PHARMACOGENETIC VARIATION AT *CYP2D6*, *CYP2C9*, AND *CYP2C19*:

Population Genetic and Forensic Aspects

Johanna Sistonen

Department of Forensic Medicine
University of Helsinki
Finland

ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Medical Faculty of the University of Helsinki, in the auditorium of the Department of Forensic Medicine on November 21st, 2008, at 12 noon.

Helsinki 2008

SUPERVISOR

Professor Antti Sajantila

Department of Forensic Medicine

University of Helsinki

Helsinki, Finland

REVIEWERS

Professor Ángel Carracedo

Institute of Legal Medicine

University of Santiago de Compostela

Santiago de Compostela, Spain

Docent Mikko Niemi

Department of Clinical Pharmacology

University of Helsinki

Helsinki, Finland

OPPONENT

Professor Magnus Ingelman-Sundberg

Department of Physiology and Pharmacology

Karolinska Institutet

Stockholm, Sweden

ISBN 978-952-92-4690-8 (paperback)
ISBN 978-952-10-5094-7 (pdf)
http://ethesis.helsinki.fi

Helsinki University Print
Helsinki 2008

Voici mon secret. Il est très simple:
on ne voit bien qu'avec le cœur.
L'essentiel est invisible pour les yeux.

Antoine de Saint-Exupéry

CONTENTS

ABBREVIATIONS

ADR	adverse drug reaction
AMOVA	analysis of molecular variance
CEPH	Centre d'Etude du Polymorphisme Humain
CYP	cytochrome P450
CYP2C9	cytochrome P450 2C9
CYP2C19	cytochrome P450 2C19
CYP2D6	cytochrome P450 2D6
dbSNP	Single-Nucleotide Polymorphism Database
DME	drug-metabolizing enzyme
EHAT	(*E*)-10-hydroxyamitriptyline
EHNT	(*E*)-10-hydroxynortriptyline
EM	extensive metabolizer
G6PD	glucose-6-phosphate dehydrogenase
GC	gas chromatography
HMG-CoA	3-hydroxy-3-methylglutaryl-Coenzyme A
IM	intermediate metabolizer
kb	kilobase
LC	liquid chromatography
LD	linkage disequilibrium
MS	mass spectrometry
NAT	*N*-acetyltransferase
NCBI	National Center for Biotechnology Information
NNT	*N*-desmethylnortriptyline
PM	poor metabolizer
PPI	proton pump inhibitor
RFLP	restriction fragment length polymorphism
SNP	single-nucleotide polymorphism
TCA	tricyclic antidepressant
TPMT	thiopurine *S*-methyltransferase
UGT	uridine diphosphate glucuronosyltransferase
UM	ultra-rapid metabolizer
VKOR	vitamin K epoxide reductase
ZHAT	(*Z*)-10-hydroxyamitriptyline
ZHNT	(*Z*)-10-hydroxynortriptyline

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by Roman numerals I-V:

I Sistonen J, Fuselli S, Levo A, Sajantila A. *CYP2D6* genotyping by a multiplex primer extension reaction. *Clin Chem* 2005; 51:1291-1295.

II Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S. *CYP2D6* worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics* 2007; 17:93-101.

III Sistonen J, Fuselli S, Palo J, Chauhan N, Padh H, Sajantila A. Pharmacogenetic variation at *CYP2C9*, *CYP2C19*, and *CYP2D6* at global and micro-geographic scales. *Pharmacogenet Genomics*, in press.

IV Koski A, Sistonen J, Ojanperä I, Gergov M, Vuori E, Sajantila A. *CYP2D6* and *CYP2C19* genotypes and amitriptyline metabolite ratios in a series of medicolegal autopsies. *Forensic Sci Int* 2006; 158:177-183.

V Koski A, Ojanperä I, Sistonen J, Vuori E, Sajantila A. A fatal doxepin poisoning associated with a defective CYP2D6 genotype. *Am J Forensic Med Pathol* 2007; 28:259-261.

ABSTRACT

Pharmacogenetics deals with genetically determined variation in drug response. In this context, three phase I drug-metabolizing enzymes, CYP2D6, CYP2C9, and CYP2C19, have a central role, affecting the metabolism of about 20-30% of clinically used drugs. Since genes coding for these enzymes in human populations exhibit high genetic polymorphism, they are of major pharmacogenetic importance. The aims of this study were to develop new genotyping methods for *CYP2D6*, *CYP2C9*, and *CYP2C19* that would cover the most important genetic variants altering the enzyme activity, and, for the first time, to describe the distribution of genetic variation at these loci on global and microgeographic scales. In addition, pharmacogenetics was applied to a postmortem forensic setting to elucidate the role of genetic variation in drug intoxications, focusing mainly on cases related to tricyclic antidepressants, which are commonly involved in fatal drug poisonings in Finland.

Genetic variability data were obtained by genotyping new population samples by the methods developed based on PCR and multiplex single-nucleotide primer extension reaction, as well as by collecting data from the literature. Data consisted of 138, 129, and 146 population samples for *CYP2D6*, *CYP2C9*, and *CYP2C19*, respectively. In addition, over 200 postmortem forensic cases were examined with respect to drug and metabolite concentrations and genotypic variation at *CYP2D6* and *CYP2C19*. The distribution of genetic variation within and among human populations was analyzed by descriptive statistics and variance analysis and by correlating the genetic and geographic distances using Mantel tests and spatial autocorrelation. The correlation between phenotypic and genotypic variation in drug metabolism observed in postmortem cases was also analyzed statistically.

The genotyping methods developed proved to be informative, technically feasible, and cost-effective. Detailed molecular analysis of *CYP2D6* genetic variation in a global survey of human populations revealed that the pattern of variation was similar to those of neutral genomic markers. Most of the *CYP2D6* diversity was observed within populations, and the spatial pattern of variation was best described as clinal. On the other hand, genetic variants of *CYP2D6*, *CYP2C9*, and *CYP2C19* associated with altered enzymatic activity could reach extremely high frequencies in certain geographic regions. Pharmacogenetic variation may also be significantly affected by population-specific demographic histories, as seen within the Finnish population. When pharmacogenetics was applied to a postmortem forensic setting, a correlation between amitriptyline metabolic ratios and genetic variation at *CYP2D6* and *CYP2C19* was observed in the sample material, even in the presence of confounding factors

typical for these cases. In addition, a case of doxepin-related fatal poisoning was shown to be associated with a genetic defect at *CYP2D6*.

Each of the genes studied showed a distinct variation pattern in human populations and high frequencies of altered activity variants, which may reflect the neutral evolution and/or selective pressures caused by dietary or environmental exposure. The results are relevant also from the clinical point of view since the genetic variation at *CYP2D6*, *CYP2C9*, and *CYP2C19* already has a range of clinical applications, *e.g.* in cancer treatment and oral anticoagulation therapy. This study revealed that pharmacogenetics may also contribute valuable information to the medicolegal investigation of sudden, unexpected deaths.

REVIEW OF THE LITERATURE

1 Pharmacogenetics

Pharmacogenetics deals with genetically determined variation in drug response. Nowadays, it is well recognized that therapeutic failures or severe adverse drug reactions (ADRs) can have a genetic component. Pharmacogenetics as a distinct discipline dates back to the 1950s, when the landmark discoveries were made (Meyer 2004). Alf Alving and coworkers observed that the antimalarial drug primaquine induced intravascular hemolysis in about 10% of African Americans, but rarely in Caucasians (Hockwald et al. 1952). A few years later, in 1956, this was shown to be caused by a deficiency of glucose-6-phosphate dehydrogenase (G6PD) (Carson et al. 1956). Inherited variation in response to succinylcholine, which is used as a muscular relaxant in anesthesia, was also described. Prolonged neuromuscular paralysis was demonstrated to be due to a deficiency in the metabolizing enzyme pseudocholinesterase (Lehmann and Ryan 1956; Kalow and Staron 1957). At the beginning of the 1950s, isoniazid was introduced in the treatment of tuberculosis. Soon after, individual differences were observed in the metabolism of the drug, and people could be classified as rapid or slow acetylators, the latter of which suffered more frequently from peripheral neuritis related to isoniazid toxicity (Hughes et al. 1954; Evans et al. 1960). Although acetylation polymorphism was the target of intense research and one of the best examples of individual differences in drug response, it was not until 40 years later that the actual molecular mechanism was characterized (Blum et al. 1991).

Based on these key discoveries, Arno Motulsky wrote a paper in 1957 on the genetic basis of adverse reactions to drugs, which was the true beginning of a distinct discipline (Motulsky 1957). A few years later, the term “pharmacogenetics” was introduced by Friedrich Vogel (Vogel 1959). Several new examples of inherited variation in drug response were later described, but it was the discovery of debrisoquine/sparteine polymorphism of drug oxidation in the late 1970s (Mahgoub et al. 1977; Eichelbaum et al. 1979) that excited researchers. Two groups independently observed unexpected adverse reactions to these drugs, and subsequent studies showed that both drugs are metabolized by the same enzyme, a cytochrome P450 (CYP) mono-oxygenase, which was later designated as CYP2D6. The coding gene *CYP2D6* was also the first polymorphic gene affecting drug response to be cloned and characterized (Gonzalez et al. 1988). Since CYP2D6 affects the metabolism of numerous commonly used drugs and is highly polymorphic, it has become one of the model traits of pharmacogenetics.

Over the years, pharmacogenetics has been increasingly recognized by physicians, geneticists, and the pharmaceutical industry. The research has been extended to cover genetic variation not only of drug-metabolizing enzymes (DMEs) but also of drug transporters and receptors. While pharmacogenetics is defined as "*the study of variations in DNA sequence as related to drug response*", a new term "pharmacogenomics" has been introduced to define "*the study of variations of DNA and RNA characteristics as related to drug response*" (The United States Food and Drug Administration, http://www.fda.gov/Cder/Guidance/8083fnl.pdf; The European Medicines Agency, http://www.emea.europa.eu/pdfs/human/ich/43798606en.pdf). The aim of pharmacogenomic research is to individualize drug treatment by identifying the optimal drug and dose for each individual based on genetic information, thereby reducing ADRs and costs of treatment. However, currently, pharmacogenomics is just beginning to make its way into the clinical practice, and it remains to be seen how extensively it will affect drug treatment in the future.

2 Drug-Metabolizing Enzymes

Drug metabolism, or more generally xenobiotic metabolism, protects the human body against the potential harmful effects of foreign compounds introduced into the body. Metabolism can be divided into phase I and phase II reactions, which usually increase the water solubility of the substrates, thus enhancing their removal. In phase I reactions, such as oxidation, reduction, and hydrolysis, the functional groups of the foreign compounds are modified. The majority of phase I enzymes belong to the CYP enzyme family (Evans and Relling 1999). Phase II enzymes, such as uridine diphosphate glucuronosyltransferases (UGTs), *N*-acetyltransferases (NATs), thiopurine *S*-methyltransferase (TPMT), glutathione *S*-transferases, and sulfotransferases, conjugate the substrates with endogenous substituents (Evans and Relling 1999).

Most DMEs have both cytosolic and membrane-bound forms (Nebert and Dalton 2006). However, some DMEs are always bound in membranes, predominantly in the endoplasmic reticulum, mitochondria, and occasionally in the plasma membrane, whereas few DMEs are found only in the cytoplasm. Hydrophobic chemicals are presumably attracted to membranes in which, for example, most phase I DMEs reside. Generally DMEs show great flexibility in binding substrates, a function essential to detoxication of new potentially harmful compounds entering the body.

Although the majority of drug metabolism occurs in the liver, DMEs are also present in other tissues, such as the mucosa of the small intestine, kidney, lung, brain, and skin (Krishna and Klotz 1994). Among these, the intestinal mucosa is probably the most important extrahepatic site of drug metabolism (Lin and Lu 2001; Paine et al. 2006). If a drug is administered orally, it may undergo metabolism in the small intestine and in the liver before reaching the systemic circulation. This process termed first-pass metabolism can significantly affect the bioavailability and consequently the effects of a drug (Thummel et al. 1997).

3 CYP Enzymes

3.1 General Characteristics

CYPs constitute a superfamily of heme-thiolate enzymes; over 7000 individual members found in different organisms are currently known (http://drnelson.utmem.edu/cytochromeP450.html) (Nelson 2006). The term cytochrome P450 (CYP) is derived from a pigment (P) that has a 450-nm spectral peak when reduced and bound to carbon monoxide. CYP enzymes are usually hydrophobic and associated with membranes, hindering early studies, and it was not until the 1980s that the first CYPs were isolated and characterized (Nebert and Russell 2002). The CYP nomenclature is based on evolutionary relationships and the proteins are classified in families (≥ 40% amino acid sequence identity) indicated by a number, and in subfamilies (≥ 55% amino acid sequence identity) indicated by a letter (Nelson 2006). Currently, there are 781 different CYP families, 110 of which have been identified in animals (http://drnelson.utmem.edu/cytochromeP450.html). Humans have 57 functional *CYP* genes arranged into 18 families.

In humans, all CYP enzymes are bound in membranes, predominantly in the endoplasmic reticulum and mitochondria (Guengerich 2003). CYPs are associated with the oxidative metabolism of both endogenous and exogenous compounds in the human body. The reaction mechanism is based on the activation of molecular oxygen by the heme group in a process that involves the delivery of two electrons to the P450 system. This is followed by cleavage of the dioxygen bond, yielding water and an activated iron-oxygen species that reacts with substrates through a variety of mechanisms (Guengerich 2007). The majority of CYP enzymes are present in families CYP1-CYP4; the CYP1, CYP2, and CYP3 enzymes are

primarily associated with the metabolism of exogenous compounds, whereas the other CYPs mainly have endogenous roles (Table 1). It is estimated that CYPs in families 1-3 are responsible for about 75% of all phase I metabolism of clinically used drugs (Evans and Relling 1999). CYPs exhibiting important endogenous functions are well conserved, while almost all CYPs involved in xenobiotic metabolism are functionally polymorphic (Ingelman-Sundberg 2004). The clinically most important polymorphism is seen with genes coding for CYP2D6, CYP2C9, and CYP2C19 (Ingelman-Sundberg 2004).

Table 1. Human CYP enzymes classified based on major substrate class (Guengerich 2008).

Xenobiotics	Sterols	Fatty acids	Eicosanoids	Vitamins	Unknown
1A1	1B1	2J2	4F2	2R1	2A7
1A2	7A1	4A11	4F3	24A1	2S1
2A6	7B1	4B1	4F8	26A1	2U1
2A13	8B1	4F12	5A1	26B1	2W1
2B6	11A1		8A1	26C1	3A43
2C8	11B1			27B1	4A22
2C9	11B2				4F11
2C18	17A1				4F22
2C19	19A1				4V2
2D6	21A2				4X1
2E1	27A1				4Z1
2F1	39A1				20A1
3A4	46A1				27C1
3A5	51A1				
3A7					

3.2 CYP2D6

CYP2D6 (OMIM 124030) has become one of the model traits of pharmacogenetics since it is highly polymorphic and responsible for the metabolism of about 20-25% of prescribed drugs, including antidepressants, neuroleptics, β-blockers, and antiarrhythmics (Table 2) (Ingelman-Sundberg 2005). The *CYP2D6* gene spans a 4.2-kilobase (kb) region located on chromosome 22q13.1 and is part of the *CYP2D* cluster together with highly homologous *CYP2D8P* and *CYP2D7P* pseudogenes (Fig. 1) (Kimura et al. 1989; Gough et al. 1993). Like other members of the *CYP2* gene family, the *CYP2D6* gene consists of nine exons and eight introns.

CYP2D6 is a polypeptide of 497 amino acids. Like other drug-metabolizing CYPs, it is hydrophobic and bound to the endoplasmic reticulum with an N-terminal sequence, while the catalytic domain of the enzyme is on the cytoplasmic surface. This has hindered structural studies of the protein, and it was not until recently that the x-ray crystal structure of CYP2D6 was solved by introducing solubilizing mutations to the protein (Rowland et al. 2006). The lengths and orientations of individual secondary structural elements were found to be very similar to those seen before in CYP2C9 (Williams et al. 2003). CYP2D6 has a well-defined active site cavity above the heme group, containing many important residues that have been implicated in substrate recognition and binding, including Asp-301, Glu-216, Phe-483, and Phe-120. Typical CYP2D6 substrate molecules contain basic nitrogen and a planar aromatic ring, features found in many central nervous system and cardiovascular drugs that act on the G protein-coupled receptor superfamily of proteins (Rowland et al. 2006).

CYP2D6 is expressed mainly in the liver, but also at lower levels in several extrahepatic tissues (Zanger et al. 2001; Bieche et al. 2007). Although CYP2D6 is expressed at relatively low levels also in the liver relative to other CYP isoforms, it is one of the most important enzymes contributing to drug metabolism along with CYP3A4, CYP2C9, and CYP2C19 (Ingelman-Sundberg 2004). Dissimilar to all other drug-metabolizing CYPs, there are no inducers described for CYP2D6. Possible mechanisms for the regulation of CYP2D6 expression have been suggested to include copy number variation (*i.e.* whole-gene duplication and multiplication) and DNA methylation (Ingelman-Sundberg 2005; Ingelman-Sundberg et al. 2007).

Table 2. Common drug substrates of CYP2D6, CYP2C9, and CYP2C19 according to therapeutic class (Desta et al. 2002; Zanger et al. 2004; Kirchheiner and Brockmöller 2005; Rettie and Jones 2005).

CYP2D6		CYP2C9	CYP2C19
Analgesica, Antitussives	**Antiemetics**	**Angiotensin II blockers**	**Anticonvulsants, hypnosedatives, muscle relaxants**
Codeine	Ondansetron	Irbesartan	Diazepam
Dextromethorphan	Tropisetron	Losartan	Phenytoin
Ethylmorphine			
Tramadol	**Antiestrogen**	**Anticonvulsant**	**Antidepressants**
	Tamoxifen	Phenytoin	Amitriptyline
Antiarrhythmics			Citalopram
Flecainide	**Antipsychotics**	**Antidiabetics**	Clomipramine
Mexiletine	Haloperidol	Glibenclamide	Imipramine
Propafenone	Perphenazine	Glimepiride	Moclobemide
	Risperidone	Glipizide	
Antidepressants	Thioridazine	Nateglinide	**Anti-infectives**
Amitriptyline	Zuclopenthixol		Proguanil
Doxepin		**Anti-inflammatories**	Voriconazole
Fluoxetine	**β-blockers**	Celecoxib	
Fluvoxamine	Metoprolol	Diclofenac	**Proton pump inhibitors**
Imipramine	Propranolol	Ibuprofen	Omeprazole
Maprotiline	Timolol	Piroxicam	Lansoprazole
Mianserin		Tenoxicam	Pantoprazole
Nortriptyline			Rabeprazole
Paroxetine		**HMG-CoA reductase inhibitor**	
Venlafaxine		Fluvastatin	**β-blocker**
			Propranolol
		Oral anticoagulant	
		(*S*)-Warfarin	

HMG-CoA, 3-hydroxy-3-methylglutaryl-Coenzyme A.

