>
<td>10.7</td>
<td>2.25</td>
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</tr>
<tr>
<td>2</td>
<td>6.594</td>
<td>15.7</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.371</td>
<td>10.1</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.6</td>
<td>7.53</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>7.345</td>
<td>10</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>16.36</td>
<td>13</td>
<td>47.5</td>
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</tr>
<tr>
<td>13</td>
<td>10.25</td>
<td>12.7</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.07</td>
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<td>*</td>
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<td>3.67</td>
<td>1.13</td>
<td>*</td>
</tr>
<tr>
<td>21</td>
<td>0.19</td>
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<td>9.13</td>
<td>*</td>
</tr>
<tr>
<td>24</td>
<td>1.33</td>
<td>4.4</td>
<td>4.5</td>
<td>*</td>
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<tr>
<td>25</td>
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<td>8.8</td>
<td>13.5</td>
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<td>36</td>
<td>17</td>
<td>22.7</td>
<td>10</td>
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<td>2.5</td>
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<td>5</td>
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<td></td>
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<td>7.961</td>
<td>9</td>
<td>43.3</td>
<td>*</td>
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<td>9</td>
<td>*</td>
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<td>2.5</td>
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<td>0.71</td>
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<td>0.771</td>
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<tr>
<td>49</td>
<td>1.31</td>
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<td>7</td>
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<td>50</td>
<td>1.612</td>
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<td>0.7</td>
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<td>1.75</td>
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<td>54</td>
<td>0.652</td>
<td>10.3</td>
<td>1</td>
<td>*</td>
</tr>
<tr>
<td>55</td>
<td>0.751</td>
<td>4.8</td>
<td>7</td>
<td>*</td>
</tr>
<tr>
<td>56</td>
<td>1.69</td>
<td>3.33</td>
<td>5.5</td>
<td>*</td>
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<tr>
<td>57</td>
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<tr>
<td>59</td>
<td>6.521</td>
<td>6.33</td>
<td>16.5</td>
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</tr>
<tr>
<td>60</td>
<td>0.753</td>
<td>18</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

| Mean ±SD  | 2.96 ± 4.18                 | 9.07 ± 5.49                      | 12.05 ± 14.92                    | Total = 21      |
| Range     | 0.019 - 7                   | 2.2 - 22.7                       | 0 - 56                           |                  |

Note: Subject reported use of cocaine is the average use during the most recent month and the first month as represented by the hair sample for each subject.
Figure 10: Frequency Distribution Histogram of Benzoylcegonine Concentrations (ng/mg) in Hair Samples (n=37)
Figure 11: Linear regression model for BZ concentrations vs. Self-report for All subjects (n=37)
\[ R^2 = 0.0567 \]

Figure 12: Linear regression model for BZ concentrations vs. Self-report for Reliable Subjects Only (n=21)
\[ R^2 = 0.634 \]
4. DISCUSSION

4.1 Discussion of Results

4.1.1 Subject Characteristics

DSM-III-R criteria for drug dependence were revised to DSM-IV criteria by the American Psychiatric Association in 1994 (see Appendix 1). These changes did not affect the determination of drug dependence in this study, as all 60 subjects were found to meet both DSM-III-R and DSM-IV cocaine dependence criteria as determined by the criteria assessed by the cocaine questionnaire (Appendix 4).

There are several potential limitations which must be considered in this study in terms of the characteristics of the subjects in the cocaine-dependent population. Ten of the 60 subjects also met DSM-IV criteria for opiate dependence. As indicated on their cocaine questionnaires, 7 of the 10 subjects limited their use of opiates to heroin. The remaining three subjects indicated use of several opiates, including codeine, oxycodone and hydrocodone. Heroin, like cocaine, is not a substrate of CYP2D6. This study was designed to assess whether the PM phenotype protection against drug dependence was limited to substrates of CYP2D6, thus being linked to metabolism, or whether it provides some other general protection factor against drug dependence. Hence, the three subjects who indicate use of other opiates which are substrates of CYP2D6 could have been excluded from the study as their use of opiates which are substrates of CYP2D6 conflicts with the purpose of this study. However, even if this is done the frequency of EMs and PMs between cocaine and never dependent controls is still significant using a chi-square test. That is, the metabolic risk factor hypothesis for the apparent protection against dependence offered by the PM genotype for these subjects cannot be ruled out.
Due to the diversity of ethnic backgrounds found in Toronto, the study was not restricted to white Caucasians. Although 50 of the subjects were Caucasian (both parents Caucasian), 5 had one Caucasian and one non-Caucasian parent (3 African, 2 Native American), 4 were of African descent and one was of Indo-Pakistani descent. Due to the variation in the frequencies of PMs found in various cultural groups, each ethnic group should be assessed separately. It is possible that the absence of the PM genotype in some of these subjects is simply a function of their ethnicity. Thus, to eliminate another possible confounding variable, perhaps these subjects should have been excluded from the study.

As Tables 6, 7 and 8 show, the cocaine dependent subjects seem to be polysubstance users. Although dependence was not assessed by DSM-IV criteria for substances other than opiates and cocaine, it is possible that dependency was met for other substances, eg. tobacco, marijuana. In this study it was important to control strictly for dependency to non-substrates of CYP2D6 in order to test the hypothesis. It is difficult to ascertain with complete certainty, from the data obtained for the subjects in the cocaine dependent population, that the subjects were not dependent on substrates of CYP2D6.

The control group used in this study was assessed to have never been dependent on cocaine. This population may or may not have tried cocaine. Perhaps a better control group would be subjects who have tried cocaine, but never progressed to chronic use. According to the trait marker hypothesis, if the PM genotype does offer protection against dependency, the prevalence of PMs in a population like this should be fairly high. However, 100% prevalence of the PM genotype would not be expected since there would be other factors which are also involved with the development of cocaine dependency. A control population like this may provide more conclusive evidence to support the trait marker hypothesis.
4.1.2 Phenotyping

The HPLC assay used in this study allows analysis of DEX and its metabolites from urine in a single run within 20 minutes with high precision, accuracy and recovery. The HPLC method used exhibited recovery rates similar to those reported for other assays (Park et al., 1984; Johansson et al., 1988; Chen et al., 1990).

The assay was used to determine the CYP2D6 phenotype of sixty cocaine dependent subjects and 210 control subjects following the oral ingestion of 30 mg dextromethorphan and the collection of urine for eight hours. Log ODMRs less than -0.5 were considered EMs and ratios greater than -0.3 were considered PMs. Based on these criteria 7% of the control population were found to be PMs, in contrast with 0% in the cocaine dependent population.

The percentages of EMs (93%) and PMs (7%) in the control population displayed a bimodal distribution and are consistent with previously published reports (Evans et al., 1980; Steiner et al., 1985). The frequency distribution histograms of the log ODMR for the cocaine dependent group exhibited similar patterns with EMs in the control group, taking into account that the number of cocaine subjects was less than that of the control subjects (lower bar graphs) (Figures 7 and 8). There was a significant difference found between the proportion of EMs and PMs in the cocaine dependent and control populations as determined by chi-square analysis. Thus, the absence of CYP2D6 (PM) may be a reliable phenotypic trait marker for the protection against the development of cocaine dependence.

It is interesting to note that the mean total urinary recoveries in the EM population of the cocaine dependent group was found to be significantly higher than that of the EM population of the control group as assessed by chi-square analysis (Table 10). It appears that the metabolism and excretion of DEX to each of its metabolites is enhanced in the cocaine dependent EMs. Since most
of the cocaine dependent subjects are polysubstance abusers, this effect may be a factor of drug interactions with the enzymes responsible for metabolizing DEX, CYP3A and CYP2D6.