CYP2D6 exhibits high genetic diversity, the highest measured in a set of 11 genes coding for DMEs (Solus et al. 2004). Currently, over 60 major *CYP2D6* genetic variants have been described (www.cypalleles.ki.se/cyp2d6.htm). These include point mutations, single or multiple base insertions and deletions, gene conversions, and whole-gene deletion and duplication. Actually, *CYP2D6* gene duplication was the first stable active gene amplification described in humans. Johansson *et al*. (Johansson et al. 1993) demonstrated 13 active gene copies in a father and his two children with very rapid metabolism of debrisoquine. Subsequently, *CYP2D6* gene duplications involving a varying number of copies and different variants have been identified (Aklillu et al. 1996; Gaedigk et al. 2007). The most common *CYP2D6* genetic variants are presented in Table 3. Genetic variation at *CYP2D6* affects the hepatic expression and function of the enzyme (Zanger et al. 2001), and the genetic variants can be associated with null, decreased, normal, or increased activity (Table 3). For the decreased-function variants *CYP2D6*10*, *CYP2D6*17*, and *CYP2D6*29*, the effect has been shown to be substrate-dependent (Wennerholm et al. 2001; Wennerholm et al. 2002; Bogni et al. 2005; Shen et al. 2007).

Genetic variation at *CYP2D6* has considerable phenotypic effects. There can be over 10-fold difference among individuals in the required dose of a substrate drug to achieve the same plasma concentration (Kirchheiner et al. 2004). When a sample of individuals from a population is challenged with a CYP2D6 probe substrate, four different phenotypic classes emerge: poor (PMs), intermediate (IMs), extensive (EMs), and ultra-rapid metabolizers (UMs). Bimodal or trimodal distribution of the metabolic ratios can usually be seen, in which the PM phenotype represents a separate subgroup, while no clear distinction exists between the other phenotypic classes (Zanger et al. 2004).

Since CYP2D6 is highly polymorphic and the altered activity variants are common in different populations, it probably does not have a major endogenous role in the human body. However, since CYP2D6 is expressed at significant levels in specific cell types and in certain areas of the brain (Siegle et al. 2001), and it has been shown to be involved in the endogenous formation of serotonin and dopamine (Hiroi et al. 1998; Miller et al. 2001; Yu et al. 2003a; Yu et al. 2003b), a possible role in modulating the levels of neurotransmitters has been suggested. Interestingly, it was also recently shown *in vivo* that the *CYP2D6* genotype affects serotonin concentration in platelets (Kirchheiner et al. 2005). Despite these new findings, the importance of CYP2D6 in endogenous metabolism and its role in neurophysiology remain largely unclear.

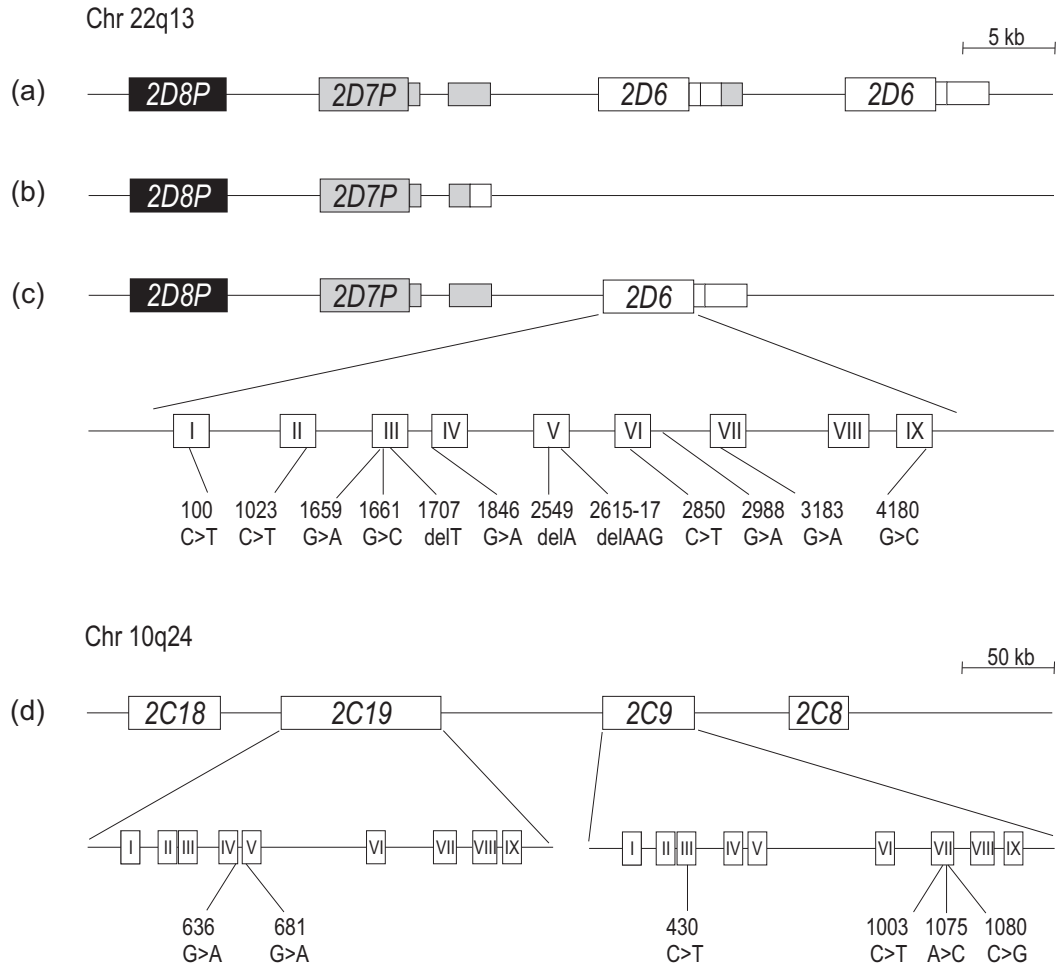

Figure 1. *CYP2D* cluster on chromosome 22q13 contains only one active gene (*CYP2D6*) and two pseudogenes (*CYP2D8P* and *CYP2D7P*). *CYP2D6* can be, however, duplicated in the genome (a) or completely deleted (b). Important genetic polymorphisms affecting CYP2D6 enzymatic activity are shown (c). *CYP2C* cluster consists of four genes and spans almost 400 kb on chromosome 10q24 (d). Important polymorphisms affecting CYP2C9 and CYP2C19 enzymatic activities are shown. I-IX indicate exons of *CYP2D6*, *CYP2C9*, and *CYP2C19*.

3.3 CYP2C9

CYP2C9 (OMIM 601130) is one of the main CYPs expressed in the liver, accounting for about 10% of total hepatic CYP expression (Läpple et al. 2003; Bieche et al. 2007). It is also expressed, albeit at a lower level, in the small intestine, possibly contributing to first-pass metabolism of substrate drugs (Läpple et al. 2003). Over 100 currently used drugs have been identified as substrates of CYP2C9, corresponding to about 15% of commonly prescribed drugs (Kirchheiner and Brockmöller 2005). These include nonsteroidal anti-inflammatory drugs, oral antidiabetics, angiotensin antagonists, oral anticoagulants, and anticonvulsants (Table 2) (Rettie and Jones 2005). Many of these substrate drugs have a narrow therapeutic index.

CYP2C9 is part of the *CYP2C* gene cluster on chromosome 10q24 along with three other *CYP2C* genes (Fig. 1) (Gray et al. 1995). It spans over 50 kb and consists of nine exons and large intronic regions. CYP2C9 was the first human CYP protein whose three-dimensional structure was resolved, both unliganded and in complex with a typical substrate drug warfarin (Williams et al. 2003). The binding mode of warfarin suggested that CYP2C9 may undergo an allosteric mechanism during its function. The crystal structure also showed an unexpectedly large active site that may simultaneously bind multiple ligands during its function, providing a possible molecular basis for understanding complex drug-drug interactions (Williams et al. 2003). Typical CYP2C9 substrates are weak acidic compounds with a hydrogen bond acceptor (Lewis 2004).

In contrast to CYP2D6, the expression of CYP2C9 can be induced by foreign chemicals, such as rifampicin and phenobarbital, through transcriptional factors (Gerbal-Chaloin et al. 2001; Ferguson et al. 2002). These nuclear receptors, namely the pregnane X receptor and the constitutive androstane receptor, sense the concentration of xenobiotics in the cytosol and can consequently induce the expression of specific DMEs to lower the concentration.

Several *CYP2C9* genetic variants with mutations in the regulatory and coding regions of the gene have been described (www.cypalleles.ki.se/cyp2c9.htm; Table 3). Two of these variants, namely *CYP2C9*2* and *CYP2C9*3*, both associated with decreased activity of the enzyme, can be considered the most important ones since they have significant functional effects as well as appreciable high population frequencies (Kirchheiner and Brockmöller 2005). The effect of *CYP2C9*2* on enzymatic activity seems to be more substrate-specific, whereas the

catalytic activity of *CYP2C9*3* is reduced for most substrates (Kirchheiner and Brockmöller 2005).

In addition to being one of the key DMEs, CYP2C9 has an important endogenous role. It is involved in the regulation of vascular homeostasis by converting arachidonic acid to its epoxyeicosatrienoic acid metabolites, which are associated with vasodilatation, angiogenesis, and anti-inflammatory effects (Fleming 2008). On the other hand, CYP2C9-related arachidonic acid metabolism generates reactive oxygen species (Fleming et al. 2001), which may contribute to cardiovascular injury and disease (Chehal and Granville 2006). In addition, CYP2C9 is a key enzyme in the liver, involved in linoleic acid epoxidation, producing leukotoxins, which together with their diols have many cytotoxic effects (Draper and Hammock 2000). Thus, genetic variation at *CYP2C9* may influence not only drug metabolism, but also physiologic processes (Kirchheiner and Brockmöller 2005).

3.4 CYP2C19

CYP2C19 (OMIM 124020) is one of the most important enzymes contributing to the metabolism of clinically used drugs, although relative to other CYP isoforms, it is expressed at low levels and almost exclusively in the liver and the small intestine (Läpple et al. 2003; Bieche et al. 2007). CYP2C19 substrate drugs include proton pump inhibitors (PPIs), antidepressants, anticonvulsants, hypnosedatives, muscle relaxants, and antimalarial drugs (Table 2) (Desta et al. 2002). These substrates are usually amides or weak bases with two hydrogen bond acceptors (Lewis 2004). *CYP2C19* gene is located in the same *CYP2C* gene cluster as *CYP2C9*, and it is fairly large gene, spanning over a 90-kb genomic region that consists of nine exons and large intronic regions (Fig. 1).

The three-dimensional structure of CYP2C19 has not yet been resolved, but it can be predicted to a great extent from the structure of CYP2C9 (Williams et al. 2003). The two enzymes differ by 43 residues out of 490, and the differences in substrate selectivity may be more due to the structure of the substrate-access channel than the amino acids within their active sites (Williams et al. 2003). The expression of CYP2C19 can be induced, similarly as CYP2C9, in response to xenobiotics through the activation of nuclear receptors (Gerbal-Chaloin et al. 2001; Chen et al. 2003).

CYP2C19 exhibits high genetic polymorphism (www.cypalleles.ki.se/cyp2c19.htm), including two common defective variants (Table 3). Single-base substitutions in the coding sequence of *CYP2C19*2* and *CYP2C19**3 lead to splicing defect and premature stop codon, respectively, and therefore to null function of the enzyme. These variants together are responsible for the majority of the CYP2C19-related PM phenotypes in different populations (Xie et al. 2001). Interestingly, a common novel variant, *CYP2C19*17*, associated with ultra-rapid drug metabolism was recently described (Sim et al. 2006). Mutation in the 5'-flanking region of the gene was shown to increase the rate of CYP2C19 transcription, leading to higher metabolic activity, possibly contributing to therapeutic failures in drug treatment with, for example, proton pump inhibitors and antidepressants (Sim et al. 2006; Rudberg et al. 2008).

The relatively high frequencies of nonfunctional *CYP2C19* variants in some populations indicate that the enzyme does not have a major endogenous role. Indeed, for the few endogenous substrates identified, such as farnesol and melatonin, CYP2C19-mediated metabolism represents only a minor pathway (DeBarber et al. 2004; Ma et al. 2005).

Table 3. Most common *CYP2D6, CYP2C9,* and *CYP2C19* genetic variants.

Variant[a]	Defining nucleotide change(s)	NCBI dbSNP[b]	Effect on protein	Enzyme activity
*CYP2D6*2*	2850C>T, 4180G>C	rs16947, rs1135840	R296C, S486T	normal
*CYP2D6*3*	2549delA		frameshift	none
*CYP2D6*4*	1846G>A	rs3892097	splicing defect	none
*CYP2D6*5*	whole-gene deletion		*CYP2D6* deleted	none
*CYP2D6*6*	1707delT	rs5030655	frameshift	none
*CYP2D6*9*	2615-2617delAAG		K281del	decreased
*CYP2D6*10*	100C>T	rs1065852	P34S	decreased
*CYP2D6*17*	1023C>T, 2850C>T	rs28371706, rs16947	T107I, R296C	decreased
*CYP2D6*29*	1659G>A, 1661G>C, 3183G>A		V136I, V338M	decreased
*CYP2D6*39*	1661G>C, 4180G>C	rs1135840	S486T	normal
*CYP2D6*41*	2988G>A		aberrant splicing	decreased
*CYP2D6*1xN*	whole-gene duplication		Nx active genes	increased
*CYP2D6*2xN*	whole-gene duplication (+2850C>T, 4180G>C)		Nx active genes	increased
*CYP2D6*4xN*	whole-gene duplication (+1846G>A)		Nx inactive genes	none
*CYP2D6*10xN*	whole-gene duplication (+100C>T)		Nx decreased-activity genes	decreased
*CYP2D6*41xN*	whole-gene duplication (+2988G>A)		Nx decreased-activity genes	decreased
*CYP2C9*2*	430C>T	rs1799853	R144C	decreased
*CYP2C9*3*	1075A>C	rs1057910	I359L	decreased
*CYP2C9*5*	1080C>G	rs28371686	D360E	decreased
*CYP2C9*11*	1003C>T	rs28371685	R335W	decreased
*CYP2C19*2*	681G>A	rs4244285	splicing defect	none
*CYP2C19*3*	636G>A	rs4986893	premature stop codon	none

[a]Nomenclature according to the Human Cytochrome P450 (*CYP)* Allele Nomenclature Committee (http://www.cypalleles.ki.se/).

[b]Reference identifier in the Single-Nucleotide Polymorphism Database (dbSNP) provided by the National Center for Biotechnology Information (NCBI).

4 Genetic Variation at *CYP2D6, CYP2C9,* and *CYP2C19* in Human Populations

CYP2D6, CYP2C9, and *CYP2C19* exhibit high levels of genetic polymorphism in human populations. Variants associated with altered enzymatic activity can reach surprisingly high frequencies, and substantial differences in the variation between populations have been described. For example, CYP2D6-related PM phenotype is most important in Caucasian populations (frequency 5-10%), predominantly accounted for by the high frequency of nonfunctional variant *CYP2D6*4* (Bradford 2002). By contrast, in Asian and African populations, the IM phenotypic group plays the major role, reflecting high frequencies of decreased-function variants *CYP2D6*10* and *CYP2D6*17*, respectively (Bradford 2002). Extremely high frequencies of *CYP2D6* active gene duplication carriers, exhibiting ultra-rapid metabolism, have been described in Ethiopian (29%) and Spanish (10%) populations (Aklillu et al. 1996; Bernal et al. 1999).

Similarly, Caucasian populations are characterized by the highest frequencies of the common decreased-function variants of *CYP2C9*, while the altered activity variants in other populations are rarer (Garcia-Martin et al. 2006). *CYP2C19* also shows a striking pattern of genetic variation; the frequency of null function variants *CYP2C19*2* and *CYP2C19*3* increases steeply in Asian populations (41%), reaching its maximum in Melanesian populations (up to 90%), indicating that over half of the people in some populations completely lack CYP2C19 enzymatic activity (Kaneko et al. 1999; Shimizu et al. 2003). In addition to the differences shown by the common variants of these genes, there are many rare population/region-specific variants that also contribute to the genetic variation seen both within and among populations.

5 Factors Affecting the Genetic Diversity at *CYP* Genes

5.1 Evolution of the Gene Superfamily

CYP enzymes have been discovered in both prokaryotes and eukaryotes, and it is clear that they first evolved to serve critical life functions (Nelson 1999). The earliest P450-mediated reactions may have been reductase and isomerase functions because of the relatively anaerobic conditions in the earth's environment (Nebert and Dieter 2000). When the level of atmospheric oxygen increased, detoxification of oxygen, partly carried out by CYP enzymes,

became important as a defence mechanism for survival against oxidant stress toxicity (Nebert and Dieter 2000).

Evolution of *CYP* genes in animals during the past 1000 million years has been strongly affected by the interaction of animals with plants (Gonzalez and Nebert 1990). Plants need animals for their reproductive cycles, but at the same time must maintain a defence system for survival. When animals started ingesting plants, they had to evolve new genes and metabolites to make them less palatable or more toxic, and animals responded with new DME genes to adapt to the constantly changing plants (Gonzalez and Nebert 1990). This is reflected in the "explosion" of new genes in the animal *CYP2* family, with over 50 gene duplication events starting around 400 million years ago, when animals first came onto land and began exploiting terrestrial plant forms (Nebert 1997).

This coevolution has led to the diversity of *CYP* gene superfamily seen in both animal and plant species. In animals, the role of DMEs has been more recently expanded to include the activation and detoxification of innumerable environmental pollutants, carcinogens, and drugs, which are, in fact, generally derived from naturally occurring plant metabolites (Nebert 1997).

5.2 Neutral Evolution in Human Populations

The genetic variation observed at *CYP* genes in humans may reflect the chance effects of mutation and genetic drift, as expected under neutral evolution. The neutral theory of molecular evolution postulates that the vast majority of polymorphisms within species are the result of random drift of neutral mutations rather than natural selection (Kimura 1968). Indeed, demographic models of human history alone may explain diversity patterns observed at random genome markers. Based on the analysis of 783 microsatellite loci in a worldwide sample of human populations, the pattern of genetic variation was best explained by a serial founder effect originating in Africa, followed by population expansions (Ramachandran et al. 2005). These results are in line with the standard model of modern human evolution, also known as the "Out of Africa" model (Cann et al. 1987). This model proposes that a small population of about 1000 individuals, most likely from East Africa, expanded throughout much of Africa (around 100 000 years ago), which was followed by a second expansion (60 000 - 40 000 years ago) into Asia and from there to the other continents (Cavalli-Sforza and

Feldman 2003). While the majority of genetic variation among human populations is determined by genetic drift due to the serial founder effect, the local variation may be produced by population-specific history or selection (Ramachandran et al. 2005).

Genetic diversity observed in a particular population can be strongly affected by the demographic history. The Finnish population, which is considered a genetic isolate, represents an excellent example. Settlement in Finland began about 10 000 years ago, soon after deglaciation of Fennoscandia. The initial colonization came from the south and south-east and was followed by waves of settlers from the south (Baltic region) and the west (Scandinavia), about 4500 years ago and later (Norio 2003b). Settlement was concentrated in the south-western and southern coastal parts of Finland, while the eastern, central, and northern parts of the country were permanently settled as late as the 16th and 17th centuries by people from the Savo region in South-East Finland (Norio 2003a; 2003b). Intense genetic drift, arising due to founder effects associated with colonization events and the resulting low effective population sizes in local sub-isolates, has played an important role in the history of the population. At the genomic level, this can be seen in, for instance, the pattern of inherited diseases in Finland (Peltonen et al. 1999; Norio 2003a; 2003c), the strong, partly hereditary, east-west difference in coronary heart disease mortality (Juonala et al. 2005), and the Y-chromosomal variation (Hedman et al. 2004; Lappalainen et al. 2006; Palo et al. 2007; Palo et al. 2008).

5.3 Selective Pressures

In addition to neutral processes, natural selection may contribute to the high levels of polymorphism exhibited by *CYP* genes in human populations. Based on the phylogenetic analysis of *CYP* genes from ten vertebrate species, genes coding for enzymes with major endogenous roles were shown to be evolutionarily stable, whereas enzymes mainly involved in the metabolism of foreign compounds were unstable, often revealing gene duplications and deletions (Thomas 2007). Many of these unstable *CYP* genes are subject to changes in their amino acid sequence over time via positive selection (Gotoh 1992; Thomas 2007). The diversification of genes in response to changes in xenobiotic exposure occurs therefore through a combination of gene duplication and selection-driven divergence in sequence. Substantial variability in DME variant frequencies between populations might thus reflect differences in dietary or environmental exposure that have evolved over thousands of years. Indeed, dietary selection pressure has been suggested to account for the extremely high

occurrence of functional *CYP2D6* gene duplications in North-East African populations (Aklillu et al. 2002; Ingelman-Sundberg 2005).

Another adaptive explanation for the presence of *CYP* genetic variants at relatively high frequencies in human populations may be balancing selection. It favors the diversity of alleles present in a population, resulting in an excess of intermediate-frequency variants. Under balancing selection, heterozygotes usually have a survival advantage over both homozygotes. One of the best examples is the G6PD deficiency and resistance to malaria (Verrelli et al. 2002). G6PD deficiency, affecting around 400 million people worldwide, is strongly associated with the distribution of malarial endemicity. The oxidative stress imposed by the deficiency in the red blood cells probably also creates a toxic environment for the *Plasmodium* parasites that cause malaria. Although G6PD deficiency may have detrimental effects, the benefit that it provides in the presence of malaria suggests that it may be maintained in populations by balancing selection (Verrelli et al. 2002). Since balancing selection is typically observed at loci involved in interaction with exogenous substances (Garrigan and Hedrick 2003; Ferrer-Admetlla et al. 2008), it may also affect the *CYP* genes involved in xenobiotic metabolism.

6 Clinical Pharmacogenetics

6.1 From Genotypes to Phenotypes

Predicting phenotype from genotype is a tool to personalize drug therapy, *i.e.*, to administer the optimal drug and dosage for each patient. Traditionally, information on an individual's metabolic capacity has been obtained through phenotyping, involving measurement and interpretation of drug concentrations. Genotyping is, however, becoming an increasingly important tool in clinical practice as well as in drug development, offering several advantages over traditional phenotyping: (i) results are not influenced by physiologic factors or concurrent medication; (ii) it can be performed less invasively without predisposing an individual to a drug and potential adverse effects; and (iii) it can provide predictive value for multiple drugs, rather than only a single drug (McElroy et al. 2000; Ensom et al. 2001). Although the availability of various commercial genotyping platforms has made genotype information readily accessible, prediction of phenotype from genotype remains a challenge (Gaedigk et al. 2008).

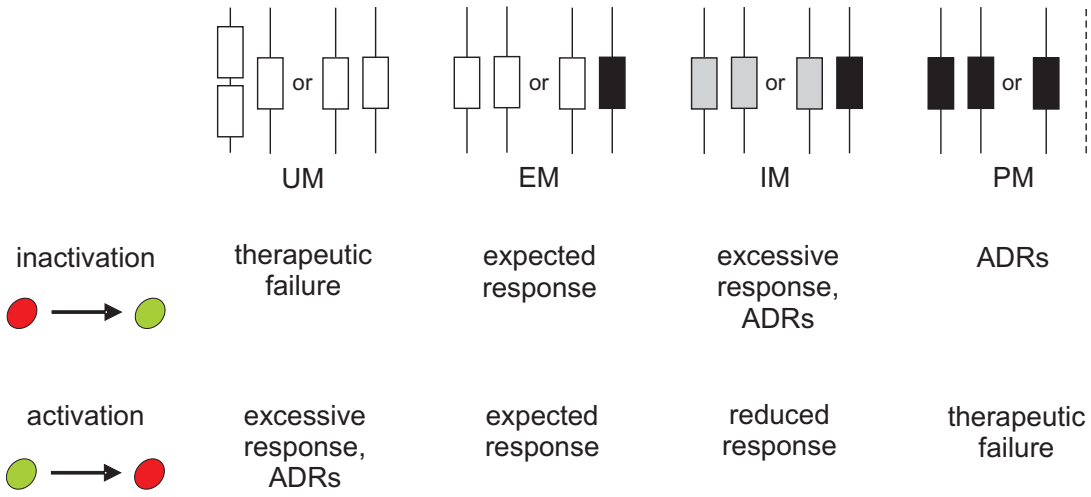

Figure 2. Scheme of the traditional classification of phenotypes based on genotypes and their clinical consequences depending on the type of reaction catalyzed by the polymorphic enzyme. Null variants are represented by black boxes, decreased-function variants by gray boxes, and fully functional variants by white boxes. The dash line indicates a whole-gene deletion. Red represents an active drug molecule and green an inactive molecule. UM: ultra-rapid metabolizer; EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer. Modified in part from (Zanger et al. 2004).

Several different systems to translate genotype data into a phenotype prediction have been used in a variety of clinical settings. Traditional classification of phenotypes is based on the assumption of dominance, in which the phenotype is determined by the most efficient variant in the genotype. Following the example of CYP2D6 genotype-phenotype relationships, four phenotypic classes can be defined: PMs, lacking the functional enzyme; IMs, carrying two decreased-function variants or a combination of one decreased-function variant and one nonfunctional variant; EMs, possessing at least one fully functional variant; and UMs, carrying active gene duplication or another mutation that increases enzyme activity (*e.g.* promoter polymorphism) in conjunction with a functional variant (Fig. 2) (Zanger et al. 2004). As the number of known genetic variants associated with a range of enzyme activities has been growing, new quantitative systems to more precisely identify the effect of individual variants on the phenotype have also been introduced (Steimer et al. 2004; Gaedigk et al. 2008).

Translation of genotype into a qualitative measure of phenotype is challenging for many reasons. Based on phenotyping studies, only the subgroup of individuals completely lacking the enzyme activity (PMs) can usually be identified, while substantial overlap exists in activity within and between the other phenotypic groups (Zanger et al. 2004; Gaedigk et al. 2008). Subjects with identical genotypes may also exhibit different phenotypic activities depending on ancestry, which may be explained by population-specific factors, including unidentified sequence variations at the encoding gene or variations within other genes impacting the enzyme activity, as well as by nongenetic factors, such as diet, altering the enzyme activity (Aklillu et al. 2002; Gaedigk et al. 2002; Gaedigk et al. 2008). In addition, the functional consequences of the genetic variation may be substrate-specific, as shown by, for example, common decreased-function variants *CYP2C9*2* and *CYP2D6*17* (Wennerholm et al. 2002; Kirchheiner and Brockmöller 2005). These population- and substrate-specific factors should be considered in improved phenotype prediction, which rather than assigning an individual to a particular phenotypic class gives the probability of the subject being present in each of the defined phenotypic classes (Gaedigk et al. 2008).

Prediction of phenotypes from genotypes has the potential to identify individuals at specific risk for having undesired drug effects or therapeutic failure due to altered enzymatic activity (Fig. 2), which would enable dose adjustment or change of therapeutic strategy (Kirchheiner 2008). Although considerable challenges remain in predicting phenotype as well as in transforming this information into clinical guidelines for drug treatment of individual patients, there are already some promising examples of how genetic variation in drug metabolism can be taken into account in clinical practice to improve therapeutic outcome (Table 4).

Table 4. Examples of drugs for which pharmacogenomic information regarding DMEs is included in the drug label (Frueh et al. 2008).

Biomarker	Drug(s)
CYP2D6 variants	Atomoxetine, fluoxetine, tamoxifen, metoprolol
CYP2C9 variants	Celecoxib, warfarin
CYP2C19 variants	Esomeprazole, omeprazole, voriconazole
NAT variants	Isoniazid, rifampin
TPMT variants	Azathioprine, mercaptopurine
UGT1A1 variants	Irinotecan

6.2 Clinical Applications Involving CYP2D6, CYP2C9, and CYP2C19

6.2.1 Cancer Treatment

Genetic variation at *CYP2D6* has important therapeutic implications in cancer treatment. Tamoxifen is used to treat estrogen receptor-positive breast cancer. It can be considered a classic pro-drug, requiring metabolic activation to antiestrogenic metabolites endoxifen and 4-hydroxytamoxifen in reactions catalyzed by CYP2D6 (Goetz et al. 2008). CYP2D6 enzyme activity has been shown to affect tamoxifen treatment outcomes such that patients with impaired CYP2D6 metabolism have a higher risk of breast cancer recurrence, shorter relapse-free periods, and worse event-free survival rates than patients with extensive CYP2D6 metabolism (Goetz et al. 2005; Borges et al. 2006; Schroth et al. 2007). *CYP2D6* genotyping has been suggested to be used as a predictive marker for the individualization of the therapy; patients with predicted PM or IM phenotypes, who would derive little benefit from tamoxifen, can be identified and considered for alternative therapy (Goetz et al. 2008). *CYP2D6* genetic polymorphism can also affect the efficacy of antiemetic drugs, which are often used for nausea and vomiting induced by cancer chemotherapy. Serotonin type 3 receptor antagonists tropisetron and ondansetron, metabolized by CYP2D6, show lack of a therapeutic effect in CYP2D6-related UMs, who would greatly benefit from genotype-based dose adjustment or change in therapeutic strategy to avoid severe emesis (Kaiser et al. 2002).

6.2.2 Oral Anticoagulation Therapy

Oral anticoagulants are widely used for the treatment and prevention of thromboembolic disorders, including deep vein thrombosis, acute myocardial infarction, and stroke (Baglin et al. 2006). Typical anticoagulants (*e.g.* warfarin, acenocoumarol, and phenprocoumon) act as vitamin K antagonists by inhibiting the liver microsomal enzyme, vitamin K epoxide reductase (VKOR), which is essential in the vitamin K cycle and formation of clotting factors (Au and Rettie 2008). Although these drugs are highly effective, clinical use is complicated by their narrow therapeutic index combined with wide interindividual variability in the dose required for adequate anticoagulation. In addition, there is a substantial related risk for serious adverse effects, such as hemorrhage, possibly leading to severe morbidity or death (Au and Rettie 2008). Variability in the response to anticoagulants can be attributed to environmental

factors, such as age, weight, liver function, magnitude of dietary intake of vitamin K, and drug interactions, as well as to genetic factors (Wadelius and Pirmohamed 2007).

Warfarin is the most widely used anticoagulant worldwide, and the genetic variation affecting drug response has been extensively studied. Warfarin is administered as a racemic mixture of *R*- and *S*-enantiomers, the latter of which is predominantly responsible for the anticoagulant effect, and metabolized by CYP2C9 (Kaminsky and Zhang 1997). Both common decreased-function variants, *CYP2C9*2* and *CYP2C9*3*, have a substantial effect on the intrinsic clearance of *S*-warfarin, leading to lower required drug dose and to an increased risk of adverse bleeding events (Kirchheiner and Brockmöller 2005; Sanderson et al. 2005; Limdi et al. 2008). Recent identification of the gene *VKORC1*, encoding the warfarin target receptor VKOR (Li et al. 2004; Rost et al. 2004), has further improved the understanding of variability in warfarin dose requirements. Mutations within the noncoding regions of *VKORC1*, reducing the protein expression level, have been identified as a major determinant of warfarin sensitivity (Rieder et al. 2005; Oldenburg et al. 2007). Around 25% of the variance in warfarin dose can be explained by genetic variation at *VKORC1*, whereas *CYP2C9* and known clinical factors (*e.g.* age, gender, weight, drug-drug interactions) account for about 10% and 20% of the total variability, respectively (Au and Rettie 2008; Wadelius et al. 2008). Several new dosing algorithms taking into account these factors have been proposed to improve the efficacy and safety of warfarin treatment (Wu 2007). Importantly, prospective randomized controlled studies have already shown that the incorporation of genotype information will lead to a better clinical outcome in anticoagulation therapy (Anderson et al. 2007; Caraco et al. 2008).

6.2.3 Proton Pump Inhibitor Therapy

PPIs, such as omeprazole, lanzoprazole, and rabeprazole, are widely used for the treatment of acid-related diseases, including gastroesophageal reflux disease and peptic ulcer, as well as for the eradication of *Helicobacter pylori* in combination with antibiotics (Horn 2000). PPIs are mainly metabolized by CYP2C19 in the liver, and the clinical outcome of drug therapy has been shown to depend on genetic variation at the encoding gene (Furuta et al. 2007b). Plasma concentrations of the drugs and the concomitant intragastric pH levels are significantly affected by *CYP2C19* genotype status such that the best acidic inhibition and therapeutic response is attained in PMs, while EMs often experience lack of a therapeutic

effect with standard drug dosages (Furuta et al. 1999; Shirai et al. 2001). In addition, recently described ultra-rapid CYP2C19-related metabolism may also be an important factor contributing to therapeutic failures in drug treatment with PPIs, especially in populations of European ancestry, in which the causative variant *CYP2C19*17* is fairly common (Sim et al. 2006; Hunfeld et al. 2008). *CYP2C19* genotype-guided PPI therapy has been suggested to improve the efficacy of the drugs (Furuta et al. 2007b), which was recently also shown in a randomized controlled trial in the treatment of *H. pylori* infection (Furuta et al. 2007a).

6.2.4 Psychiatric Drug Therapy

Neuropsychiatric conditions, such as major depressive disorders and schizophrenia, are among the most important causes of death and disability worldwide (Lopez et al. 2006). Despite the availability of a wide range of different antidepressants and antipsychotics, a high proportion of patients will not respond sufficiently to treatment (Kirchheiner et al. 2004). Genetic variation has been identified as an important factor underlying the variation in psychiatric drug response. The meta-analysis by Kirchheiner *et al*. (Kirchheiner et al. 2004) of 36 commonly used antidepressants and 38 antipsychotics showed that genetic variation in metabolizing enzymes CYP2D6 and CYP2C19 strongly affected the pharmacokinetics of about one-third of the drugs.

Tricyclic antidepressants (TCAs) have been the basis of antidepressive therapy for over four decades. Amitriptyline, which is one of the oldest TCAs, remains widely used because of higher efficacy and lower cost of therapy compared with newer antidepressants (Barbui and Hotopf 2001). However, amitriptyline is also well known for its relatively narrow therapeutic range (Schulz and Schmoldt 2003) and high toxicity at increased concentrations, leading to severe adverse effects. The main CYPs involved in amitriptyline metabolism are CYP2C19, catalyzing the major demethylation pathway to an active compound nortriptyline, and CYP2D6 mediating the main hydroxylation reactions of both compounds (Fig. 3) (Breyer-Pfaff 2004). Genetic variation at these enzymes has been shown to correlate with the serum concentrations of amitriptyline and nortiptyline, as well as with the occurrence of side-effects related to amitriptyline therapy (Steimer et al. 2004; 2005).

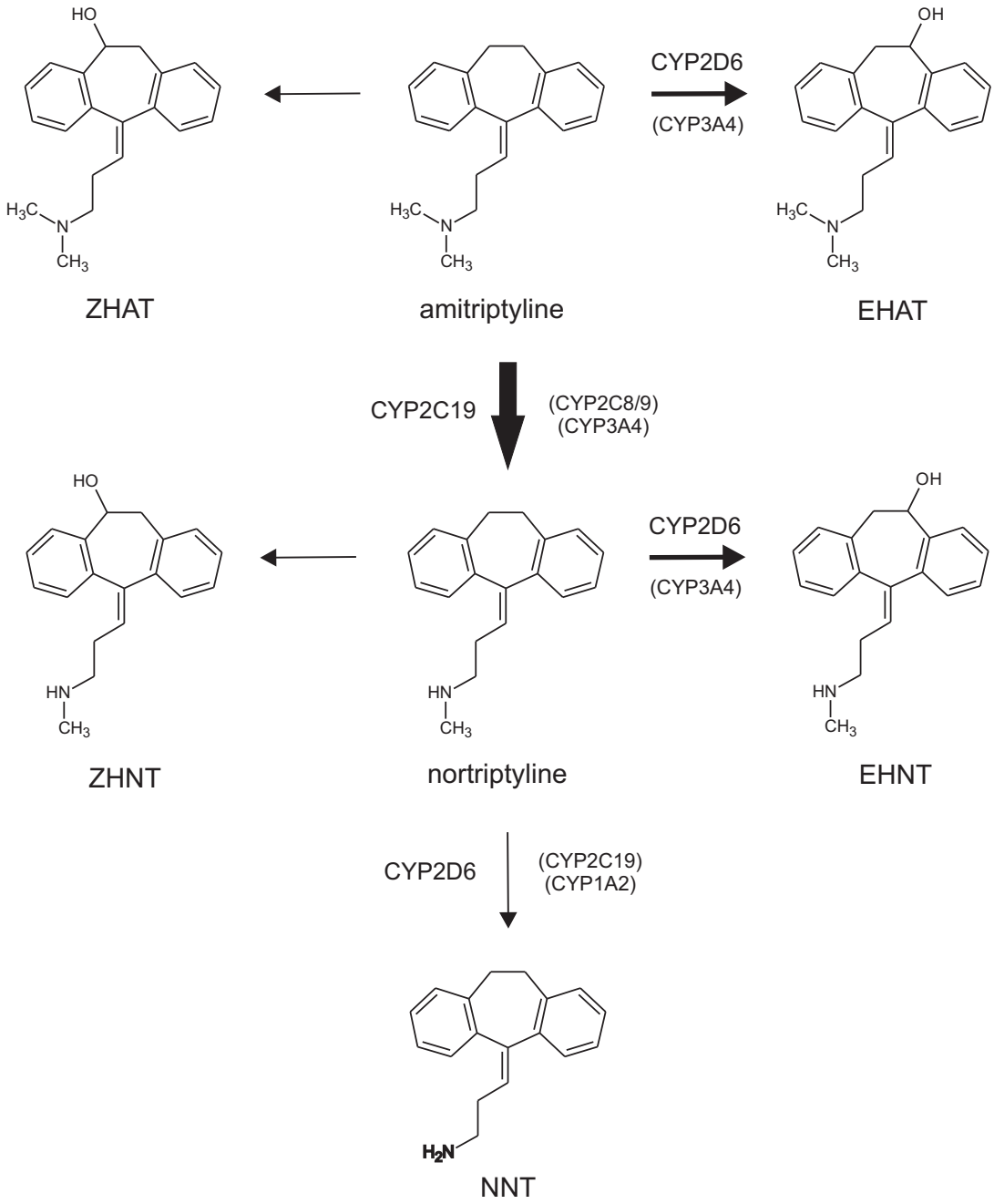

Figure 3. Selected biotransformation pathways of amitriptyline and the main CYP enzymes involved. The relative contribution of each reaction to the overall metabolism of amitriptyline is shown by the thickness of the arrow, and the principal CYP isoforms responsible are highlighted. NNT: *N*-desmethylnortriptyline; EHAT: (*E*)-10-hydroxyamitriptyline; ZHAT: (*Z*)-10-hydroxyamitriptyline; EHNT: (*E*)-10-hydroxynortriptyline; ZHNT: (*Z*)-10-hydroxynortriptyline.