The lower end of the range of log ODMRs for the EMs in the cocaine dependent subjects seemed to be lower than that of the control population (-4.34 vs. -3.62) (Table 10, Figures 7 and 8). Using a chi-square test the difference between the medians of the log ODMRs of the EMs in the two populations was not significant. Several other researchers have found values for control populations within the range described here for the cocaine-dependent EM group (Chen et al., 1990; Droll K, 1996).

The effects of smoking, gender and age were not controlled for as they have been found to have no influence on the log ODMR (Cholerton et al., 1996; Hildebrand et al., 1989; Steiner, et al., 1985,1988). Drug intake and the collection of urine for eight hours were conducted without supervision and thus could be a source of error due to incomplete sampling. However, the log ODMR is not affected by incomplete sampling, since it has been found to be constant at different intervals after dosing (Kupfer et al., 1986).

4.1.3 Genotyping

The cocaine dependent population was genotyped for the CYP2D6*3 and CYP2D6*4 defective mutant alleles, as well as the CYP2D6*I wildtype allele. Using PCR analysis, 58% of the cocaine dependent subjects were found to be homozygous dominant (CYP2D6*I/CYP2D6*I) and 42% were heterozygous dominant (CYP2D6*I/mut), yielding EM genotypes in all 59 subjects (100%) (Table 11). This was in contrast to several control populations from the literature yielding average EM genotypes of 95% and PM genotypes of 5% (Table 12). The complete absence of PMs in the cocaine dependent population suggests that the PM genotype may serve as a genotypic trait
marker for the protection against the risk of cocaine dependence.

Three variant alleles, *CYP2D6*3, *CYP2D6*4 and *CYP2D6*5, have been reported to account for about 90% of the mutant CYP2D6 alleles in Caucasians (Saxena et al., 1994), and there are several additional mutant alleles that have been identified (Daly et al., 1996). Although the deletion variant *CYP2D6*5 and other mutant alleles were not studied, CYP2D6 phenotyping was also used to categorize subjects as EMs and PMs. PM subjects that may have been misclassified by the genotyping method would have been identified by the phenotyping procedure. Mutant allele frequencies (*CYP2D6*3 and *CYP2D6*4) (Table 13) may not be completely accurate (may be slightly high) using this technique since all variant alleles were not tested.

4.1.4 Hair Analysis

A spot urine sample was initially used to verify use by using TLC to measure cocaine, BZ and ecgonine methyl ester, however these tests came out negative for all samples assessed due to the short half-life of these compounds. Thus, the traditional urine analysis for cocaine detection was replaced in this study by hair analysis. Unfortunately, although attempts were made to contact all subjects who completed the study prior to the use of the hair analysis method, only 37 of the 60 subjects had hair samples taken. This drug testing method was useful in confirming cocaine use with all hair samples tested having measurable BZ concentrations. This method is especially useful for testing of former users (within 2 years) of cocaine, whose use could not be verified by any other method (ie. urine and blood). Hair remains positive for BZ throughout its entire life cycle, and thus depending on the length of hair it could verify use of cocaine for many months in the past. It was necessary to verify cocaine use because of the financial incentive being provided to subjects.

BZ was used as a measure of cocaine use in hair samples rather than cocaine itself because
external exposure may affect levels of cocaine detectable in hair. It has been shown that when hair is contaminated with smoke from the equivalent of 5000 lines of crack in a small unventilated room, minimal cocaine is detectable even after washing, but no BZ could be found (Koren et al., 1991). Since this population is probably exposed to cocaine in its environment, BZ hair concentration is a much more accurate measure of use.

The data suggest that accumulation of BZ in hair does not have good correlation to subject reported use in the population studied. This confirms the previously mentioned inaccuracy of self report. However, when a subset of the sample that was determined to have fairly accurate recall was isolated the correlation to self report was much better. However this conclusion could be attributed to the result in a single subject. The inaccuracy of recall may be due to the length of time that was being studied (depending on the length of the hair sample) or due to the impurity of cocaine used. However in Toronto, cocaine is usually over 95% pure and contains very little adulteration (Forman et al., 1992).

Thus, BZ concentrations in hair were found to be a better verification of cocaine use than urine tests and self-reported history.

4.2 Conclusions

In the cocaine dependent group, there were no genotypically (homozygous for CYP2D6 variant alleles) or phenotypically CYP2D6 PMs. This resulted in a significant difference in the frequency of the PM genotype between the cocaine and control population (0% vs 7%), as well as other control populations from the literature.

According to the metabolic risk factor hypothesis, since cocaine is not a substrate, EMs and PMs should be equally represented. However PMs are underrepresented in the cocaine dependent
group. This result suggests that the CYP2D6 defective genotype confers some type of selective trait upon PMs that protects them from developing dependency. A CYP2D6 PM appears less likely to develop dependency on cocaine than an EM who has the active enzyme. Thus, this study shows that CYP2D6 PM phenotype and genotype may serve as a genotypic trait marker for protection against developing cocaine dependence.

4.3 General Discussion

Since cocaine is not metabolized by CYP2D6, the apparent protective role of its absence may be as a marker of some general propensity to become dependent. At present there is no explanation for the protection offered by the PM genotype. Several possible reasons, however, could be suggested. The variant alleles of the PM genotype may be linked to alleles that protect against the development of cocaine dependency by affecting dopamine reinforcement, alleles that cause rapid metabolism to inactive metabolites or alleles that protect from addictive or dependent behaviour in general.

Recently, the CYP2D6 enzyme (or a homolog) has been found in the brain of rats and humans (Fonne-Pfister et al., 1987) and in the brain of dogs (Niznik et al., 1990; Tyndale et al., 1991). Evidence for the presence of CYP2D6 in the brain has been found from radioligand binding, western blotting, immunoprecipitation techniques, activity studies (Niznik et al., 1990; Fonne-Pfister et al., 1987) and molecular studies (Tyndale et al., 1991). CYP2D6 messenger ribonucleic acid has been found in human caudate (Tyndale et al., 1991). Although the amount of CYP2D6 enzyme in the brain is very low, its absence may be of great significance. CYP2D6 may be involved with drug metabolism in the CNS. The oxidation of pro-drugs to their active metabolites may actually occur near receptor sites in the brain. Recent work with codeine suggests its conversion to morphine in
the brain may significantly affect the CNS morphine levels (Chen et al., 1990). An interesting observation is that (-)-cocaine binds both the dopamine transporter and CYP2D6 in the brain. CYP2D6 appears to be an important binding site for cocaine in brain (Niznik et al., 1990). Cocaine displayed a Ki of 74 nM for the canine striatal CYP2D6, which is higher than that observed for the dopamine transporter (Niznik et al., 1990; Javitch et al., 1983). It would be interesting to know how the absence of this binding site in PM subjects might affect the action of cocaine. It has been suggested that the absence of CYP2D6 might deprive cocaine of a site of action, or it might allow an increased presence and action of cocaine elsewhere (Niznik et al., 1990).

GBR-12909 is a dopamine transporter blocker that binds with high affinity in canine brain membranes equally to the dopamine transporter and to CYP2D6 (Niznik et al., 1990). Compounds that interact with the neuronal dopamine transporter appear to display significant overlap in substrate specificity with CYP2D6 (Niznik et al., 1990). The significance of the binding similarities between CYP2D6 and the dopamine transporter is unknown. It may have no significance at all, however, in view of the fact that there are similar binding affinities and also since cocaine binds both the dopamine transporter and CYP2D6 in the brain, it would be interesting to explore this association as a potential reason for the PM genotype serving as a trait marker for protection against drug dependence.