In a study by Chou *et al*. (Chou et al. 2000), the influence of *CYP2D6* genetic variability was examined in 100 consecutive psychiatric patients by evaluating ADRs, hospital stays, and total costs over a one-year period. They found that when considering medication primarily dependent on CYP2D6 enzyme for their metabolism, patients exhibiting PM phenotype had higher number of ADRs and longer duration of hospitalization. In addition, the cost of treating patients with extremes in CYP2D6 activity (PMs and UMs) was on average $4000 to $6000 per year greater than the cost of treating other patients with the same medication. The application of pharmacogenetics in psychiatric clinical practice seems promising, and the first guidelines on the dose adjustments for specific antidepressants and antipsychotics based on *CYP2D6* and *CYP2C19* genotypes are already available (Kirchheiner et al. 2004). However, future prospective studies are necessary to evaluate the actual outcome and benefit of pharmacogenetic individualization of psychiatric drug therapy.

7 Postmortem Pharmacogenetics

Genetic variation related to drug response can cause severe ADRs or even fatal intoxications. In the case of CYP enzymes, poor drug metabolism can lead to accumulation of a drug in the body and subsequent toxic effects. Already in 1997, Swanson *et al.* (Swanson et al. 1997) speculated that the death of two young subjects resulting from TCA imipramine and desipramine intoxication could be due to a genetic defect in drug metabolism. A very low metabolic ratio of imipramine to its active metabolite desipramine and the absence of evidence suggesting an acute overdose led the authors to conclude that the intoxication in both cases had been chronic, and potential mechanisms included genetically determined PM phenotype of CYP2D6, which is the major enzyme catalyzing hydroxylation of both compounds, and drug interactions.

However, the case described by Sallee *et al.* (Sallee et al. 2000) was the first in which genetically determined poor drug metabolism was shown to lead to fatal drug intoxication. In this case, a nine-year-old boy, who had a history of extreme behavioral problems and had been treated with a combination of psychotherapeutic agents, died of fluoxetine intoxication. Extremely high concentration of fluoxetine and its major active metabolite norfluoxetine found from several tissues in postmortem toxicologic evaluation led to a legal investigation of the adoptive parents of the child. Thorough examination of the case revealed that the child had a completely defective *CYP2D6* gene, resulting in a compromised ability to metabolize CYP2D6 substrates, such as fluoxetine. In addition, despite experiencing over a 10-month period signs and symptoms suggestive of metabolic toxicity, including three hospitalizations, the child had been prescribed an increasing dose of fluoxetine; the final dose of 100 mg/day was higher than doses normally used in adults.

Ultra-rapid drug metabolism can also be associated with severe or fatal ADRs if the enzyme catalyzes the conversion of a pro-drug into an active compound. Two case reports involving CYP2D6 and codeine have recently been described (Gasche et al. 2004; Koren et al. 2006). In the case described by Koren *et al.* (Koren et al. 2006), a breastfed neonate was found dead at the age of 13 days. Postmortem analysis revealed that the baby died of morphine intoxication. He got the morphine in the breast milk of the mother, who had been prescribed codeine after birth for episiotomy pain. Codeine is *O*-demethylated to morphine in a reaction catalyzed by CYP2D6, and the mother was later found to carry an active *CYP2D6* gene duplication associated with increased codeine metabolism and formation of morphine, which was lethal to the neonate.

Postmortem pharmacogenetics is a relatively new area of research. It has thus far been focused on genetic variation at CYP enzymes in relation to drug intoxications. In 1999, *CYP* genotyping was for the first time shown to be feasible in postmortem sample material (Druid et al. 1999). In this study, 22 suspected overdose cases with drugs metabolized by CYP2D6 and a control group of 24 cases were genotyped for nonfunctional variants *CYP2D6*3* and *CYP2D6*4*. No PM subjects among the cases were identified, and the authors concluded that drug-drug interactions constitute a more frequent and important problem in interpreting forensic toxicology results than genetic variability in drug metabolism. Interestingly, in two subsequent studies by the same group on fatal drug intoxications, PM subjects were found to be underrepresented among the cases due to significantly lower frequency of *CYP2D6*4* than in the general population (Holmgren et al. 2004; Zackrisson et al. 2004). However, no explanation was offered for this observation.

Genetic variation in drug metabolism has been shown to be correlated with the observed phenotype, defined as parent drug to metabolite ratios, in postmortem sample material (Levo et al. 2003), and *CYP* genotyping has been used to aid interpretation of postmortem toxicology results in oxycodone- (Jannetto et al. 2002), methadone- (Wong et al. 2003), and fentanyl-related deaths (Jin et al. 2005). However, most of the postmortem pharmacogenetic studies have been performed on a limited number of samples detecting only a few genetic variants, and often without considering the relevant metabolic ratios or background information of the cases. While pharmacogenetics in a postmortem setting is a challenging and exciting new area of research, it remains to be seen to what extent it will contribute to medicolegal investigations in the future.

AIMS OF THE STUDY

The aim of this study was to describe genetic variation at *CYP2D6, CYP2C9,* and *CYP2C19* in different human populations on a global scale and to apply pharmacogenetics to a postmortem forensic setting.

Specific aims of the study were as follows:

1. To develop a *CYP2D6* genotyping method that covers the most important mutations affecting enzymatic activity (I), and to apply the same method to genotype *CYP2C9* and *CYP2C19* (III).

2. To consistently genotype *CYP2D6* for the first time in a global survey of human populations and to analyze the distribution of its genetic variation (II).

3. To describe and compare genetic variation at *CYP2C9*, *CYP2C19*, and *CYP2D6* on a global scale (III).

4. To describe genetic variation at *CYP2C9, CYP2C19*, and *CYP2D6* within the Finnish population (III).

5. To estimate the correlation between amitriptyline metabolic ratios and *CYP2D6* and *CYP2C19* genotypes in postmortem sample material (IV).

6. To determine whether accidental or undetermined fatal drug intoxications can be attributed to genetic polymorphism at *CYP2D6* or *CYP2C19* in selected cases (IV, V).

MATERIALS AND METHODS

1 Samples

1.1 Population Genetic Studies (II, III)

Samples belonging to the Human Genome Diversity Cell Line Panel (Cann et al. 2002) were used in Studies II and III. This sample set was obtained from the Centre d'Etude du Polymorphisme Humain (CEPH) in Paris. It includes the DNA of 1064 individuals originating from 52 globally distributed populations, which were in some of the analyses grouped into large geographic regions following the original CEPH documents (http://www.cephb.fr/HGDP-CEPH-Panel, Study II) or the United Nations classification of geographic regions (http://unstats.un.org/unsd/methods/m49/m49regin.htm, Study III). In addition, 56 Western Finnish (Kankaanpää region), 86 Eastern Finnish (Suomussalmi region), and 202 Western Indian (Gujarat state) unrelated healthy volunteers were included in Study III.

1.2 Postmortem Cases (IV, V)

All cases included were autopsied in Finland during 2000-2002, and the toxicological analyses were performed at the Laboratory of Toxicology, Department of Forensic Medicine, University of Helsinki. Study IV included 202 consecutive toxicology cases where at least 0.2 mg/l of amitriptyline was detected in a broad drug and alcohol screen and where a sufficient amount of blood for metabolite analysis and genotyping was available.

In Study V, 11 cases (from 2002) of fatal CYP2D6 substrate (amitriptyline, doxepin, dextromethorphan, fluoxetine, fluvoxamine, codeine, oxycodone, paroxetine, tramadol, or venlafaxine) poisonings with the manner of death denoted as accidental or undetermined were included. To facilitate the interpretation of the results, a control group of 34 doxepin cases where nordoxepin was found in the same year was also included.

2 Genotyping

2.1 DNA Extraction (III-V)

DNA was extracted from blood samples using an E.Z.N.A.™ SE Blood DNA Kit (Omega Bio-Tek, Inc., Doraville, GA, USA) or by standard protocols involving digestion of leucocytes with proteinase K, followed by phenol-chloroform extraction and ethanol precipitation (Sambrook et al. 1989).

2.2 Detected Genetic Variants

Among the several polymorphic positions known at *CYP2C9*, *CYP2C19*, and *CYP2D6* (http://www.cypalleles.ki.se/), we selected 4 (*CYP2C9*), 2 (*CYP2C19*), and 12 (*CYP2D6*) variable sites either highly represented in different human populations or, even if rare, known to be responsible for low or null metabolic activity of the corresponding enzyme (Fig. 1, Table 3). *CYP2D6* whole-gene deletion and duplications were also included in the genotyping.

2.3 *CYP2D6* Genotyping (I-V)

CYP2D6 genotyping was based on long PCR and single-nucleotide primer extension reactions. The entire *CYP2D6* gene (5.1 kb) was amplified in a long PCR reaction, and the purified fragment was subsequently used as a template to detect 12 polymorphic positions of the gene. Detection was based on multiplex extension of unlabeled oligonucleotide primers with fluorescently labeled dideoxynucleotide triphosphates (SNaPshot™; Applied Biosystems, Foster City, CA, USA). Two additional long PCR reactions were used to analyze the whole-gene deletion and duplication, and the phase of the gene duplication in heterozygous genotypes was defined based on the SNaPshot result. For details of the *CYP2D6* genotyping, see Studies I and II.

2.4 *CYP2C9* and *CYP2C19* Genotyping (III-V)

A modification of the above-mentioned method was used to genotype *CYP2C9* and *CYP2C19*. Two fragments covering exons 2-3 (0.6 kb) and exon 7 (0.4 kb) of *CYP2C9* as well as a fragment covering exons 4-5 (1.9kb) of *CYP2C19* were amplified and used as templates in a SNaPshot multiplex reaction to detect the polymorphic positions (Fig. 1, Table 3). For details of the genotyping, see Study III.

3 Collection of Data from the Literature (III)

In Study III, data on genetic variation at *CYP2C9*, *CYP2C19*, and *CYP2D6* were collected from the literature. Published articles (from 1991 to 2007) were retrieved from the PubMed database provided by the National Center for Biotechnology and Information (http://www.pubmed.gov). About 900 articles were reviewed, and data from 186 articles were used in the study. Inclusion criteria were that the subjects be apparently unrelated, randomly selected volunteers of defined ethnicity. Data from controls of case-control studies were used when the above criteria were fulfilled. Populations were classified according to geographic origin following the United Nations classification of geographic regions (http://unstats.un.org/unsd/methods/m49/m49regin.htm).

4 Definition of CYP2D6 Phenotype Classes (II)

To describe the CYP2D6 phenotypic diversity in different geographic regions, phenotypes were predicted from genotypes. Conventional classification of phenotypes, in which the phenotype is determined by the most efficient haplotype in the genotype, was used. The prediction of enzyme activity of each haplotype was based on results obtained from previously published studies (for reference, see http://www.cypalleles.ki.se/cyp2d6.htm). In this way, four phenotypic categories were defined, namely PM, IM, EM, and UM (see also Fig. 2 on p. 26). Specifically, two decreased-function variants or a combination of one decreased-function variant and one null-function variant were classified as IMs, whereas UM was defined as a carrier of an active gene duplication in conjunction with a functional variant.

5 Analysis of Drug Concentrations

5.1 Drug Screening (IV, V)

Each postmortem case was submitted to a comprehensive toxicological analysis of blood and urine samples performed using a multi-technique approach. Urine samples were screened for approximately 700 drugs by immunoassay, liquid chromatography-tandem mass spectrometry (LC-MS/MS), and LC coupled with time-of-flight mass spectrometry (Pelander et al. 2003). Simultaneously, blood samples were quantitatively monitored for 200 drugs by three techniques: gas chromatography-mass spectrometry (GC-MS) for acidic/neutral drugs, GC with electron capture detection for benzodiazepines, and GC with nitrogen phosphorus detection for basic drugs (Rasanen et al. 2003). Confirmation and additional determinations were carried out using GC-MS and LC-MS/MS in both urine and blood. The screening approach covered the majority of psychotropic drugs available on licit and illicit markets, with a special emphasis on abused substances.

5.2 Metabolite Analysis (IV)

Major amitriptyline metabolites nortriptyline, *N*-desmethylnortriptyline (NNT), (*E*)-10-hydroxyamitriptyline (EHAT), (*Z*)-10-hydroxyamitriptyline (ZHAT), (*E*)-10-hydroxynortriptyline (EHNT), and (*Z*)-10-hydroxynortriptyline (ZHNT) were analyzed using LC-MS/MS. An internal standard (imipramine) was added to blood samples, and the analytes were extracted into butyl acetate/2-propanol at pH 9. The organic phase was separated and evaporated, and the residue was reconstituted into LC eluents. Separation was achieved on a C18 column with gradient elution, and the detection was performed by tandem mass spectrometry in multiple reaction monitoring mode. Limit of quantitation was 0.001 mg/l, and concentrations above 5 mg/l were quantitated in 1:10 dilutions.

6 Statistical Methods

6.1 Analyses of Genetic Variation (II, III)

CYP2D6 haplotypes were inferred from genotypes using the software PHASE v. 2.1 (Stephens et al. 2001; Stephens and Donnelly 2003). Linkage disequilibrium (LD) between pairs of polymorphic sites was tested in each geographic region by calculating two statistics, $|D'|$ (Lewontin 1964) and R^2 (Hill and Robertson 1968), using the software DnaSP v. 3.99 (Rozas et al. 2003). The phylogenetic relationships of *CYP2D6* haplotypes were summarized with a maximum parsimony network (Templeton et al. 1992) using TCS software (Clement et al. 2000). Genetic distances between populations, namely F_{ST} values (based on genetic variant frequencies or predicted phenotypes) and Φ_{ST} values (based on haplotypes), were estimated, and the Analysis of Molecular Variance (AMOVA; (Excoffier et al. 1992)) was used to quantify the genetic diversity at three levels: within populations, among populations within regions, and among regions. Locus-by-locus AMOVA was performed to assess whether a specific variant is responsible for the *CYP2D6* genetic distance between Finnish subpopulations and other European populations. Arlequin v. 3.11 (Excoffier et al. 2005) was used for estimation of pairwise genetic distances and for the AMOVA. Geographic patterns of *CYP2D6* genetic diversity were assessed by comparing genetic and geographic distance matrices using Mantel tests (Mantel 1967) and by spatial autocorrelation analysis (PASSAGE; (Rosenberg 2001)).

6.2 Analyses of Amitriptyline Metabolism (IV)

The logarithms of the main metabolite ratios were used for calculation of medians, confidence intervals for medians, and differences between medians. Mann-Whitney U-test was applied to assess differences between median metabolite ratios. Univariate analysis of variance was carried out for the most relevant logarithmic metabolite ratios using gender, age, and number of functional copies of *CYP2D6* and *CYP2C19* as covariates. MINITAB v. 13.31 (Minitab Inc., State College, PA, USA) was used for calculations involving medians and proportions, and SPSS v. 10.0.7 (SPSS Inc., Chicago, IL, USA) for univariate analysis of variance.

RESULTS

1 Methodological Development (I-III)

A new genotyping method, based on long PCR and single-nucleotide primer extension reactions, was developed for *CYP2D6*. The method allowed identification of the most important altered activity variants (Table 3). A novel and interesting feature of this method was the possibility to determine the phase of *CYP2D6* gene duplication in heterozygous genotypes by taking advantage of the quantitative nature of the SNaPshot reaction. To validate the method, a sample of individuals representing different detected variants, including gene duplications, was genotyped by PCR-restriction fragment length polymorphism (RFLP) analysis (for details, see Study I), (Sachse et al. 1997; Levo et al. 2003). In addition, polymorphic positions 2988G>A and 3183G>A, which were not included in the PCR-RFLP protocol, were verified by sequencing. Concordance was 100% between the new method and conventional methods. The same method was applied to genotype the most important variable sites of *CYP2C9* and *CYP2C19* (Table 3). SNaPshot-based genotyping proved to be robust and accurate, and the results were easy to interpret (Fig. 4).

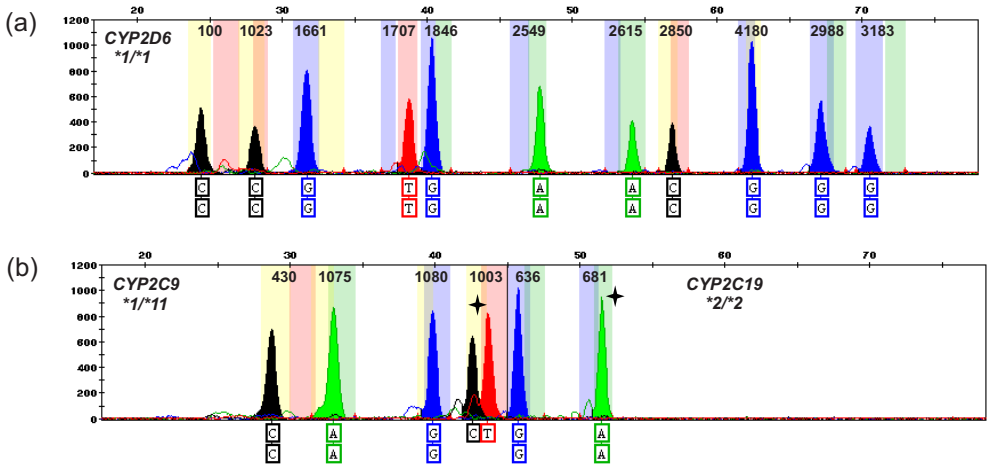

Figure 4. *CYP2D6* (a), and *CYP2C9* and *CYP2C19* (b) SNaPshot genotyping results. Numbers above the peaks indicate the detected polymorphic positions of the corresponding genes and the stars indicate mutations (*CYP2C9* 1003C>T; *CYP2C19* 681G>A). Detection primer for position 1080 of *CYP2C9* is complementary to the coding strand, and therefore, nucleotide G in the electropherogram corresponds to nucleotide C in the coding sequence. Defined genotypes are indicated.