Cocaine inhibits dopamine reuptake (Boja et al., 1989; Bosy et al., 1989) and increases the levels of dopamine in synaptic regions of the dopamine neuronal terminals (Carboni et al., 1989). DA neurotransmission in the nucleus accumbens has been shown to be essential for the acute reinforcing effects of cocaine (Parsons et al., 1993, 1996), because lesions of DA terminals in this region abolish cocaine self-administration (Caine and Koob, 1994), whereas the local administration of DA antagonists increase cocaine intake (Maldonado et al., 1993; Caine et al., 1995). Other
support for this conclusion comes from structure-activity studies, studies of antagonism of the reinforcing effects of these drugs, and anatomical studies (Koob and Bloom, 1988). In view of the well established importance of the dopaminergic system in cocaine reinforcement and because of the molecular relationship previously described between the dopamine transporter and CYP2D6 in the brain, the absence of CYP2D6 could protect against development of cocaine dependence by affecting brain functions important in maintaining drug reinforced behavior.

It is possible that individuals with the PM phenotype have an unpleasant first experience so they never progress to continuous use. So the PM phenotype could be offering protection by adversely affecting individuals to prevent them from becoming chronic users.

Another possibility is that certain CYP2D6 linked personality traits may affect drug taking behaviour, hence reducing the risk of PM individuals to developing cocaine dependency. The CYP2D6 PM phenotype has been proposed by researchers (Bertilsson et al., 1989; Llerena.. 1993) to explain personality traits related to alertness, high vitality, efficiency, ease of decision making and anxiety. Such CYP2D6-related personality traits have also been suggested by other researchers to potentially be associated with drug taking behaviours (Boustead et.al., 1997; Tyndale et al, 1997).

It is possible that the absence of PMs in the cocaine dependent population is a drug selection phenomenon. Drug abusing PMs may be more likely to choose a drug of abuse whose deactivation is CYP2D6-dependent, further providing evidence to support the metabolic risk factor hypothesis. This may reduce their chances of abusing another drug, thus creating a situation where there is a complete absence of PM individuals in a drug-using population of a non-substrate of CYP2D6. In this case the lack of PMs demonstrated in this study may actually be support for the metabolic risk factor hypothesis.

On the other hand, the sample size for this study is modest. 100% of the cocaine dependent
subjects carried at least one CYP2D6 wildtype allele (thus being classified as EMs), however, with a larger sample size I may not have obtained such conclusive results. Based on the allele frequencies for the cocaine dependent group (Table 13), the PM frequency should be 4%. Although, a statistically significant difference was found between the EM and PM proportions in the cocaine dependent and control populations, it is possible that with a larger sample size for the cocaine dependent population, PMs would be found. Thus the results of this study may be due merely to a chance phenomenon.

Alternatively, the complete absence of PMs in the cocaine dependent population may not be due to a protective effect at all, but rather the PM genotype may be a risk factor to those who abuse or develop dependency to cocaine. In this case, PMs who use cocaine would either not survive, or would be intolerant to the effects of cocaine, and thus would be absent from a cocaine using population. This could be due to the linkage of variant alleles with an enzyme that produces a toxic metabolite of cocaine, linkage to alleles that attenuate the effects of cocaine to dangerous levels or linkage to alleles which prevent detoxification of cocaine. Hence, in a cocaine using population, the PM genotype may increase the likelihood of experiencing adverse reactions or may simply be a risk factor to surviving. For this suggestion to be possible, deaths due to cocaine at doses which do not approach overdose levels should be looked at in an epidemiological-type study. These deaths should be related to the individual's CYP2D6 phenotype if possible. Since phenotyping data would probably not be available, reactions to substrates of CYP2D6 used by those individuals may provide some insight.
4.4 Future Studies

A similar study involving a larger sample size should be conducted in order to confirm the results of this study. The limitations presented by this study should be controlled for, in terms of opiate and other drug dependency and ethnic variation of subjects. An ideal control group could be used consisting of non-cocaine dependent subjects who have tried cocaine at least once.

The trait marker hypothesis could be further confirmed by using subjects dependent on other non-substrate drugs of abuse, eg. alcohol. The absence of CYP2D6 may serve as a trait marker for protection against drug dependent behaviour in general. However, nicotine also is not a substrate of CYP2D6 (Flamang et al., 1992; Benowitz et al., 1996), and studies have shown that CYP2D6 genotype does not influence whether an individual smokes (Cholerton et al., 1996).

If the presence of variant alleles does provide some protection against developing cocaine dependence, it could be postulated that heterozygote EMs would also have some protection. With a larger sample size, differences in cocaine dependent behaviour between heterozygote and homozygote EMs could be studied.

It is important to realize that this study had a majority of Caucasian subjects. Therefore, these findings may not apply to other populations. Further studies are required on individual populations.

A study actually administering cocaine and studying the reactions of EMs and PMs to cocaine use would be very interesting. Responses of the EMs and PMs could be compared.

In vivo pharmacogenetic studies have suggested that the monkey may be an animal model for the human polymorphism of CYP2D6 (Jacq et al., 1988b). A high degree of similarity has been observed between CYP2D6 of monkey and human liver microsomes. Self-administration of drugs by monkeys is a well-validated model of human drug taking behaviour (Griffiths, et al., 1979).
Thus, the monkey may be a useful non-human primate model of the CYP2D6 polymorphism to investigate the role of CYP2D6 in drug dependency, especially in studies that can't meet ethical approval with human subjects.

It has been suggested that CYP2D6 dependent personality traits may be responsible for certain drug taking behaviours (Boustead et al., 1997; Tyndale et al., 1997). It would be interesting to study the relationship between drug taking personality traits and the EM and PM phenotype.
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APPENDIX 1
The Clinical Research and Treatment Institute, a fully affiliated teaching hospital of the University of Toronto and the Addiction Research Foundation, is conducting a research project which involves regular cocaine users.

- COMPENSATION -

All information will be kept strictly confidential.

For Further Details, Call 595-6107.
APPENDIX 2
COCaine STUDY

Information for Telephone Screening

1. Complete the telephone screening questionnaire.

2. If they are eligible, give them the following information:

We are interested in the way people metabolize (or break down) cocaine. There may be a difference in the way people handle cocaine.

To determine this, we do a simple test with dextromethorphan (the DM in cough syrups). We ask you to take 1 caplet at bedtime and collect your urine for the next 8 hours in a bottle we provide. You may experience a little drowsiness from the DM, but this will be gone by morning.

We also ask for blood to be taken in order to confirm the results of the urine test. A hair sample is also taken for the same reason.

The other part of the study is a survey we ask you to complete. This asks questions about your use of cocaine.

So, two visits to the Addiction Research Foundation are required. At Visit 1 all the instructions will be explained, I will give you the DM capsule and the urine bottle, you will fill out the cocaine questionnaire and provide a spot urine sample. At Visit 2 you will return the urine bottle, and the blood and hair samples will be taken. Also some questions will be asked regarding your pattern of cocaine use.

All of this information is kept strictly confidential. You will receive $40.00 upon completion of all parts of the study.

3. If they are interested, determine when they will be coming in.
COCAINE USE
TELEPHONE SCREENING QUESTIONNAIRE

Name: ________________________________

Telephone #: (home) __________________________
(work) __________________________

Best time to call: __________________________

Date: __________________________

How old are you? (18-70) _________ years

Date of Birth: ______________

Male/Female? _________

When did you first use cocaine? __________________________

Are you currently using cocaine? yes_____ or no_____ 

If no, when did you stop using cocaine? __________________________

STOP if more than 2 years ago.

What is the maximum number of times you have ever used cocaine in one month? ______________

STOP if < 10.

Is cocaine your primary substance of abuse or dependence ie. largest amounts, largest consequences? yes_____ or no_____ 

STOP if No.

In your lifetime have you ever been dependent on opiates (met DSM-III-R for opiate dependence)?

yes_____ or no_____ or don’t know____

STOP if Yes.

Are you allergic to dextromethorphan? yes_____ or no_____ 

STOP if Yes.

SIN #: ______________________

OHIP #: ______________________

_________ appears to be eligible for the study

_________ does not appear to be eligible for the study

reason: __________________________

Appointment date: __________________________

Subject #: ______________
CONSENT FORM

I ______________________, hereby consent to participate in the research project entitled "Dextromethorphan Oxidation Phenotype Distribution (Human Study IC)" being conducted at the Clinical Research and Treatment Institute of the Addiction Research Foundation and University of Toronto under the direction of E.M. Sellers, M.D., Ph.D. and S.V. Otton, Ph.D. The purpose of this research, the procedures to be followed, and possible risks of this research have been explained to me by ______________________.

In consenting to participate, I understand that:

1. The purpose of this study is to determine my pattern of metabolizing a substance called dextromethorphan (Contac® Coughcaps™ DM).

2. Dextromethorphan is a widely used anti-cough medication which is available without a prescription. Dextromethorphan has been given safely in the dose used in this study to millions of individuals without risk or hazard. At the dose I will receive (30 mg), the side effect that might occur is a slight degree of drowsiness.

3. As part of this study, I will provide information about myself including medical history, current medication use and non-prescription drug use by answering a cocaine questionnaire. I will also provide a spot urine specimen.

4. As part of this study, I will swallow a capsule containing 30 mg dextromethorphan. Before doing this I will empty my bladder. After taking the drug I will collect all my urine for the next 8 hours in the container provided.

5. I will return the urine container to the study site.
6. As part of the study, a blood sample (20 ml) will be taken from a vein in my arm for analysis of the portion of my DNA which determines my drug metabolizing capacity. DNA is the chemical which carries information on human inheritance. The person collecting the blood will be experienced in the procedure and I can expect little and brief pain associated with inserting the needle. Afterward, there is some chance of slight bruising or inflammation, but this is a routine procedure that presents very low risk to me.

7. I may decline to answer any particular questions asked of me. If this refusal makes my participation in this study of no scientific value, my participation can be terminated.

8. I may withdraw from the study at any time and for any reason. If I should withdraw from the study this will not in any way jeopardize my right to present or future treatment at the Addiction Research Foundation.

9. I understand that I may be approached in the future and asked to participate in related research projects. I am entirely at liberty to decline and will not be subject to any coercion.

10. I can expect no therapeutic benefit from participating in this study.

11. If at any time during or after the study I would like treatment for alcohol or drug abuse, this will be arranged at the Clinical Research and Treatment Institute of the Addiction Research Foundation.

12. The data I provide will be kept strictly confidential and secure, available only to the researchers involved in this study and for the purpose set out in paragraph #1 above. Neither my name nor any pieces of identifying information will be kept together with the other data that I may provide. My records will be treated with the same confidentiality afforded medical records.
13. The results of this study may be published and if so will be published in such a manner that I will not be identifiable. Published reports will refer to group data and not to a particular identifiable individual.

14. In consideration of the inconvenience and time involved in this study, I will receive $40.00 upon submission of my urine and blood samples.

15. I have had an opportunity to ask questions, and my questions have been satisfactorily answered.

16. I will be given a copy of the consent form at the time I sign it.

Dated at Toronto, this _________________ day of __________________, 19____.

_________________________ __________________________
Signature

_________________________ __________________________
Signature

_________________________ __________________________
Signature

_________________________ __________________________
Address

_________________________
Print Name

_________________________ _________________
Witness Signature Date

This consent form was read in my presence by ___________________ who has informed me that he/she carefully considered and understood each point above. I hereby confirm that the study will be conducted in accordance with the conditions and procedures set out above.

_________________________
Print Name

_________________________
Classification

_________________________
Signature
APPENDIX 4
1. Year of Birth: ____________

2. Sex:  1. _____ Female  2. _____ Male

3. a) Country of Birth: __________________________  (specify) ____________
   
   b) Which of the following ethno-cultural groups describe your family's ethnic origins as far back as you are aware? (Circle those that apply)

   (01) African  (13) English  (25) Metis
   (02) American (U.S.A.)  (14) French Canadian  (26) Native Indian
   (03) Arab  (15) French  (27) Other Native American
   (04) Black-African  (16) German  (28) Other South East Asian
   (05) Black-Caribbean  (17) Greek  (29) Polish
   (06) Black-North American  (18) Indo-Pakistani  (30) Portuguese
   (07) Canadian  (19) Inuit  (31) Scottish
   (08) Caribbean  (20) Irish  (32) South American
   (09) Central American  (21) Italian  (33) Spanish
   (10) Chinese  (22) Japanese  (34) Ukrainian
   (11) Dutch  (23) Jewish  (35) Vietnamese
   (12) East European  (24) Korean  (36) Other-specify: __________________________

4. Are you currently taking any medications?  1. _____ Yes  2. _____ No

   If yes, answer appropriately for the following reasons and indicate drug name(s) next to the corresponding reason(s).

<table>
<thead>
<tr>
<th>Reason</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Seizure Disorder</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Asthma</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Cough/Cold/Allergy</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Stomach</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Constipation</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Pain</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Infection</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Birth Control</td>
<td>1. __</td>
<td>2. ___</td>
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<tr>
<td>Depression</td>
<td>1. __</td>
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</tr>
<tr>
<td>Anxiety</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
<tr>
<td>Other</td>
<td>1. __</td>
<td>2. ___</td>
</tr>
</tbody>
</table>
5. How many times have you used cocaine ("C", coke, flake, snow, freebase crack, rock) in your lifetime? (Check the one that best describes you)

1. ___ I have taken cocaine between 1 and 10 times
2. ___ I have taken cocaine more than 10 times, but less than 100
3. ___ I have taken cocaine 100 times or more

6. My attitude towards cocaine use is most closely described as: (Check only one)

1. ___ I never wanted to even try cocaine
2. ___ I was indifferent to whether I tried cocaine or not
3. ___ I wanted to try cocaine but to resist becoming a regular user
4. ___ I wanted to try cocaine even if I became a regular user

7. Here are some reasons why people try using cocaine. Which of them, if any, were true in your case? (Answer all)

A friend or relative offered it me
A dealer offered me a free sample
I wanted to do what other people were doing
I was curious about its effects
I was told it would make something else (like sex or music) more pleasant
Other __________________________

1. ___ Yes 2. ___ No 1. ___ Yes 2. ___ No 1. ___ Yes 2. ___ No

8. About how old were you when you first used cocaine? _____ yrs

9. About how old were you when you started using cocaine regularly (i.e. more than 10 times per month)? _____ yrs

10. How have you used cocaine? (Answer all)

Snorting 1. ___ Yes 2. ___ No
Smoking (freebase, crack) 1. ___ Yes 2. ___ No
IV 1. ___ Yes 2. ___ No

IF YOU ARE A FORMER COCAINE USER
BUT DO NOT USE COCAINE NOW, SKIP TO QUESTION 37

2
11. At the present time do you take cocaine daily?
   1. ___ yes   2. ___ no

12. How many days in a month do you use cocaine? (Estimate as accurately as possible) __________

13. Apart from times that you might be trying to stop cocaine use, are there months when you don't take any cocaine?
   1. ___ never   2. ___ sometimes   3. ___ frequently

14. What is the highest number of days you have taken cocaine in a month? __________

15. What is the number of days you prefer to use cocaine each month? __________

16. How much money in dollars do you spend on cocaine in an average month? __________ dollars

17. How many grams of cocaine do you use in an average month? __________ grams

18. How likely do you think it is that taking cocaine will lead to health problems for you? (Check only one)
   1. ___ very likely
   2. ___ somewhat likely
   3. ___ somewhat unlikely
   4. ___ very unlikely
   5. ___ don't know
19. When was your last use of cocaine?