2 Pharmacogenetic Variation on a Global Scale

2.1 *CYP2D6* (II)

2.1.1 Haplotypic and Phenotypic Variation

CYP2D6 haplotypes were statistically inferred from the genotypes of 1060 individuals belonging to the 52 global populations. Most of the 21 inferred haplotypes corresponded to previously described combinations of SNPs (http://www.cypalleles.ki.se/cyp2d6.htm). Three new haplotypes bear only one detected SNP, namely 4180G>C, 1661G>C, or 1661G>C, in a duplicated gene.

Subsaharan African populations displayed the highest diversity, with eight frequent (> 5%) polymorphic positions. By contrast, only three to six variable sites reached > 5% frequency in other regions. When pairs of polymorphic sites were tested for the presence of LD, the statistic |*D'*| was 1 for 78 out of 82 comparisons, with the four exceptions in Subsaharan Africa and the Middle East (for details, see Study II). Subsaharan Africa was also the only region where most of the R^2 values were below 0.3 and the association was nonsignificant for some pairwise comparisons; all tests reached statistical significance in the other geographic regions. The generally high values of LD and the significance of the association tests indicate that intra-locus recombination has not played a relevant role in shaping the *CYP2D6* molecular variation, at least after human migration out of Africa.

CYP2D6 haplotypes were represented in a network, showing also the geographic distribution (Fig. 5a). The phylogenetic relationships of different variants were clearly defined. Fully functional haplotypes *CYP2D6*1* and *CYP2D6*2* were the most frequent genetic variants, being widely distributed in different geographic regions. However, also altered activity variants reached relatively high frequencies in different areas of the world. Decreased-function variants *CYP2D6*10* and *CYP2D6*17* were common in Asian and African populations, respectively, while *CYP2D6*41* was most frequent in Middle Eastern and Central/South Asian populations. The only common null-function variant, *CYP2D6*4*, was most frequent in European populations, whereas the increased-function variant *CYP2D6*2xN* reached an extremely high frequency (28.3%) in North Africa.

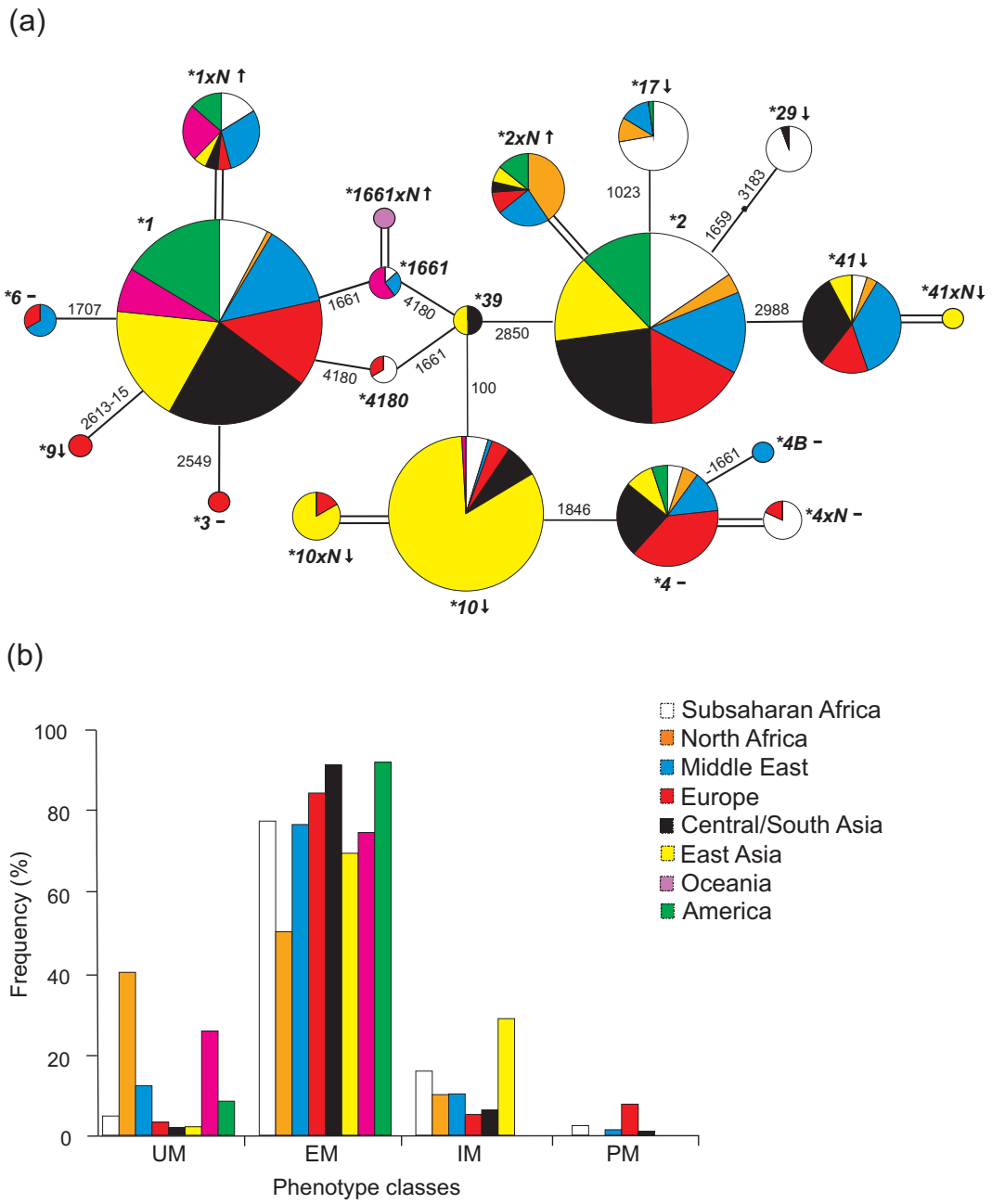

Figure 5. *CYP2D6* haplotype and phenotype diversity in different geographic regions. (a) Haplotypes are represented in a network. The size of the circle is proportional to the haplotype frequency in the whole dataset of 1060 individuals. Mutations separating haplotypes are indicated. Double lines correspond to gene duplication. The altered enzymatic activity related to a haplotype is represented as follows: increased (↑), decreased (↓), null (-). (b) Frequency of CYP2D6 phenotype classes is shown in different geographic regions. Phenotypes are predicted from genotypes as described in Materials and Methods. UM: ultra-rapid metabolizers; EM: extensive metabolizers; IM: intermediate metabolizers; PM: poor metabolizers.

To describe CYP2D6 phenotypic diversity within the same geographic regions, phenotypes of the 1060 individuals were predicted from genotypes, as described in Materials and Methods. Interestingly, the most common altered metabolic activity group was UM in North Africa (40.0%), Oceania (25.6%), the Middle East (12.2%), and America (8.3%), whereas PMs were common only in Europe (7.6%) (Fig. 5b). Frequent decreased-function variants *CYP2D6*10*, *CYP2D6*17*, and *CYP2D6*41* led to higher number of IMs in East Asia, Africa, and the Middle East than in other regions.

2.1.2 Analysis of Molecular Variance

In the AMOVA analysis, both *CYP2D6* haplotypes and phenotypes showed similar results. Most of the diversity was observed within populations (haplotypes 89.8%; phenotypes 90.5%). When all 52 populations included in Study II were analyzed based on seven geographic regions, the differences between regions accounted for 9.3% (haplotypes) or 6.5% (phenotypes) of the total variance. This result is consistent with estimates based on 377 autosomal microsatellite markers typed in the same CEPH sample set (Rosenberg et al. 2002; Excoffier and Hamilton 2003), and based on other neutral autosomal markers (Barbujani et al. 1997; Jorde et al. 2000; Romualdi et al. 2002).

2.1.3 Geographic Patterns of Genetic Diversity

Matrices of *CYP2D6* genetic and geographic distances between populations included in Study II were compared by means of a Mantel test. Since the aim was to determine whether the *CYP2D6* genetic variation has been shaped by human migrations and subsequent demographic effects, the geographic distances of populations were estimated considering the likely routes of human migration out of Africa, following the criteria of Ramachandran *et al*. (Ramachandran et al. 2005). The correlation was almost significant, but explained only a small fraction of the total variation ($r = 0.18$; $P = 0.05$). To test whether *CYP2D6* genetic diversity corresponds to that inferred from neutral markers, the *CYP2D6* genetic distance matrix was compared with a genetic distance matrix estimated using 377 autosomal microsatellites typed in the same sample set (Rosenberg et al. 2002). A positive and statistically significant correlation was observed ($r = 0.37$; $P < 0.01$), also when controlling

for the geographic distance (r = 0.21; P < 0.05). This indicates that the observed correlation between genetic variation at *CYP2D6* and neutral markers was not due to the effect of geographic location of the samples.

Spatial autocorrelation analysis of single *CYP2D6* haplotypes revealed clear worldwide clines for variants *CYP2D6*4*, *CYP2D6*10*, *CYP2D6*17*, and in part, *CYP2D6*41* (Fig. 6), all of them associated with null or decreased metabolism. These variants, each showing its maximum frequency in a different geographic region (Europe, East Asia, Subsaharan Africa, and Western-Central Asia, respectively), decrease in frequency with distance from the maximum frequency region, suggesting that these regions were the likely centers of origin for these haplotypes.

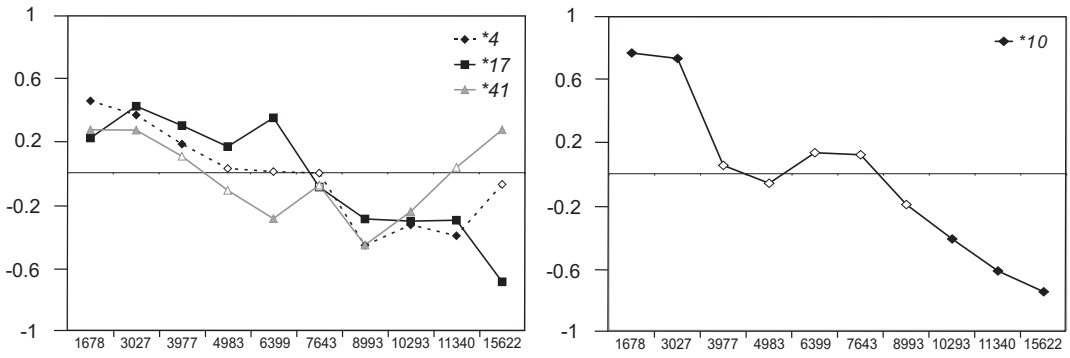

Figure 6. Spatial autocorrelation analysis of frequent *CYP2D6* altered activity haplotypes in populations from the Old World, included in Study II. X-axis: higher limit of geographic distance classes (in kilometers). Y-axis: autocorrelation index I. Filled symbols indicate significant values.

2.2 *CYP2C9* (III)

CYP2C9 genetic variation data were created for four decreased-function variants in 129 population samples by genotyping new samples as well as by collecting data from the literature. The most common *CYP2C9* genetic variants, *CYP2C9*2* and *CYP2C9*3*, were found in the highest frequencies in Northern African and European populations (Fig. 7a). Interestingly, the frequency of *CYP2C9*2* decreased rapidly when moving from Europe towards the East, and it was practically zero in Eastern Asian populations. *CYP2C9*3* occurred more evenly in different geographic regions. *CYP2C9*5* and *CYP2C9*11* were rarer variants, mainly found in African populations.

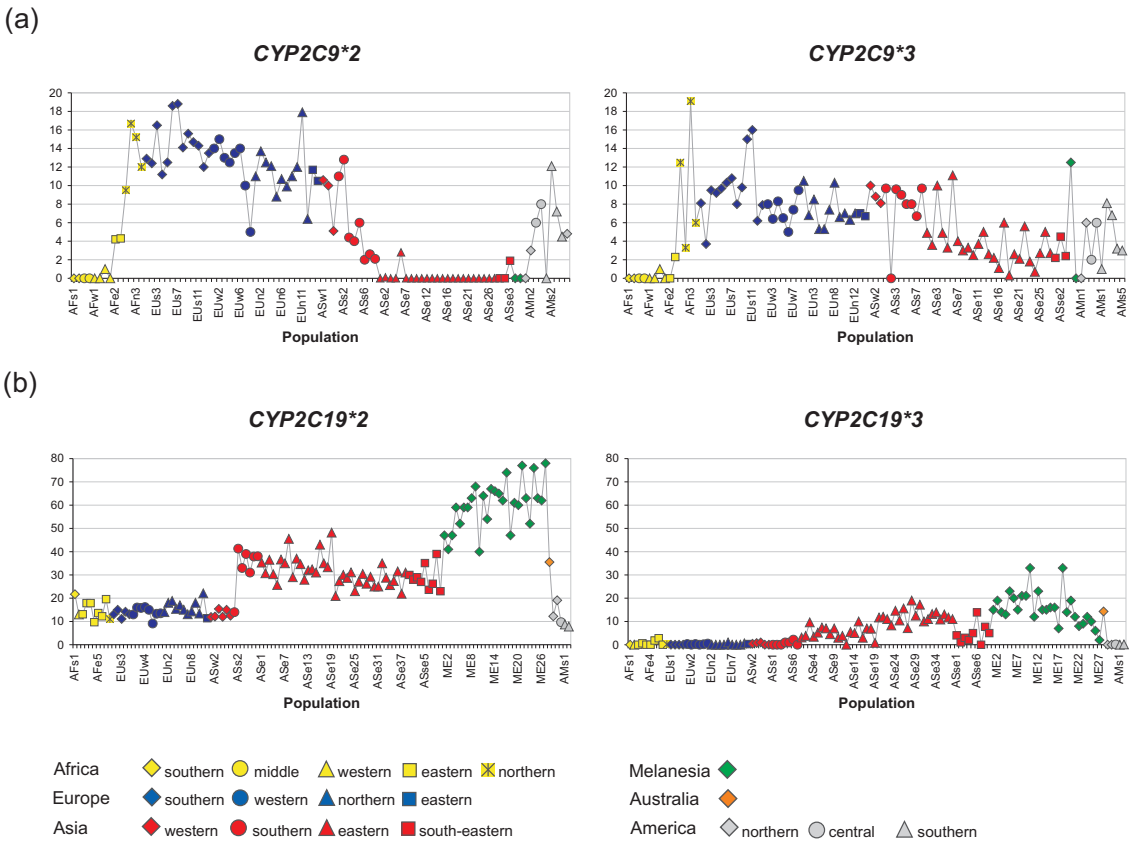

Figure 7. Frequencies of the most common *CYP2C9* (a) and *CYP2C19* (b) genetic variants in worldwide distributed populations. For details of the population samples and the data, see Study III.

2.3 *CYP2C19* (III)

The geographic pattern revealed by *CYP2C19* polymorphism differed substantially from those shown by *CYP2D6* and *CYP2C9*. The null-function variant *CYP2C19*2* was found in all 146 populations studied worldwide, with a minimum frequency of about 10% (Fig. 7b). *CYP2C19*2* frequency increased steeply when moving from Western Asia and Iran to India and reached its maximum (> 75%) in Melanesian populations. The frequency distribution of *CYP2C19*3* showed a similar trend, as the frequency increased in Eastern Asia and reached its maximum (33%) in Melanesia (Fig. 7b). However, outside these regions, *CYP2C19*3* was rare.

3 Pharmacogenetic Variation within the Finnish Population (III)

To gain insight into the pharmacogenetic variation within the Finnish population, two regional samples were genotyped for *CYP2D6*, *CYP2C9*, and *CYP2C19* (Fig. 8). A significant overall difference was present in *CYP2C9* variant frequencies between the two subpopulations (F_{ST} = 0.028; P = 0.008). *CYP2C9*2* was much more frequent in the Western (17.9%) than in the Eastern (6.4%) subpopulation. In addition, *CYP2C9*11* was found only in the Eastern sample, albeit at a low frequency (1.2%). *CYP2C19* also showed differences in frequencies of the variants between the two samples, but the difference was not significant (F_{ST} = 0.019; P > 0.05).

By contrast, the Finnish subpopulations were homogeneous with respect to variation at *CYP2D6*. However, for this gene, Finns showed a population-specific variation pattern compared with other European populations. Based on locus-by-locus AMOVA analysis, the difference was mainly due to polymorphisms 100C>T and 1846G>A, both carried by the null-function variant *CYP2D6*4*, which was indeed observed at a much lower frequency in Finns (8.5%) than in European populations on average (17.2%; Study II). In addition, the active gene duplications (*CYP2D6*1xN*, *CYP2D6*2xN*) leading to ultra-rapid CYP2D6-mediated metabolism were more frequent in Finns (4.6%) than in other Northern European populations (about 1%, (Dahl et al. 1995; Bathum et al. 1998)). Together these findings suggest a higher CYP2D6-related metabolic rate in Finns than in other European populations (Sachse et al. 1997; Bernal et al. 1999; Bozina et al. 2003; Gaikovitch et al. 2003; Fuselli et al. 2004; Arvanitidis et al. 2007; Buzkova et al. 2008).

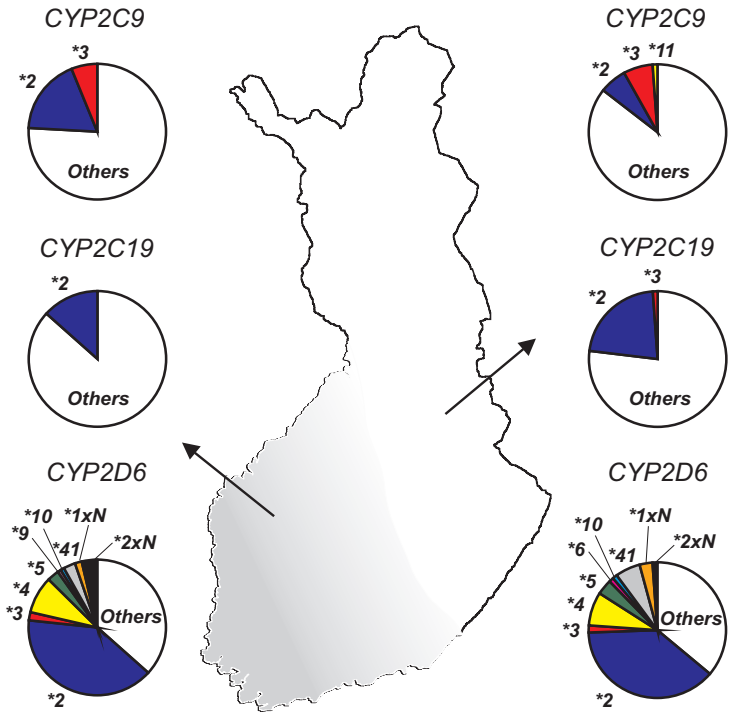

Figure 8. *CYP2C9*, *CYP2C19*, and *CYP2D6* genetic variation in Western and Eastern Finland, roughly corresponding to the early and late settlement areas of the country.