   YR   MO   DA

20. Over the past seven days, check the boxes on the days which you have used cocaine?

   __  __  __  __  __  __  __
   -7  -6  -5  -4  -3  -2  yesterday

21. Have you ever tried to stop using cocaine?

   1. ___ Yes  2. ___ No

   IF NO, SKIP TO QUESTION 24

22. If yes, how many of these would you consider serious attempts?

   1. ___ none
   2. ___ a few
   3. ___ about half
   4. ___ most
   5. ___ all

23. What is the longest period that you have been able to stop using cocaine since you started to use it regularly? (Answer only the line that corresponds to the time unit that applies to you)

   A  ___  hours
   B  ___  days
   C  ___  weeks
   D  ___  months
   E  ___  years

24. Have you ever had any of the following withdrawal symptoms when you stopped taking cocaine? (Answer all)

   Anxiety or irritability  1. ___ Yes  2. ___ No
   Fatigue  1. ___ Yes  2. ___ No
   Trouble sleeping  1. ___ Yes  2. ___ No
   Feeling down or depressed  1. ___ Yes  2. ___ No
   Difficulties concentrating  1. ___ Yes  2. ___ No
   Other (specify)__________________________  1. ___ Yes  2. ___ No
25. Do you feel that you take cocaine to prevent the above symptoms or make them go away?
   1. ___ Yes   2. ___ No

26. Do you find that when you start taking cocaine you end up taking much more of it than you were planning?
   1. ___ Yes   2. ___ No

27. Do you spend a lot of time taking cocaine or doing whatever you have to do to get it?
   1. ___ Yes   2. ___ No

28. Do you ever use cocaine while doing something that may be dangerous if done under the influence of cocaine (i.e. driving)?
   1. ___ Yes   2. ___ No

   Briefly describe: ________________________________________________________

29. Do you ever use cocaine while doing something important, like being at school or work or taking care of children?
   1. ___ Yes   2. ___ No

30. Do you ever miss something important, like school or work or an appointment, because you are using cocaine or spending time getting cocaine?
   1. ___ Yes   2. ___ No

31. Do you ever use cocaine so often that you use it instead of working or spending time on hobbies or with your family and friends?
   1. ___ Yes   2. ___ No
32. Does your use of cocaine cause problems with other people, such as family members or people at work?
   1. __ Yes  2. __ No
   Briefly describe:__________________________________________

33. Does your use of cocaine cause psychological problems, like making you depressed?
   1. __ Yes  2. __ No
   Briefly describe:__________________________________________

34. Does your use of cocaine cause physical problems or make physical problems worse?
   1. __ Yes  2. __ No
   Briefly describe:__________________________________________

35. Does cocaine have the same effect on you now as when you first started using it?
   1. __ Yes  2. __ No
   Briefly describe:__________________________________________

36. Do you find that you need to use more cocaine to get high than you did when you first started using it?
   1. __ Yes  2. __ No
   GO TO QUESTION 59

IF YOU WERE A FORMER COCAINE USER
BUT DO NOT USE COCAINE NOW, PLEASE CONTINUE

37. When did you stop using cocaine?
   Month ___  Year ___
3. I am likely to return to cocaine use
2. I may return to cocaine use
1. I am confident I will not return to cocaine use

Which statement best reflects your feelings about future use? (Check only one)

3. How many grams of cocaine did you use in an average month?

2. How much money in dollars did you spend on cocaine in an average month?

1. What is the highest number of days you have taken cocaine in a month?

3. Sometimes
2. Never
1. Frequently

3. While you were still using cocaine regularly, apart from times that you may have been trying to stop cocaine use, were there months when you didn't take any cocaine?

39. (Estimate as accurately as possible)

3. While you were still using cocaine regularly, how many days did you take cocaine each month?

3. Yes
2. No
1. No

3. While you were still using cocaine regularly, did you take cocaine daily?
45. Why did you stop taking cocaine? (Answer all)

<table>
<thead>
<tr>
<th>Reason</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>It began to make me feel sick</td>
<td></td>
<td></td>
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<tr>
<td>I worried about the long-term health problems (i.e. physical or psychological problems)</td>
<td>1</td>
<td>2</td>
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<tr>
<td>I stopped enjoying it</td>
<td></td>
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<tr>
<td>My friends stopped using it</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>It cost too much money</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Religious or ethical reasons</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>My parents or other people important to me disapproved of my cocaine use</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>It is illegal</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other (Specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

46. Did you ever have any of the following withdrawal symptoms when you stopped taking cocaine? (Answer all)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety or irritability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
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<tr>
<td>Trouble sleeping</td>
<td></td>
<td></td>
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<tr>
<td>Feeling down or depressed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulties concentrating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

47. Did you ever feel that you could take cocaine to prevent the above symptoms or make them go away?

1. ___ Yes 2. ___ No

48. While you were still using cocaine regularly, did you find that when you started taking cocaine you ended up taking much more of it than you were planning?

1. ___ Yes 2. ___ No

49. Did you spend a lot of time taking cocaine or doing whatever you had to do to get it?

1. ___ Yes 2. ___ No

50. Did you ever use cocaine while doing something that may be dangerous if done under the influence of cocaine (i.e. driving)?

1. ___ Yes 2. ___ No

Briefly describe: ___________________________________________
51. Did you ever use cocaine while doing something important, like being at school or work or taking care of children?
   1. ___ Yes    2. ___ No

52. Did you ever miss something important, like school or work or an appointment, because you were using cocaine or spending time getting cocaine?
   1. ___ Yes    2. ___ No

53. Did you ever use cocaine so often that you used it instead of working or spending time on hobbies or with your family and friends?
   1. ___ Yes    2. ___ No

54. Did your use of cocaine cause problems with other people, such as family members or people at work?
   1. ___ Yes    2. ___ No
   Briefly describe: ________________________________

55. Did your use of cocaine cause psychological problems, like making you depressed?
   1. ___ Yes    2. ___ No
   Briefly describe: ________________________________

56. Did your use of cocaine cause physical problems or make physical problems worse?
   1. ___ Yes    2. ___ No
   Briefly describe: ________________________________

57. Did cocaine have the same effect on you right before you stopped taking cocaine as when you first started using it?
   1. ___ Yes    2. ___ No
   Briefly describe: ________________________________
58. Did you find that you needed to use more cocaine to get high right before you stopped taking cocaine than you did when you first started using it?
   1. Yes  2. No

   ************************************************************

ALCOHOL

59. In your life, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one)
   1. None
   2. 1-14 drinks
   3. 15-28 drinks
   4. 29-56 drinks
   5. 57-84 drinks
   6. 85-112 drinks
   7. > 112 drinks

   IF ANSWER IS NONE, SKIP TO QUESTION 65

60. In the last 12 months, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one).
   1. None
   2. 1-14 drinks
   3. 15-28 drinks
   4. 29-56 drinks
   5. 57-84 drinks
   6. 85-112 drinks
   7. > 112 drinks

61. In the last 30 days, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one).
   1. None
   2. 1-14 drinks
   3. 15-28 drinks
   4. 29-56 drinks
   5. 57-84 drinks
   6. 85-112 drinks
   7. > 112 drinks
62. Was there ever a period in your life when you drank too much?
   1. ___ Yes  2. ___ No

63. Has alcohol ever caused problems for you?
   1. ___ Yes  2. ___ No

64. Has anyone ever objected to your drinking?
   1. ___ Yes  2. ___ No

65. If you NEVER used alcohol regularly (i.e., less than 10 times in your lifetime), indicate why. (Please check all that apply)
   A ___ Did not like the effect
   B ___ Concerned about health risk
   C ___ Not available
   D ___ Cultural reasons
   E ___ Too expensive
   F ___ Prohibition on religious grounds
   G ___ Other: Please specify _______________________

CANNABIS (Hash, Marijuana)

66. In your life, on how many occasions (if any) have you used cannabis? (Check only one)
   1. ___ Never
   2. ___ 1 - 2 times
   3. ___ 3 - 5 times
   4. ___ 6 - 9 times
   5. ___ 10-19 times
   6. ___ 20-39 times
   7. ___ 40-99 times
   8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 69
67. In the last 12 months, on how many occasions (if any) have you used cannabis? (Check only one)

1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times

68. In the last 30 days, on how many occasions (if any) have you used cannabis? (Check only one)

1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times

69. If you NEVER used cannabis regularly (i.e., less than 10 times in your lifetime) indicate why. (Please check all that apply)

A _____ Did not like the effect  
B _____ Concerned about health risk  
C _____ Not available  
D _____ Cultural reasons  
E _____ Too expensive  
F _____ Because it is illegal  
G _____ Other (specify) __________________________

-------------------------------

BARBITURATES

Seconal, Tuinal, Amytal, Fiorinal, "downers"  
(Circle those you have used)

70. In your life, on how many occasions (if any) have you used a barbiturate? (Check only one)

1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 73
71. **In the last 12 months**, on how many occasions (if any) have you used a barbiturate?  
(Check only one)  
1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times  

72. **In the last 30 days**, on how many occasions (if any) have you used a barbiturate?  
(Check only one)  
1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times  

73. If you *NEVER* used a barbiturate regularly (i.e., less than 10 times in your lifetime) indicate why.  
(Please check all that apply)  
A ___ Did not like the effect  
B ___ Concerned about health risk  
C ___ Not available  
D ___ Cultural reasons  
E ___ Too expensive  
F ___ Not needed (i.e., Never prescribed)  
G ___ Other (specify) ____________________________________________________________  

--------------------------------------------------------------------------------------

**ANXIOLYTICS/TRANQUILLIZERS**

diazepam (Valium), lorazepam (Ativan), alprazolam (Xanax), chlordiazepoxide (Librium), triazolam (Halcion) (Circle those you have used)

74. **In your life**, on how many occasions (if any) have you used an anxiolytic/tranquilizer?  
(Check only one)  
1. ___ Never  
2. ___ 1 - 2 times  
3. ___ 3 - 5 times  
4. ___ 6 - 9 times  
5. ___ 10-19 times  
6. ___ 20-39 times  
7. ___ 40-99 times  
8. ___ 100+ times  

*IF ANSWER IS NEVER, SKIP TO QUESTION 77*
75. In the last 12 months, on how many occasions (if any) have you used an amnolytic/tranquilizer? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

76. In the last 30 days, on how many occasions (if any) have you used an amnolytic/tranquilizer? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

77. If you NEVER used an amnolytic/tranquilizer regularly (i.e., less than 10 times in your lifetime) indicate why: (Please check all that apply)

A _____ Did not like the affect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Not needed
G _____ Other (specify)________________________

**********************************************************************************************************************************************

STIMULANTS (Other than cocaine)

Methamphetamine (Ice), diet pills (Ionamin, Tenuate), caffeine tablets (Wake-Ups), "Bennies", Ritalin, decongestants (Sudafed, Oradea)
(Circle those you have used)

78. In your life, on how many occasions (if any) have you used a stimulant? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 81
79. **In the past 12 months**, on how many occasions (if any) have you used a stimulant? (Check only one)

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<tr>
<td>1.</td>
<td>Never</td>
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<td>2.</td>
<td>1 - 2 times</td>
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<td>3.</td>
<td>3 - 5 times</td>
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<td>4.</td>
<td>6 - 9 times</td>
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<td>5.</td>
<td>10-19 times</td>
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<td>6.</td>
<td>20-39 times</td>
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<tr>
<td>7.</td>
<td>40-99 times</td>
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<tr>
<td>8.</td>
<td>100+ times</td>
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</table>

80. **In the past 30 days**, on how many occasions (if any) have you used a stimulant? (Check only one)

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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Never</td>
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<tr>
<td>2.</td>
<td>1 - 2 times</td>
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<td>3.</td>
<td>3 - 5 times</td>
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<td>4.</td>
<td>6 - 9 times</td>
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<tr>
<td>5.</td>
<td>10-19 times</td>
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<tr>
<td>6.</td>
<td>20-39 times</td>
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<tr>
<td>7.</td>
<td>40-99 times</td>
<td></td>
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<tr>
<td>8.</td>
<td>100+ times</td>
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</tbody>
</table>

81. If you never used stimulants regularly (ie. less than 10 times in your lifetime) indicate why. (Please check all that apply)

- A ______ Did not like the effect
- B ______ Concerned about health risk
- C ______ Not available
- D ______ Cultural reasons
- E ______ Too expensive
- F ______ Because it is illegal
- G ______ Other (specify) __________________________

TOBACCO

82. **In your life, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period?** (Check only one)

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<tbody>
<tr>
<td>1.</td>
<td>None</td>
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<tr>
<td>2.</td>
<td>&lt; 1 pack</td>
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<tr>
<td>3.</td>
<td>1 - 2 packs</td>
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<td>4.</td>
<td>3 - 5 packs</td>
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<tr>
<td>5.</td>
<td>6 - 9 packs</td>
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</tr>
<tr>
<td>6.</td>
<td>10-19 packs</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7.</td>
<td>20-39 packs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8.</td>
<td>40+ packs</td>
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</tbody>
</table>

**IF ANSWER IS NONE, SKIP TO QUESTION 85**
83. In the last 12 months, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period? (Check only one)
   1. ___ None
   2. ___ < 1 pack
   3. ___ 1 - 2 packs
   4. ___ 3 - 5 packs
   5. ___ 6 - 9 packs
   6. ___ 10-19 packs
   7. ___ 20-39 packs
   8. ___ 40 + packs

84. In the last 30 days, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period? (Check only one)
   1. ___ None
   2. ___ < 1 pack
   3. ___ 1 - 2 packs
   4. ___ 3 - 5 packs
   5. ___ 6 - 9 packs
   6. ___ 10-19 packs
   7. ___ 20-39 packs
   8. ___ 40 + packs

85. If you NEVER used cigarettes regularly (ie. never more than 10 cigarettes/day for a week) indicate why. (Please check all that apply)
   A _____ Concerned about health risk
   B _____ Not available
   C _____ Cultural reasons
   D _____ Too expensive
   E _____ Tried, but just could not seem to get to like them
   F _____ Other (specify) ______________________