4 Amitriptyline Metabolism in Relation to *CYP2D6* and *CYP2C19* Genotypes (IV)

In the 202 amitriptyline-related postmortem cases, six amitriptyline metabolites were analyzed along with *CYP2D6* and *CYP2C19* genotypes. When metabolite ratios were compared with the number of active genes, a correlation was found between the rate of *trans*-hydroxylation (*i.e.* EHNT/ZHNT, EHAT/ZHAT, nortriptyline/EHNT, amitriptyline/EHAT, and nortriptyline/EHAT) and the number of functional copies of *CYP2D6*, and between the rate of *N*-demethylation (*i.e.* amitriptyline/nortriptyline, EHAT/EHNT, ZHAT/ZHNT, nortriptyline/EHAT, and nortriptyline/ZHAT) and the number of functional copies of *CYP2C19* (Fig. 9). Several median metabolite ratios differed significantly between different *CYP2D6* and *CYP2C19* genotype groups.

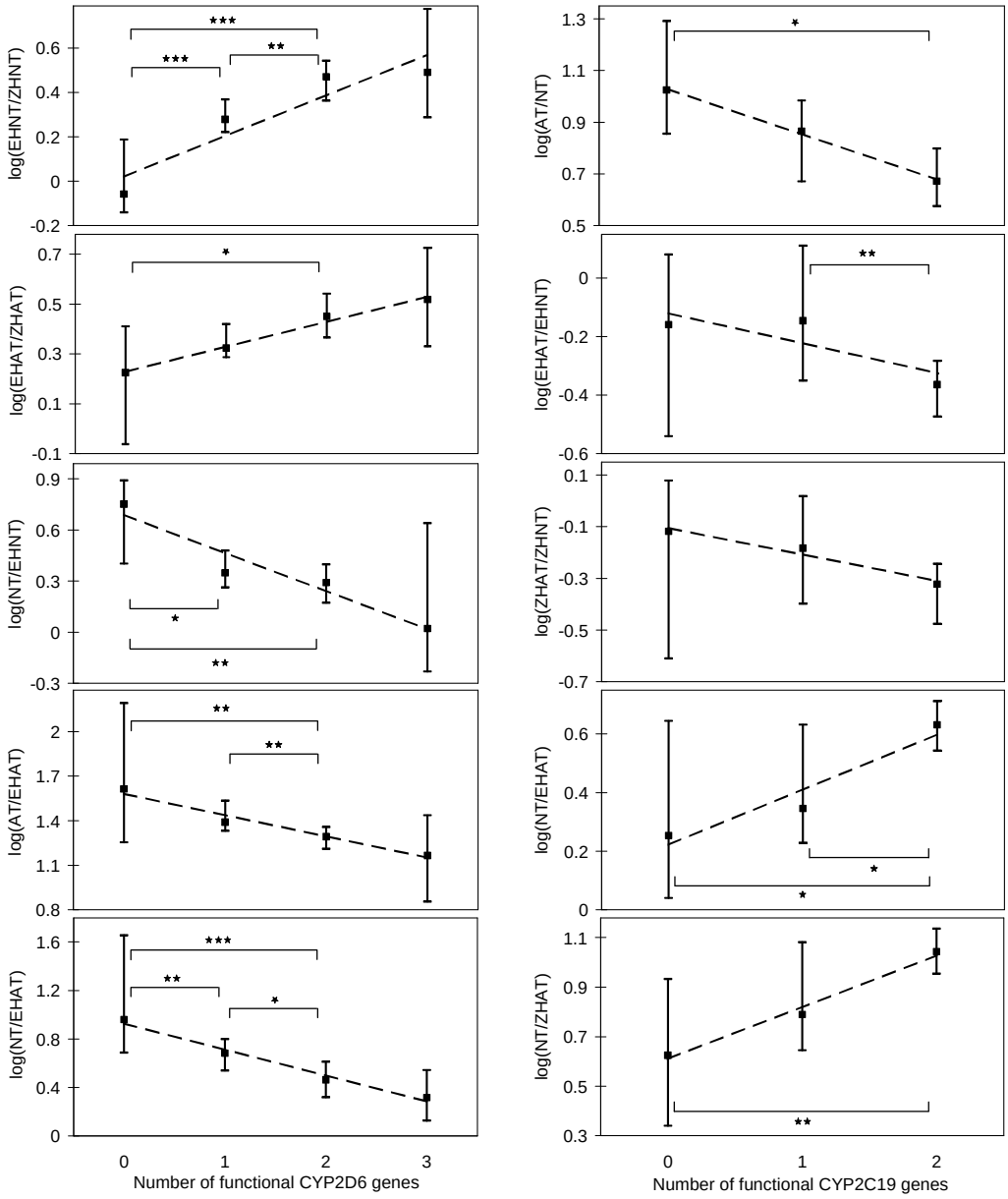

Figure 9. Relevant metabolite ratios in amitriptyline metabolism plotted against the number of functional *CYP2D6* and *CYP2C19* genes. Logarithmic transformations of median metabolite ratios are shown with 95% confidence intervals. AT = amitriptyline; NT = nortriptyline. See also Fig. 3 on page 31. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Figure modified from (Koski 2005).

5 Genetic Variation Associated with Fatal Drug Intoxications (IV, V)

The possibility of fatal drug poisoning occurring due to a combination of drug treatment and a genetic defect in drug metabolism was examined in Studies IV and V. Sixty-three fatal amitriptyline poisoning cases were included in Study IV. The manner of death had been judged as accidental in 17 and undetermined in seven cases. However, none of these 24 poisonings was associated with a nonfunctional *CYP2D6* or *CYP2C19* genotype. Interestingly, in one suicide case, an exceptionally high amitriptyline concentration of 60 mg/l coincided with a defective *CYP2D6* genotype (**4/*4*).

When fatal CYP2D6 substrate poisonings with the manner of death denoted as accidental or undetermined were genotyped (Study V), a case of doxepin-related poisoning was observed to coincide with a defective *CYP2D6* genotype (**3/*4*). In this case, a 43-year-old Finnish man had been found dead in his home, and the forensic toxicology samples taken at autopsy revealed 2.4 mg/l of doxepin and 2.9 mg/l of nordoxepin in femoral venous blood, while the therapeutic blood concentration of doxepin is 0.01-0.2 mg/l (Schulz and Schmoldt 2003). The high concentration of the active metabolite nordoxepin was not consistent with acute intoxication, and the doxepin-to-nordoxepin ratio of 0.83 was the lowest found among the 35 nordoxepin-positive postmortem cases analyzed the same year. The defective genotype may therefore have contributed to the death, possibly involving a repeatedly high dosage of doxepin.

DISCUSSION

1 Methodological Considerations

Genotyping can be used as a tool to personalize drug therapy, *i.e.* to administer the optimal drug and dosage for each patient. Predicting phenotype from genotype offers several advantages over the experimental determination of phenotype: (i) results are not influenced by physiologic factors or concurrent medication; (ii) it can be performed less invasively, without predisposing an individual to a drug and potential adverse effects; and (iii) it can provide predictive value for multiple drugs, rather than merely a single drug (McElroy et al. 2000; Ensom et al. 2001). The availability of technically feasible and cost-effective genotyping methods is important in facilitating the translation of pharmacogenetic data into clinical practice to improve drug efficacy and safety.

New genotyping methods based on a combination of PCR and multiplex single-nucleotide primer extension reactions were developed for *CYP2D6*, *CYP2C9*, and *CYP2C19*, all of which exhibit clinically important genetic polymorphisms. The methods developed, which covered the most important genetic variants that alter enzyme activity (Table 3), proved to be rapid and cost-effective. Samples could be processed in 96-well plates, and after the PCR, the final genotypes could be obtained in five hours. The cost per *CYP2D6* genotype was estimated to be ~5 € (from long PCR to capillary electrophoresis) at the time of the study, which was less than one-half the cost of the corresponding genotyping based on laborious but widely used RFLP analysis (Arvanitidis et al. 2007; Zand et al. 2007).

A novel and interesting feature of the *CYP2D6* genotyping method was the possibility to determine the phase of gene duplication in heterozygous genotypes by taking advantage of the quantitative nature of the SNaPshot reaction. This is particularly useful in distinguishing, for example, the genotypes *CYP2D6*1/*4xN* and *CYP2D6*1xN/*4*, the former producing only a single full-function allele and the latter producing at least twice the amount of enzyme.

A wide variety of different SNP-genotyping methods are currently available, but their disadvantages often include low throughput, high cost, or the requirement of special laboratory facilities (Syvänen 2001). The *CYP* genotyping methods developed here are technically feasible and cost-effective, therefore being suitable for many applications in both routine and research investigations. In addition, one of the advantages of the SNaPshot

technique is the possibility to extend the assay to also cover alternative or newly described SNPs in the targeted genomic region.

2 Pharmacogenetic Variation in Human Populations

CYP2D6, *CYP2C9*, and *CYP2C19* exhibit high levels of genetic polymorphism in human populations. Since these genes code for enzymes affecting the metabolism of 20-30% of clinically used drugs, they are of major pharmacogenetic importance (Desta et al. 2002; Ingelman-Sundberg 2005; Kirchheiner and Brockmöller 2005). In this study, the global genetic variation at these loci was investigated for the first time in a systematic way by genotyping new population samples as well as by collecting data from the literature.

Genetic diversity at *CYP2D6* was examined in detail by genotyping 12 highly informative variable sites, as well as whole-gene deletion and duplications, in a global survey of 52 populations originating from all continents (Cann et al. 2002). All of the results suggested that the diversity observed at *CYP2D6* reflects the same combination of gene flow and drift events that shaped the diversity of most other genomic regions. High *CYP2D6* genetic variances within populations were in good agreement with estimates based on neutral autosomal markers (Barbujani et al. 1997; Jorde et al. 2000; Romualdi et al. 2002; Rosenberg et al. 2002). The lowest level of LD observed in Africa was consistent with the results of studies, suggesting that through their longer evolutionary history, African populations have had a greater potential for recombination to reduce the LD generated by new mutations or founder effects (Gabriel et al. 2002; Tishkoff and Verrelli 2003). In addition, the geographic patterns of *CYP2D6* genetic diversity were best described as clinal, being very similar to those shown by autosomal microsatellites (Serre and Paabo 2004; Ramachandran et al. 2005) and protein markers (Cavalli-Sforza et al. 1994).

Although the spatial patterns of *CYP2D6* diversity appeared clinal and most of the variants were geographically dispersed over all continents, some mutations altering the enzyme activity occurred at very high frequencies in specific areas of the world (Fig. 10a). In particular, decreased-function variants *CYP2D6*10*, *CYP2D6*17*, and *CYP2D6*41* were common in Asian, African, and Western Asian populations, respectively, while null-function variant *CYP2D6*4* was common in European populations. The highest frequency of active gene duplications described thus far (28.3%) was found in a Northern African Mozabite

population, in which about 40% of the people were predicted to exhibit the UM phenotype. The data on genetic variation at *CYP2C9* and *CYP2C19* collected in this study showed a similar high occurrence of altered activity variants in specific regions (Fig. 10b-c). Especially the pattern of variation seen at *CYP2C19* was striking: extremely high frequencies of null-function variants indicated that over half of the people in some populations completely lack the enzymatic activity.

These findings are relevant from the clinical point of view since CYP2D6, CYP2C9, and CYP2C19 are involved in the metabolism of many commonly used drugs. The first clinical applications to take into account the genetic variation to improve therapeutic outcome have already been introduced. These include genetic variation at *CYP2D6* in cancer treatment (Goetz et al. 2008), *CYP2C9* in oral anticoagulation therapy (Au and Rettie 2008), *CYP2C19* in PPI therapy (Furuta et al. 2007b), and *CYP2D6* together with *CYP2C19* in psychiatric drug therapy (Kirchheiner et al. 2004).

The findings do, however, raise questions concerning the evolution of the three loci studied, each of which showed a distinct geographic pattern of variation. *CYP2D6* genetic diversity on a global scale was shown to parallel that described for neutral markers and may be explained by demographic models of human history, consisting of a founder effect due to "Out of Africa" migration, followed by population expansions (Ramachandran et al. 2005). Genetic variation observed at *CYP2D6*, *CYP2C9*, and *CYP2C19* may thus reflect the chance effects of mutation and drift, as expected under neutral evolution. However, the high level of genetic polymorphism at these loci and the local high frequencies of altered activity variants may also be the result of natural selection.

CYP genes coding for enzymes involved mainly in the metabolism of foreign compounds have been shown to be evolutionarily unstable, often possessing gene duplications and deletions. Many of these genes are subject to positive selection to change their amino acid sequence over time in response to changes in xenobiotic exposure (Thomas 2007). Substantial variability in *CYP* variant frequencies might thus reflect differences in dietary or environmental exposure that have evolved over thousands of years. Indeed, dietary selection pressure has been suggested to account for the local high occurrence of active *CYP2D6* gene duplications in North East African populations (Aklillu et al. 2002). Another adaptive explanation for the presence of *CYP* genetic variants at relatively high frequencies in human populations may be balancing selection, which favors the diversity of alleles present in a population. Since balancing selection is typically observed at loci involved in interaction with

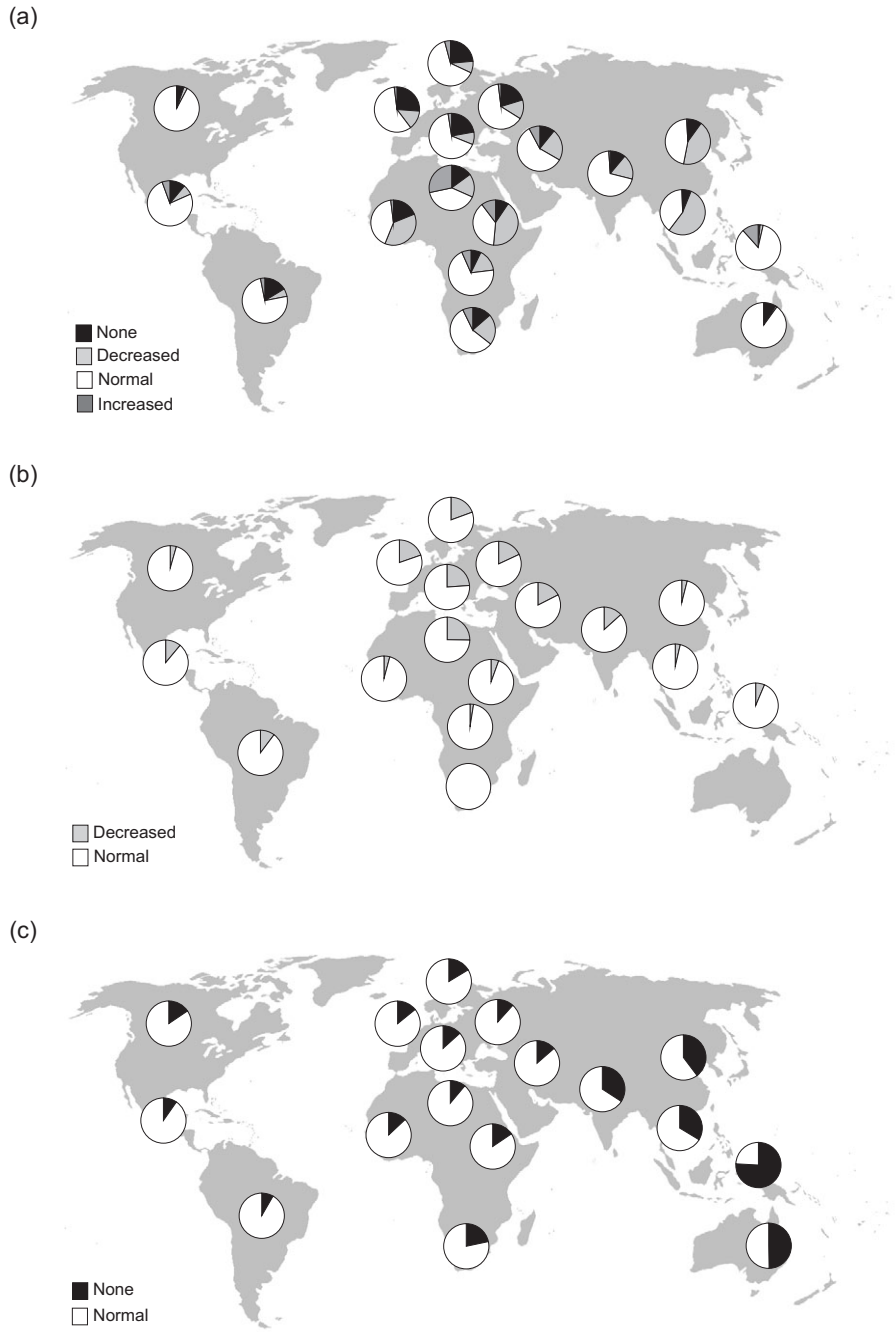

Figure 10. World maps showing the distribution of *CYP2D6* (a), *CYP2C9* (b), and *CYP2C19* (c) altered activity variants in different geographic regions. Variants were grouped based on the phenotypic effect as follows: *CYP2D6* none (**3, *4, *5, *6, *4xN*); *CYP2D6* decreased (**9, *10, *17, *29, *41, *10xN, *41xN*); *CYP2D6* increased (**1xN, *2xN*); *CYP2C9* decreased (**2, *3, *5, *11*); and *CYP2C19* none (**2, *3*). All other variants were considered to have normal activity. For data on individual variants in different populations and geographic regions, see Study III.

exogenous substances (Garrigan and Hedrick 2003; Ferrer-Admetlla et al. 2008), it may also affect the genes belonging to the *CYP2* family. However, more detailed molecular studies are needed to elucidate the evolutionary history of *CYP2D6*, *CYP2C9*, and *CYP2C19*.

Pharmacogenetic variation was also examined at a microgeographic scale by analyzing two regional samples from Finland, representing the early settlement (Western Finland) and the late settlement (Eastern Finland) areas of the country. The same differentiation between the subpopulations observed for neutral markers, such as Y-chromosomal short tandem repeats (Lappalainen et al. 2006; Palo et al. 2007), was observed at *CYP2C9* (F_{ST} = 0.028) and *CYP2C19* (F_{ST} = 0.019), although the latter was not statistically significant. This may be explained by the demographic history of the Finnish population; the Eastern subpopulation has been more affected by recurring founder effects and small local effective population sizes than the Western subpopulation, resulting in the diversity differences seen at different genomic markers. However, the subpopulations were completely homogeneous with respect to variation at *CYP2D6*, which instead showed a population-specific pattern, suggesting a higher CYP2D6-related metabolic rate than in other European populations. These results indicate that the pattern of pharmacogenetic variation can be population-specific and may be significantly affected by the population's demographic history.