86. In your life, on how many occasions have you used an opiate alone or in combination? (Check only one)
   1. ___ Never
   2. ___ 1 - 2 times
   3. ___ 3 - 5 times
   4. ___ 6 - 9 times
   5. ___ 10-19 times
   6. ___ 20-39 times
   7. ___ 40-99 times
   8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 123
87. In the past 12 months, on how many occasions have you used an opiate alone or in combination? (Check only one)

1. Never
2. 1 - 2 times
3. 3 - 5 times
4. 6 - 9 times
5. 10-19 times
6. 20-39 times
7. 40-99 times
8. 100+ times

88. In the past 30 days, on how many occasions have you used an opiate alone or in combination? (Check only one)

1. Never
2. 1 - 2 times
3. 3 - 5 times
4. 6 - 9 times
5. 10-19 times
6. 20-39 times
7. 40-99 times
8. 100+ times

89. What is the maximum number of times you have ever used opiates in one month?

1. 1 - 9 times
2. 10 + times

IF ANSWER IS 1 - 9 TIMES, SKIP TO QUESTION 123

IF ANSWER IS 10+ TIMES BUT YOU DO NOT USE OPIOATES ANYMORE, SKIP TO QUESTION 108

IF ANSWER IS 10+ TIMES AND YOU ARE STILL USING OPIOATES, PLEASE CONTINUE

90. Which opiate do you use the most?

Specify ____________________________________

91. How does the opiate you use most affect you?

I get a 'high' or pleasurable effect 1. ___ Yes 2. ___ No
I get a therapeutic effect 1. ___ Yes 2. ___ No
I don't get any effect 1. ___ Yes 2. ___ No
92. Have you ever tried to stop using opiates?
   1. ___ Yes     2. ___ No

   IF NO, SKIP TO QUESTION 95

93. If yes, how many of these would you consider serious attempts?
   1. ___ none
   2. ___ a few
   3. ___ about half
   4. ___ most
   5. ___ all

94. What is the longest period that you have been able to stop using opiates since you started to use them regularly? (Answer only the line that corresponds to the time unit that applies to you)
   A ___ hours
   B ___ days
   C ___ weeks
   D ___ months
   E ___ years

95. Have you ever had any of the following withdrawal symptoms when you stopped taking opiates?
    (Answer all)
    Anxiety or irritability 1. ___ Yes     2. ___ No
    Fatigue                  1. ___ Yes     2. ___ No
    Trouble sleeping         1. ___ Yes     2. ___ No
    Feeling down or depressed 1. ___ Yes     2. ___ No
    Difficulties concentrating 1. ___ Yes     2. ___ No
    Other (specify)________________________ 1. ___ Yes     2. ___ No

96. Do you feel that you take opiates to prevent the above symptoms or make them go away?
   1. ___ Yes     2. ___ No

97. Do you find that when you start taking opiates you end up taking much more than you were planning?
   1. ___ Yes     2. ___ No
96. Do you spend a lot of time taking opiates or doing whatever you have to do to get them?
   1. ___ Yes   2. ___ No

97. Do you ever use opiates while doing something that may be dangerous if done under the influence of opiates (i.e., driving)?
   1. ___ Yes   2. ___ No
   Briefly describe: ____________________________________________

98. Do you ever use opiates while doing something important, like being at school or work or taking care of children?
   1. ___ Yes   2. ___ No

99. Do you ever miss something important, like school or work or an appointment, because you are using opiates or spending time getting opiates?
   1. ___ Yes   2. ___ No

100. Do you ever use opiates so often that you use them instead of working or spending time on hobbies or with your family and friends?
   1. ___ Yes   2. ___ No

101. Does your use of opiates cause problems with other people, such as family members or people at work?
   1. ___ Yes   2. ___ No
   Briefly describe: ____________________________________________

102. Does your use of opiates cause psychological problems, like making you depressed?
   1. ___ Yes   2. ___ No
   Briefly describe: ____________________________________________
105. Does your use of opiates cause physical problems or make physical problems worse?

1. __ Yes  2. __ No

Briefly describe:______________________________

106. Do opiates have the same effect on you now as when you first started using them?

1. __ Yes  2. __ No

Briefly describe:______________________________

107. Do you find that you need to use more opiates to get high than you did when you first started using them?

1. __ Yes  2. __ No

GO TO QUESTION 124

IF YOU ARE A FORMER OPIATE USER
BUT DO NOT USE OPIATES NOW, PLEASE CONTINUE

108. Which opiate did you use the most?

Specify ______________________________

109. How did the opiate you use most affect you?

I get a 'high' or pleasurable effect  1. __ Yes  2. __ No
I get a therapeutic effect  1. __ Yes  2. __ No
I don't get any effect  1. __ Yes  2. __ No

110. Did you ever have any of the following withdrawal symptoms when you stopped taking opiates? (Answer all)

Anxiety or irritability  1. __ Yes  2. __ No
Fatigue  1. __ Yes  2. __ No
Trouble sleeping  1. __ Yes  2. __ No
Feeling down or depressed  1. __ Yes  2. __ No
Difficulties concentrating  1. __ Yes  2. __ No
Other (specify)____________________________  1. __ Yes  2. __ No
111. Did you ever feel that you could take opiates to prevent the above symptoms or make them go away?
   1. ___ Yes   2. ___ No

112. While you were still using opiates regularly, did you find that when you started taking opiates you ended up taking much more than you were planning?
   1. ___ Yes   2. ___ No

113. Did you spend a lot of time taking opiates or doing whatever you had to do to get them?
   1. ___ Yes   2. ___ No

114. Did you ever use opiates while doing something that may be dangerous if done under the influence of opiates (i.e., driving)?
   1. ___ Yes   2. ___ No
   Briefly describe: ____________________________________________________________

115. Did you ever use opiates while doing something important, like being at school or work or taking care of children?
   1. ___ Yes   2. ___ No

116. Did you ever miss something important, like school or work or an appointment, because you were using opiates or spending time getting opiates?
   1. ___ Yes   2. ___ No

117. Did you ever use opiates so often that you used them instead of working or spending time on hobbies or with your family and friends?
   1. ___ Yes   2. ___ No
118. Did your use of opiates cause problems with other people, such as family members or people at work?

1. ___ Yes  2. ___ No

Briefly describe: ____________________________________________

119. Did your use of opiates cause psychological problems, like making you depressed?

1. ___ Yes  2. ___ No

Briefly describe: ____________________________________________

120. Did your use of opiates cause physical problems or make physical problems worse?

1. ___ Yes  2. ___ No

Briefly describe: ____________________________________________

121. Did opiates have the same effect on you right before you stopped taking them as when you first started using them?

1. ___ Yes  2. ___ No

Briefly describe: ____________________________________________

122. Did you find that you needed to use more opiates to get high right before you stopped taking them than you did when you first started using them?

1. ___ Yes  2. ___ No

GO TO QUESTION 124

123. If you never used opiates regularly, indicate why. (Check all that apply)

A ___ Did not like the effect
B ___ Concerned about health risk
C ___ Not available
D ___ Cultural reasons
E ___ Too expensive
F ___ Not needed (i.e. for treating pain)
G ___ Other (specify) ________________________________
124.

We appreciate your help and would like to know why you have participated. (Please check all that apply)

A _____ Concerned about drug use
B _____ Want to stop or decrease use
C _____ Like to participate in surveys
D _____ Other (specify) ____________________________


INFORMATION FOR PARTICIPANTS

For your information we have listed a number of telephone numbers you can phone should you have any questions about any aspects of this project.