3 Pharmacogenetics in Postmortem Forensic Settings

Postmortem pharmacogenetics is a relatively new area of research that can be considered very challenging for many reasons. First, postmortem material is often of poor quality, and degradation of DNA can hamper the genotyping analyses. Second, interpretation of pharmacogenetic results may be difficult because of polypharmacy and various pathophysiological conditions, which are common findings in postmortem cases. In fact, drug interactions have been suggested to be a far greater problem in drug intoxications than genetic variation related to drug response (Druid et al. 1999; Holmgren et al. 2004). Third, postmortem redistribution may contribute to the observed drug concentrations, which do not necessarily reflect the concentrations at the time of death (Pelissier-Alicot et al. 2003). However, since fatal drug intoxications may be caused by genetic variation in drug metabolism (Sallee et al. 2000; Koren et al. 2006), postmortem pharmacogenetics can be of the utmost importance in medicolegal investigations.

The tricyclic antidepressant amitriptyline ranks among the major causes of fatal drug intoxications in Finland (Vuori et al. 2006). It has a relatively narrow therapeutic range (Schulz and Schmoldt 2003) and high toxicity at increased concentrations, leading to severe side-effects. Results of clinical studies show that genetic polymorphism at *CYP2D6* and *CYP2C19*, which encode the major enzymes involved in amitriptyline metabolism (see Fig. 3 on page 31), correlates with the serum concentrations of amitriptyline and its active metabolite nortriptyline as well as with the occurrence of side-effects related to drug therapy (Steimer et al. 2004; 2005). However, genetic variation related to amitriptyline metabolism was investigated here for the first time in a postmortem forensic setting by analyzing the concentrations of amitriptyline metabolites along with *CYP2D6* and *CYP2C19* genotypes in a series of 202 amitriptyline-related postmortem toxicology cases.

Positive correlations were found between the proportion of *trans*-hydroxylated metabolites and the number of functional copies of *CYP2D6*, and between the proportion of demethylated metabolites and the number of functional copies of *CYP2C19*. Therefore, the same correlation between phenotype and genotype observed in clinical studies was also seen in postmortem material, even in the presence of confounding factors, such as drug-drug interactions, typical for these cases. Similar results have been obtained before with respect to opioid drug tramadol metabolite ratios and genetic variation at *CYP2D6*, though in a limited number of samples (Levo et al. 2003).

In investigating accidental or undetermined fatal drug intoxication cases for a genetic defect in drug metabolism, we found a doxepin-related death coinciding with a completely defective *CYP2D6* genotype (**3/*4*). In this case, the high concentration of the active metabolite nordoxepin was not consistent with acute intoxication. In addition, the lowest doxepin-to-nordoxepin ratio found in forensic toxicology cases over a one-year period in Finland suggested that the genetic defect at *CYP2D6* had probably contributed to the accumulation of toxic substances and subsequent fatal intoxication. This case illustrated the importance of considering the concentrations of relevant metabolites in addition to the parent drug when interpreting the results obtained from forensic toxicology and genetic analyses.

While routinely performing genotyping of polymorphic *CYP*s in suspected poisoning cases is probably not worthwhile, postmortem pharmacogenetics may be of great value in specific cases, especially when applied to drugs of high toxicity, such as antidepressants and antipsychotics. When poisoning is caused by a drug that is metabolized by a polymorphic enzyme, and the concentrations of the parent drug and metabolites differ from normal

findings, genotyping may add valuable information to the interpretation of forensic toxicology results and the manner of death. Postmortem pharmacogenetics has the potential to improve medicolegal investigations of death, and at its best, integrates the latest knowledge in the fields of forensic pathology, toxicology, and genetics (Sajantila et al. 2006).

4 Future Directions in Pharmacogenetic Research

ADRs are a significant cause of morbidity, mortality, and excessive medical care costs. ADRs account for an estimated 7% of all hospital admissions (Lazarou et al. 1998; Pirmohamed et al. 2004) and rank as one of the leading causes of death in the United States (Lazarou et al. 1998). One possible cause of ADRs is genetic variation. A meta-analysis by Phillips *et al*. (Phillips et al. 2001) revealed that 59% of drugs frequently causing ADRs were metabolized by at least one enzyme with a known variant associated with poor metabolism, compared with only 7-22% of randomly selected drugs. These results suggest that pharmacogenetic information may have a major impact on reducing ADRs and improving drug therapy.

Pharmacogenetic research has thus far focused mostly on relatively simple monogenic traits involving drug metabolism (Weinshilboum and Wang 2006). These well-established examples of pharmacogenetic traits include genetic variation at butyrylcholinesterase (Kalow and Staron 1957), NAT2 (Evans et al. 1960), CYP2D6 (Mahgoub et al. 1977), and TPMT (Weinshilboum and Sladek 1980). Although these single-gene defects can have a strong effect on their drug substrates, most of the drug effects and treatment outcomes are determined by the interplay of multiple genes (Evans and Relling 2004). Therefore, pharmacogenetic research has also been increasingly focused on entire pathways encoding proteins that influence both pharmacokinetics and pharmacodynamics of a drug (Weinshilboum and Wang 2006). Research on anticoagulant warfarin represents an excellent example of the future direction of pharmacogenetic research. Genetic variation affecting warfarin response has been investigated with 29 genes involved in the action and biotransformation of the drug, and new dosing algorithms taking into account genetic and environmental factors have been developed (Wu 2007; Wadelius et al. 2008).

The latest advances in human genetics research include genome-wide association studies that use dense maps of SNPs (around 500 000 – 1 000 000) covering the human genome to detect allele-frequency differences between cases and controls (Kruglyak 2008). This enables

genome regions containing functional DNA-sequence variants that influence the disease or trait in question to be identified (Raelson et al. 2007; Scott et al. 2007). This approach may be valuable also in pharmacogenetic research in identifying genetic variants associated with drug efficacy or ADRs (Link et al. 2008; Nelson et al. 2008; Roses 2008).

Despite several well-established clinical applications and the wide variety of molecular genotyping methods available, pharmacogenetic testing is still rarely used in clinical settings. There are several factors limiting the translation of new research data into clinical practice that is generally a slow process (Lenfant 2003). Firstly, it will probably require that clinicians receive further training to be able to interpret genotype data and incorporate it into clinical decision-making (Evans and Relling 2004). Secondly, there is a need for randomized prospective clinical trials to demonstrate that pharmacogenetic testing really benefits the selection of appropriate drug and dosage for individual patients, improving therapeutic responses and/or reducing ADRs (Eichelbaum et al. 2006). To date, only a few such studies have been performed (Anderson et al. 2007; Furuta et al. 2007a; Caraco et al. 2008). Pharmacogenetic research would also greatly benefit from establishment of large-scale research networks to collect adequate numbers of samples and to share resources, tools, and statistical approaches. Some multicenter collaborations to characterize the genetic variation underlying drug response and ADRs have already being developed. These include the Pharmacogenetics Research Network (PGRN) (Giacomini et al. 2007a), the European Network of Pharmacogenetics/Genomics (Maitland-van der Zee et al. 2007), the EUDRAGENE project (Molokhia and McKeigue 2006), and the Canadian Genotypic Approaches to Therapy in Children (GATC) project (Ross et al. 2007).

Given the current pharmacogenetic knowledge and the clinical applications already available, "*It is unthinkable that selecting drugs for individual patients remain an empirical exercise*" (Giacomini et al. 2007b). This is particularly true in such cases as *CYP2D6* genetic variation and tamoxifen therapy in breast cancer, in which the pharmacogenetic approach is critical not only for reducing costs of treatment or days of hospitalization, but also for increasing duration of survival (Goetz et al. 2008; Kirchheiner 2008). Although pharmacogenetics is a challenging area of research, it has the potential to translate knowledge of human genome variability into better therapeutics.

CONCLUSIONS

The distribution of genetic variation in three drug-metabolizing enzymes, CYP2D6, CYP2C9, and CYP2C19, was examined for the first time on a global scale by genotyping new population samples with developed methods as well as by systematically collecting data from the literature. Detailed molecular analysis of *CYP2D6* genetic variation in 52 populations originating from all continents revealed a pattern of variation that was similar to those shown by neutral genomic markers. Most of the *CYP2D6* diversity was observed within populations, and the spatial pattern of variation was best described as clinal. However, genetic variants of *CYP2D6*, *CYP2C9*, and *CYP2C19* associated with altered enzymatic activity could reach extremely high frequencies in certain geographic regions. Pharmacogenetic variation may also be affected by population-specific demographic histories, which was revealed by analyzing the variation within Finns. Eastern and Western subpopulations showed a significant difference in variation at *CYP2C9*, and a population-specific pattern emerged at *CYP2D6* compared with other European populations.

Each of the genes studied uncovered a distinct variation pattern in human populations and high frequencies of altered activity variants. This may reflect neutral evolution and/or selective pressures caused by dietary or environmental exposure. These results are relevant from the clinical point of view since *CYP2D6*, *CYP2C9*, and *CYP2C19* code for enzymes of major importance in drug metabolism, and several clinical applications taking into account genetic variation to improve therapeutic outcome already exist.

Pharmacogenetics was also applied to a postmortem forensic setting. A correlation between amitriptyline metabolic ratios and genetic variation at *CYP2D6* and *CYP2C19* was observed in the sample material, even in the presence of confounding factors typical for these cases. In addition, a case of doxepin-related fatal poisoning was shown to be associated with a genetic defect at *CYP2D6*. These results suggest that pharmacogenetics may also add valuable information to medicolegal investigations of sudden unexpected deaths.

ACKNOWLEDGMENTS

This study was carried out at the Department of Forensic Medicine, University of Helsinki, in 2002-2008. I am deeply grateful to my supervisor Professor Antti Sajantila for support, advice, and long discussions on science and life in general. These years gave me the possibility to learn different aspects of research work and grow as a person, and still maintain my inspiration for science.

I thank my collaboration group at the University of Ferrara and Professor Guido Barbujani for giving me the opportunity to work with them and simultaneously learn about the Italian temperament and way of life. I am indebted to Dr. Silvia Fuselli for her expertise and encouragement and for shared moments of inspiring discussions and hard work, all of which were invaluable to me.

The Finnish Graduate School in Population Genetics is gratefully acknowledged for financial support and especially Professor Pekka Pamilo for his consistently positive attitude towards my work.

I sincerely thank Professor Erkki Vuori, Head of the Department of Forensic Medicine, for creating an enthusiastic atmosphere at the department and for his continuous support and interest in my work. I also thank Docent Ilkka Ojanperä for advice and his belief in me from the very beginning of my studies at the university; it's been a long journey that started with washing dishes!

Dr. Anna Koski-Pirilä is gratefully thanked for her collaboration and expertise in toxicology, Docent Jukka Palo and Minttu Hedman for generous help and enlightening discussions, and Airi Sinkko for skillful technical assistance and support. Dr. Antti Levo is gratefully acknowledged for creating the basis for pharmacogenetic research at the Department of Forensic Medicine.

The reviewers of my thesis, Professor Ángel Carracedo and Docent Mikko Niemi, are gratefully thanked for their expertise and insightful comments. I also thank Carol Ann Pelli for editing the language of my thesis.

All of my colleagues at the Department of Forensic Medicine and especially personnel at the Laboratory of Forensic Biology are warmly thanked for their support and for the inspiring work atmosphere.

My friends are owed a special thanks for sharing moments of the greatest joy and despair and for reminding me that there is life outside of work!

Finally, my deepest gratitude is due to my Mother, Father, Ansku, Jukka, and Sanna for their love, encouragement, and belief in me.

Helsinki, October 2008

Johanna Sistonen

REFERENCES

Aklillu E, Herrlin K, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M. Evidence for environmental influence on CYP2D6-catalysed debrisoquine hydroxylation as demonstrated by phenotyping and genotyping of Ethiopians living in Ethiopia or in Sweden. *Pharmacogenetics* 2002; 12:375-383.

Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. *J Pharmacol Exp Ther* 1996; 278:441-446.

Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. *Circulation* 2007; 116:2563-2570.

Arvanitidis K, Ragia G, Iordanidou M, Kyriaki S, Xanthi A, Tavridou A, et al. Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. *Fundam Clin Pharmacol* 2007; 21:419-426.

Au N, Rettie AE. Pharmacogenomics of 4-hydroxycoumarin anticoagulants. *Drug Metab Rev* 2008; 40:355-375.

Baglin TP, Keeling DM, Watson HG. Guidelines on oral anticoagulation (warfarin): third edition--2005 update. *Br J Haematol* 2006; 132:277-285.

Barbui C, Hotopf M. Amitriptyline v. the rest: still the leading antidepressant after 40 years of randomised controlled trials. *Br J Psychiatry* 2001; 178:129-144.

Barbujani G, Magagni A, Minch E, Cavalli-Sforza LL. An apportionment of human DNA diversity. *Proc Natl Acad Sci U S A* 1997; 94:4516-4519.

Bathum L, Johansson I, Ingelman-Sundberg M, Horder M, Brosen K. Ultrarapid metabolism of sparteine: frequency of alleles with duplicated CYP2D6 genes in a Danish population as determined by restriction fragment length polymorphism and long polymerase chain reaction. *Pharmacogenetics* 1998; 8:119-123.

Bernal ML, Sinues B, Johansson I, McLellan RA, Wennerholm A, Dahl ML, et al. Ten percent of North Spanish individuals carry duplicated or triplicated CYP2D6 genes associated with ultrarapid metabolism of debrisoquine. *Pharmacogenetics* 1999; 9:657-660.

Bieche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, et al. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues. *Pharmacogenet Genomics* 2007; 17:731-742.

Blum M, Demierre A, Grant DM, Heim M, Meyer UA. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. *Proc Natl Acad Sci U S A* 1991; 88:5237-5241.

Bogni A, Monshouwer M, Moscone A, Hidestrand M, Ingelman-Sundberg M, Hartung T, et al. Substrate specific metabolism by polymorphic cytochrome P450 2D6 alleles. *Toxicol In Vitro* 2005; 19:621-629.

Borges S, Desta Z, Li L, Skaar TC, Ward BA, Nguyen A, et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther* 2006; 80:61-74.

Bozina N, Granic P, Lalic Z, Tramisak I, Lovric M, Stavljenic-Rukavina A. Genetic polymorphisms of cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian population. *Croat Med J* 2003; 44:425-428.

Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002; 3:229-243.

Breyer-Pfaff U. The metabolic fate of amitriptyline, nortriptyline and amitriptylinoxide in man. *Drug Metab Rev* 2004; 36:723-746.

Buzkova H, Pechandova K, Slanar O, Perlik F. Frequency of single nucleotide polymorphisms of CYP2D6 in the Czech population. *Cell Biochem Funct* 2008; 26:76-81.

Cann HM, de Toma C, Cazes L, Legrand MF, Morel V, Piouffre L, et al. A human genome diversity cell line panel. *Science* 2002; 296:261-262.

Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. *Nature* 1987; 325:31-36.

Caraco Y, Blotnick S, Muszkat M. CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. *Clin Pharmacol Ther* 2008; 83:460-470.

Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956; 124:484-485.

Cavalli-Sforza LL, Feldman MW. The application of molecular genetic approaches to the study of human evolution. *Nat Genet* 2003; 33 Suppl:266-275.

Cavalli-Sforza LL, Menozzi P, Piazza A. *The history and geography of human genes*. Princeton, NJ: Princeton University Press; 1994.

Chehal MK, Granville DJ. Cytochrome p450 2C (CYP2C) in ischemic heart injury and vascular dysfunction. *Can J Physiol Pharmacol* 2006; 84:15-20.

Chen Y, Ferguson SS, Negishi M, Goldstein JA. Identification of constitutive androstane receptor and glucocorticoid receptor binding sites in the CYP2C19 promoter. *Mol Pharmacol* 2003; 64:316-324.

Chou WH, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, et al. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. *J Clin Psychopharmacol* 2000; 20:246-251.

Clement M, Posada D, Crandall KA. TCS: a computer program to estimate gene genealogies. *Mol Ecol* 2000; 9:1657-1659.

Dahl ML, Johansson I, Bertilsson L, Ingelman-Sundberg M, Sjoqvist F. Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. *J Pharmacol Exp Ther* 1995; 274:516-520.

DeBarber AE, Bleyle LA, Roullet JB, Koop DR. Omega-hydroxylation of farnesol by mammalian cytochromes p450. *Biochim Biophys Acta* 2004; 1682:18-27.

Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002; 41:913-958.

Draper AJ, Hammock BD. Identification of CYP2C9 as a human liver microsomal linoleic acid epoxygenase. *Arch Biochem Biophys* 2000; 376:199-205.

Druid H, Holmgren P, Carlsson B, Ahlner J. Cytochrome P450 2D6 (CYP2D6) genotyping on postmortem blood as a supplementary tool for interpretation of forensic toxicological results. *Forensic Sci Int* 1999; 99:25-34.

Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. *Annu Rev Med* 2006; 57:119-137.

Eichelbaum M, Spannbrucker N, Steincke B, Dengler HJ. Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur J Clin Pharmacol* 1979; 16:183-187.

Ensom MH, Chang TK, Patel P. Pharmacogenetics: the therapeutic drug monitoring of the future? *Clin Pharmacokinet* 2001; 40:783-802.

Evans DA, Manley KA, McKusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* 1960; 2:485-491.

Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999; 286:487-491.

Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. *Nature* 2004; 429:464-468.

Excoffier L, Hamilton G. Comment on "Genetic structure of human populations". *Science* 2003; 300:1877.

Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online* 2005; 1:47-50.

Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 1992; 131:479-491.

Ferguson SS, LeCluyse EL, Negishi M, Goldstein JA. Regulation of human CYP2C9 by the constitutive androstane receptor: discovery of a new distal binding site. *Mol Pharmacol* 2002; 62:737-746.

Ferrer-Admetlla A, Bosch E, Sikora M, Marques-Bonet T, Ramirez-Soriano A, Muntasell A, et al. Balancing selection is the main force shaping the evolution of innate immunity genes. *J Immunol* 2008; 181:1315-1322.

Fleming I. Vascular cytochrome p450 enzymes: physiology and pathophysiology. *Trends Cardiovasc Med* 2008; 18:20-25.

Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, et al. Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res* 2001; 88:44-51.

Frueh FW, Amur S, Mummaneni P, Epstein RS, Aubert RE, DeLuca TM, et al. Pharmacogenomic biomarker information in drug labels approved by the United States food and drug administration: prevalence of related drug use. *Pharmacotherapy* 2008; 28:992-998.

Furuta T, Ohashi K, Kosuge K, Zhao XJ, Takashima M, Kimura M, et al. CYP2C19 genotype status and effect of omeprazole on intragastric pH in humans. *Clin Pharmacol Ther* 1999; 65:552-561.