Questions related to the research project: 595-6737

Questions concerning rights of research subjects: Ethics Committee Office/Chairperson: 978-5585

In case of medical emergency: 595-6000 (ask for the on-call physician for the pharmacogenetic study).
APPENDIX 5
1. **WHAT IS CYP2D6?**

CYP2D6 is the scientific name for an enzyme found in the liver. When you take a drug, that drug doesn’t stay in your body forever. Instead, it is chemically changed by enzymes (such as CYP2D6) so that it can be easily excreted from your body, e.g. in urine.

2. **WHICH DRUGS DOES CYP2D6 ACT ON?**

CYP2D6 is known to act on about 40 different drugs, including drugs used in the treatment of heart problems (e.g. Tambocor®), depression (e.g. Tofranil®), schizophrenia (e.g. Mellaril®), pain (e.g. 222®’s, Tylenol® No. 1, 2, 3), and coughs (e.g. Ornex-DM®, Hycofan®).

3. **WHY IS CYP2D6 OF INTEREST TO RESEARCHERS?**

Some people (about 7% of the population) do not have any CYP2D6. This is because of the particular genes they have inherited from their parents. People who have no CYP2D6 handle some drugs differently compared to the rest of the population. There is no evidence that this affects their therapeutic outcome however, probably because drug dosages are routinely tailored to the individual patient’s needs.

4. **HOW IS CYP2D6 MEASURED?**

CYP2D6 can be measured in two ways: by the “Dextromethorphan Test", and by the “CYP2D6 Gene Test”.

5. **WHAT IS THE "DEXTROMETHORPHAN TEST"?**

In this test, the subject is given a low dose of a drug which is broken down by CYP2D6 and asked to collect urine over the next 8 hours. If the person has no CYP2D6, then no break-down products of the drug will be detected in the urine. The drug used is dextromethorphan. Dextromethorphan is a safe anti-cough drug which is available in all drug stores in preparations such as Sudafed-DM® and Robitussin-DM®.

6. **WHAT IS THE "CYP2D6 GENE TEST"?**

In this test, a small sample of blood (about 2 tablespoons) is taken from a vein in the arm by a nurse or doctor. In the laboratory, the DNA contained in the blood cells is collected. A portion of the DNA is analyzed in detail to see if it is capable of producing CYP2D6 enzyme.

7. **WHAT DO I HAVE TO DO?**

You will be tested with dextromethorphan. As outlined above, this involves taking a capsule containing dextromethorphan (at bedtime) and collecting all of your urine over the next 8 hours. A urine bottle will be provided. A sample of blood from a vein in your arm will also be taken on one occasion for the CYP2D6 Gene Test.
1. Complete the Consent Form and Cocaine Questionnaire and provide a spot urine sample.

2. At bedtime, empty your bladder and swallow the dextromethorphan capsule with water.

3. When you first get up the next morning (about 8 hours after you swallowed the dextromethorphan), collect ALL of your overnight urine in the bottle provided. Should you urinate more than once after taking the dextromethorphan capsule, collect your urine in the bottle each time for the next 8 hours.

4. Return collected urine at next appointment: ____________________________

5. A 20 mL blood sample will be drawn from a vein in your arm and a hair sample will be taken. Some questions will be asked regarding your pattern of cocaine use.

6. A $40.00 cheque will be ordered for you to pick up. Call Savita to find out if the cheque is ready at 1 pm on ____________________________ at 595-6737.

---

**CONTAC' COUGHCAPS' DM**

*SmithKline Beecham*

*Dextromethorphan*

*Antitussive*

**Indications:** Contac' CoughCaps™ DM are specially formulated to control dry, hacking coughs due to colds for up to 8 hours without causing drowsiness.

**Contraindications:** Hypersensitivity to any of the components. Pre-existing respiratory depression. Patients receiving or having received MAO inhibitors in the preceding 3 weeks.

**Precautions:** Before prescribing medication to suppress or modify cough, it is important to ascertain that the underlying cause of the cough is identified, that modification of the cough does not increase the risk of clinical or physiologic complications, and that appropriate therapy for the primary disease is provided.

Caution should be exercised and dosage may need to be reduced when a dextromethorphan containing product is administered with other drugs which depress the CNS (including alcohol), phenothiazines or tricyclic antidepressants. Not recommended for patients with asthma unless directed by a physician.

If cough worsens, lasts for more than 1 week or is accompanied by high fever, consult a physician. Do not exceed recommended dosage. Keep out of reach of children.

**Dosage:** Adults and children 14 years and over: 1 caplet every 8 hours as required, not to exceed 2 caplets per day. Children under 14 years: as directed by a physician. The effect of each caplet lasts for up to 8 hours. Do not take a second caplet during this time.

**Supplied:** Each orange caplet contains: 30 mg Dextromethorphan Hydrobromide, 254 mg lactose. Cartons of 10’s and 20’s.
APPENDIX 7
### ASSESSMENT TIMELINE CALENDAR

**Note:** Complete today's date, end date (day before today), and start date (as shown on interval Key Sheet). Then indicate these dates on the calendar by using dark slashes.

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**START DATE:** ____________ 1999

**END DATE:** ____________ 1999

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## Timeline of Cocaine Use

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**Notes:**
- 20 Father's Day
- 1 Canada Day
- 3 Chic Holiday
- 6 Labour Day
- 11 Thanksgiving
- 31 Halloween

**Holidays:**
- Father's Day
- Canada Day
- Chic Holiday
- Labour Day
- Thanksgiving
- Halloween
Addendum to consent form for the research project entitled “Dextromethorphan Oxidation Phenotype Distribution (Human Study 1C)”.

I __________________________ hereby consent to donate approximately 60-100 mg of my hair for the purpose of analysis to detect the presence of certain drugs.

The confidentiality provisions outlined in #12 and 13 also apply to the results of this analysis.

Dated at Toronto, this _______ day of __________________________ 19 _______.

____________________________________________
Signature

____________________________________________
Print Name

This addendum form was read in my presence by ______________________________ who has informed me that he/she carefully considered and understood the above.

____________________________________________
Print Name

____________________________________________
Signature
APPENDIX 9
Criteria for Substance Dependence

A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:

(1) Tolerance, as defined by either of the following:
   (a) a need for markedly increased amounts of the substance to achieve intoxication or desired effect
   (b) markedly diminished effect with continued use of the same amount of the substance

(2) withdrawal, as manifested by either of the following:
   (a) the characteristic withdrawal syndrome for the substance (refer to Criteria A and B of the criteria sets for Withdrawal from the specific substances)
   (b) the same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms

(3) the substance is often taken in larger amounts or over a longer period than was intended

(4) there is a persistent desire or unsuccessful efforts to cut down or control substance use

(5) a great deal of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects

(6) important social, occupational, or recreational activities are given up or reduced because of substance use

(7) the substance use is continued despite knowledge of having a permanent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption)

Criteria for Substance Abuse

A. A maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by one (or more) of the following, occurring within a 12-month period:

(1) recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home (e.g., repeated absences or poor work performance related to substance use; substance-related absences, suspensions, or expulsions from school; neglect of children or household)

(2) recurrent substance use in situations in which it is physically hazardous (e.g., driving an automobile or operating a machine when impaired by substance use)

(3) recurrent substance-related legal problems (e.g., arrests for substance-related disorderly conduct)

(4) continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance (e.g., arguments with spouse about consequences of intoxication, physical fights)

B. The symptoms have never met the criteria for Substance Dependence for this class of substance.