Furuta T, Shirai N, Kodaira M, Sugimoto M, Nogaki A, Kuriyama S, et al. Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of H. pylori. *Clin Pharmacol Ther* 2007a; 81:521-528.

Furuta T, Sugimoto M, Shirai N, Ishizaki T. CYP2C19 pharmacogenomics associated with therapy of Helicobacter pylori infection and gastro-esophageal reflux diseases with a proton pump inhibitor. *Pharmacogenomics* 2007b; 8:1199-1210.

Fuselli S, Dupanloup I, Frigato E, Cruciani F, Scozzari R, Moral P, et al. Molecular diversity at the CYP2D6 locus in the Mediterranean region. *Eur J Hum Genet* 2004; 12:916-924.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science* 2002; 296:2225-2229.

Gaedigk A, Bradford LD, Marcucci KA, Leeder JS. Unique CYP2D6 activity distribution and genotype-phenotype discordance in black Americans. *Clin Pharmacol Ther* 2002; 72:76-89.

Gaedigk A, Ndjountche L, Divakaran K, Dianne Bradford L, Zineh I, Oberlander TF, et al. Cytochrome P4502D6 (CYP2D6) gene locus heterogeneity: characterization of gene duplication events. *Clin Pharmacol Ther* 2007; 81:242-251.

Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. *Clin Pharmacol Ther* 2008; 83:234-242.

Gaikovitch EA, Cascorbi I, Mrozikiewicz PM, Brockmoller J, Frotschl R, Kopke K, et al. Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. *Eur J Clin Pharmacol* 2003; 59:303-312.

Garcia-Martin E, Martinez C, Ladero JM, Agundez JA. Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. *Mol Diagn Ther* 2006; 10:29-40.

Garrigan D, Hedrick PW. Perspective: detecting adaptive molecular polymorphism: lessons from the MHC. *Evolution* 2003; 57:1707-1722.

Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, et al. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. *N Engl J Med* 2004; 351:2827-2831.

Gerbal-Chaloin S, Pascussi JM, Pichard-Garcia L, Daujat M, Waechter F, Fabre JM, et al. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos* 2001; 29:242-251.

Giacomini KM, Brett CM, Altman RB, Benowitz NL, Dolan ME, Flockhart DA, et al. The pharmacogenetics research network: from SNP discovery to clinical drug response. *Clin Pharmacol Ther* 2007a; 81:328-345.

Giacomini KM, Krauss RM, Roden DM, Eichelbaum M, Hayden MR, Nakamura Y. When good drugs go bad. *Nature* 2007b; 446:975-977.

Goetz MP, Kamal A, Ames MM. Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. *Clin Pharmacol Ther* 2008; 83:160-166.

Goetz MP, Rae JM, Suman VJ, Safgren SL, Ames MM, Visscher DW, et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol* 2005; 23:9312-9318.

Gonzalez FJ, Nebert DW. Evolution of the P450 gene superfamily: animal-plant 'warfare', molecular drive and human genetic differences in drug oxidation. *Trends Genet* 1990; 6:182-186.

Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, et al. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 1988; 331:442-446.

Gotoh O. Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analyses of amino acid and coding nucleotide sequences. *J Biol Chem* 1992; 267:83-90.

Gough AC, Smith CA, Howell SM, Wolf CR, Bryant SP, Spurr NK. Localization of the CYP2D gene locus to human chromosome 22q13.1 by polymerase chain reaction, in situ hybridization, and linkage analysis. *Genomics* 1993; 15:430-432.

Gray IC, Nobile C, Muresu R, Ford S, Spurr NK. A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Genomics* 1995; 28:328-332.

Guengerich FP. Cytochromes P450, drugs, and diseases. *Mol Interv* 2003; 3:194-204.

Guengerich FP. Mechanisms of cytochrome P450 substrate oxidation: MiniReview. *J Biochem Mol Toxicol* 2007; 21:163-168.

Guengerich FP. Cytochrome p450 and chemical toxicology. *Chem Res Toxicol* 2008; 21:70-83.

Hedman M, Pimenoff V, Lukka M, Sistonen P, Sajantila A. Analysis of 16 Y STR loci in the Finnish population reveals a local reduction in the diversity of male lineages. *Forensic Sci Int* 2004; 142:37-43.

Hill WG, Robertson A. Linkage disequilibrium in finite populations. *Theor Appl Genet* 1968; 38:226-231.

Hiroi T, Imaoka S, Funae Y. Dopamine formation from tyramine by CYP2D6. *Biochem Biophys Res Commun* 1998; 249:838-843.

Hockwald RS, Arnold J, Clayman CB, Alving AS. Toxicity of primaquine in Negroes. *J Am Med Assoc* 1952; 149:1568-1570.

Holmgren P, Carlsson B, Zackrisson AL, Lindblom B, Dahl ML, Scordo MG, et al. Enantioselective analysis of citalopram and its metabolites in postmortem blood and genotyping for CYD2D6 and CYP2C19. *J Anal Toxicol* 2004; 28:94-104.

Horn J. The proton-pump inhibitors: similarities and differences. *Clin Ther* 2000; 22:266-280; discussion 265.

Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc* 1954; 70:266-273.

Hunfeld NG, Mathot RA, Touw DJ, van Schaik RH, Mulder PG, Franck PF, et al. Effect of CYP2C19*2 and *17 mutations on pharmacodynamics and kinetics of proton pump inhibitors in Caucasians. *Br J Clin Pharmacol* 2008; 65:752-760.

Ingelman-Sundberg M. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* 2004; 369:89-104.

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005; 5:6-13.

Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 2007; 116:496-526.

Jannetto PJ, Wong SH, Gock SB, Laleli-Sahin E, Schur BC, Jentzen JM. Pharmacogenomics as molecular autopsy for postmortem forensic toxicology: genotyping cytochrome P450 2D6 for oxycodone cases. *J Anal Toxicol* 2002; 26:438-447.

Jin M, Gock SB, Jannetto PJ, Jentzen JM, Wong SH. Pharmacogenomics as molecular autopsy for forensic toxicology: genotyping cytochrome P450 3A4*1B and 3A5*3 for 25 fentanyl cases. *J Anal Toxicol* 2005; 29:590-598.

Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjoqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci U S A* 1993; 90:11825-11829.

Jorde LB, Watkins WS, Bamshad MJ, Dixon ME, Ricker CE, Seielstad MT, et al. The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *Am J Hum Genet* 2000; 66:979-988.

Juonala M, Viikari JS, Kahonen M, Taittonen L, Ronnemaa T, Laitinen T, et al. Geographic origin as a determinant of carotid artery intima-media thickness and brachial artery flow-mediated dilation: the Cardiovascular Risk in Young Finns study. *Arterioscler Thromb Vasc Biol* 2005; 25:392-398.

Kaiser R, Sezer O, Papies A, Bauer S, Schelenz C, Tremblay PB, et al. Patient-tailored antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists according to cytochrome P-450 2D6 genotypes. *J Clin Oncol* 2002; 20:2805-2811.

Kalow W, Staron N. On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can J Biochem Physiol* 1957; 35:1305-1320.

Kaminsky LS, Zhang ZY. Human P450 metabolism of warfarin. *Pharmacol Ther* 1997; 73:67-74.

Kaneko A, Lum JK, Yaviong L, Takahashi N, Ishizaki T, Bertilsson L, et al. High and variable frequencies of CYP2C19 mutations: medical consequences of poor drug metabolism in Vanuatu and other Pacific islands. *Pharmacogenetics* 1999; 9:581-590.

Kimura M. Evolutionary rate at the molecular level. *Nature* 1968; 217:624-626.

Kimura S, Umeno M, Skoda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. *Am J Hum Genet* 1989; 45:889-904.

Kirchheiner J. CYP2D6 phenotype prediction from genotype: which system is the best? *Clin Pharmacol Ther* 2008; 83:225-227.

Kirchheiner J, Brockmöller J. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther* 2005; 77:1-16.

Kirchheiner J, Henckel HB, Franke L, Meineke I, Tzvetkov M, Uebelhack R, et al. Impact of the CYP2D6 ultra-rapid metabolizer genotype on doxepin pharmacokinetics and serotonin in platelets. *Pharmacogenet Genomics* 2005; 15:579-587.

Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol Psychiatry* 2004; 9:442-473.

Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. *Lancet* 2006; 368:704.

Koski A. *Interpretation of postmortem toxicology results: pharmacogenetics and drug-alcohol interaction*. Doctoral thesis, University of Helsinki, Helsinki; 2005.

Krishna DR, Klotz U. Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet* 1994; 26:144-160.

Kruglyak L. The road to genome-wide association studies. *Nat Rev Genet* 2008; 9:314-318.

Lappalainen T, Koivumaki S, Salmela E, Huoponen K, Sistonen P, Savontaus ML, et al. Regional differences among the Finns: a Y-chromosomal perspective. *Gene* 2006; 376:207-215.

Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *Jama* 1998; 279:1200-1205.

Lehmann H, Ryan E. The familial incidence of low pseudocholinesterase level. *Lancet* 1956; 271:124.

Lenfant C. Shattuck lecture--clinical research to clinical practice--lost in translation? *N Engl J Med* 2003; 349:868-874.

Lewis DF. 57 varieties: the human cytochromes P450. *Pharmacogenomics* 2004; 5:305-318.

Levo A, Koski A, Ojanperä I, Vuori E, Sajantila A. Post-mortem SNP analysis of CYP2D6 gene reveals correlation between genotype and opioid drug (tramadol) metabolite ratios in blood. *Forensic Sci Int* 2003; 135:9-15.

Lewontin RC. The interaction of selection and linkage. I. General considerations: heterotic models. *Genetics* 1964; 49:49-67.

Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. *Nature* 2004; 427:541-544.

Limdi NA, McGwin G, Goldstein JA, Beasley TM, Arnett DK, Adler BK, et al. Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin. *Clin Pharmacol Ther* 2008; 83:312-321.

Lin JH, Lu AY. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu Rev Pharmacol Toxicol* 2001; 41:535-567.

Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* 2008; 359:789-799.

Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006; 367:1747-1757.

Läpple F, von Richter O, Fromm MF, Richter T, Thon KP, Wisser H, et al. Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* 2003; 13:565-575.

Ma X, Idle JR, Krausz KW, Gonzalez FJ. Metabolism of melatonin by human cytochromes p450. *Drug Metab Dispos* 2005; 33:489-494.

Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of Debrisoquine in man. *Lancet* 1977; 2:584-586.

Maitland-van der Zee AH, Aitchison K, Kirchheiner J. Workshop outcomes report: initiatives to establish a European Network of pharmacogenetics/genomics and progress. *Eur J Pharm Sci* 2007; 31:151-155.

Mantel N. The detection of disease clustering and a generalized regression approach. *Cancer Res* 1967; 27:209-220.

McElroy S, Sachse C, Brockmoller J, Richmond J, Lira M, Friedman D, et al. CYP2D6 genotyping as an alternative to phenotyping for determination of metabolic status in a clinical trial setting. *AAPS PharmSci* 2000; 2:E33.

Meyer UA. Pharmacogenetics - five decades of therapeutic lessons from genetic diversity. *Nat Rev Genet* 2004; 5:669-676.

Miller GP, Hanna IH, Nishimura Y, Guengerich FP. Oxidation of phenethylamine derivatives by cytochrome P450 2D6: the issue of substrate protonation in binding and catalysis. *Biochemistry* 2001; 40:14215-14223.

Molokhia M, McKeigue P. EUDRAGENE: European collaboration to establish a case-control DNA collection for studying the genetic basis of adverse drug reactions. *Pharmacogenomics* 2006; 7:633-638.

Motulsky AG. Drug reactions enzymes, and biochemical genetics. *J Am Med Assoc* 1957; 165:835-837.

Nebert DW. Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? *Am J Hum Genet* 1997; 60:265-271.

Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 2006; 6:947-960.

Nebert DW, Dieter MZ. The evolution of drug metabolism. *Pharmacology* 2000; 61:124-135.

Nebert DW, Russell DW. Clinical importance of the cytochromes P450. *Lancet* 2002; 360:1155-1162.

Nelson DR. Cytochrome P450 and the individuality of species. *Arch Biochem Biophys* 1999; 369:1-10.

Nelson DR. Cytochrome P450 nomenclature, 2004. *Methods Mol Biol* 2006; 320:1-10.

Nelson MR, Bacanu SA, Mosteller M, Li L, Bowman CE, Roses AD, et al. Genome-wide approaches to identify pharmacogenetic contributions to adverse drug reactions. *Pharmacogenomics J* 2008; published online February 26, DOI:10.1038/tpj.2008.4.

Norio R. Finnish Disease Heritage I: characteristics, causes, background. *Hum Genet* 2003a; 112:441-456.

Norio R. Finnish Disease Heritage II: population prehistory and genetic roots of Finns. *Hum Genet* 2003b; 112:457-469.

Norio R. The Finnish Disease Heritage III: the individual diseases. *Hum Genet* 2003c; 112:470-526.

Oldenburg J, Bevans CG, Fregin A, Geisen C, Muller-Reible C, Watzka M. Current pharmacogenetic developments in oral anticoagulation therapy: the influence of variant VKORC1 and CYP2C9 alleles. *Thromb Haemost* 2007; 98:570-578.

Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE, Zeldin DC. The human intestinal cytochrome P450 "pie". *Drug Metab Dispos* 2006; 34:880-886.

Palo JU, Hedman M, Ulmanen I, Lukka M, Sajantila A. High degree of Y-chromosomal divergence within Finland - forensic aspects. *Forensic Sci Int Genetics* 2007; 1:120-124.

Palo JU, Pirttimaa M, Bengs A, Johnsson V, Ulmanen I, Lukka M, et al. The effect of number of loci on geographical structuring and forensic applicability of Y-STR data in Finland. *Int J Legal Med* 2008; 122:449-456.

Pelander A, Ojanpera I, Laks S, Rasanen I, Vuori E. Toxicological screening with formula-based metabolite identification by liquid chromatography/time-of-flight mass spectrometry. *Anal Chem* 2003; 75:5710-5718.

Pelissier-Alicot AL, Gaulier JM, Champsaur P, Marquet P. Mechanisms underlying postmortem redistribution of drugs: a review. *J Anal Toxicol* 2003; 27:533-544.

Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. *Hum Mol Genet* 1999; 8:1913-1923.

Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W. Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. *Jama* 2001; 286:2270-2279.

Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *Bmj* 2004; 329:15-19.

Raelson JV, Little RD, Ruether A, Fournier H, Paquin B, Van Eerdewegh P, et al. Genome-wide association study for Crohn's disease in the Quebec Founder Population identifies multiple validated disease loci. *Proc Natl Acad Sci U S A* 2007; 104:14747-14752.

Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW, Cavalli-Sforza LL. Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. *Proc Natl Acad Sci U S A* 2005; 102:15942-15947.

Rasanen I, Kontinen I, Nokua J, Ojanpera I, Vuori E. Precise gas chromatography with retention time locking in comprehensive toxicological screening for drugs in blood. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; 788:243-250.

Rettie AE, Jones JP. Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu Rev Pharmacol Toxicol* 2005; 45:477-494.

Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* 2005; 352:2285-2293.

Romualdi C, Balding D, Nasidze IS, Risch G, Robichaux M, Sherry ST, et al. Patterns of human diversity, within and among continents, inferred from biallelic DNA polymorphisms. *Genome Res* 2002; 12:602-612.

Rosenberg MS. *PASSAGE: Pattern analysis, spatial statistics and geographic exegesis*. 1.0. Tempe, AZ; 2001.

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, et al. Genetic structure of human populations. *Science* 2002; 298:2381-2385.

Roses AD. Pharmacogenetics in drug discovery and development: a translational perspective. *Nat Rev Drug Discov* 2008; 7:807-817.

Ross CJ, Carleton B, Warn DG, Stenton SB, Rassekh SR, Hayden MR. Genotypic approaches to therapy in children: a national active surveillance network (GATC) to study the pharmacogenomics of severe adverse drug reactions in children. *Ann N Y Acad Sci* 2007; 1110:177-192.

Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 2004; 427:537-541.

Rowland P, Blaney FE, Smyth MG, Jones JJ, Leydon VR, Oxbrow AK, et al. Crystal structure of human cytochrome P450 2D6. *J Biol Chem* 2006; 281:7614-7622.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 2003; 19:2496-2497.

Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E. Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. *Clin Pharmacol Ther* 2008; 83:322-327.

Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genet* 1997; 60:284-295.

Sajantila A, Lunetta P, Ojanperä I. Postmortem pharmacogenetics: toward molecular autopsies. In: Wong S, Linder M, Valdes R (eds). *Pharmacogenomics and proteomics: enabling the practice of personalized medicine*. Washington, DC: AACC Press; 2006:301-310.

Sallee FR, DeVane CL, Ferrell RE. Fluoxetine-related death in a child with cytochrome P-450 2D6 genetic deficiency. *J Child Adolesc Psychopharmacol* 2000; 10:27-34.

Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. Plainview, New York: Cold Spring Harbor Laboratory Press; 1989.

Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. *Genet Med* 2005; 7:97-104.

Schroth W, Antoniadou L, Fritz P, Schwab M, Muerdter T, Zanger UM, et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol* 2007; 25:5187-5193.

Schulz M, Schmoldt A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* 2003; 58:447-474.

Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007; 316:1341-1345.

Serre D, Paabo S. Evidence for gradients of human genetic diversity within and among continents. *Genome Res* 2004; 14:1679-1685.

Shen H, He MM, Liu H, Wrighton SA, Wang L, Guo B, et al. Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. *Drug Metab Dispos* 2007; 35:1292-1300.

Shimizu T, Ochiai H, Asell F, Shimizu H, Saitoh R, Hama Y, et al. Bioinformatics research on inter-racial difference in drug metabolism I. Analysis on frequencies of mutant alleles and poor metabolizers on CYP2D6 and CYP2C19. *Drug Metab Pharmacokinet* 2003; 18:48-70.

Shirai N, Furuta T, Moriyama Y, Okochi H, Kobayashi K, Takashima M, et al. Effects of CYP2C19 genotypic differences in the metabolism of omeprazole and rabeprazole on intragastric pH. *Aliment Pharmacol Ther* 2001; 15:1929-1937.

Siegle I, Fritz P, Eckhardt K, Zanger UM, Eichelbaum M. Cellular localization and regional distribution of CYP2D6 mRNA and protein expression in human brain. *Pharmacogenetics* 2001; 11:237-245.

Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006; 79:103-113.

Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C, et al. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 2004; 5:895-931.

Steimer W, Zopf K, von Amelunxen S, Pfeiffer H, Bachofer J, Popp J, et al. Allele-specific change of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP2C19 and CYP2D6 extensive and intermediate metabolizers. *Clin Chem* 2004; 50:1623-1633.

Steimer W, Zopf K, von Amelunxen S, Pfeiffer H, Bachofer J, Popp J, et al. Amitriptyline or not, that is the question: pharmacogenetic testing of CYP2D6 and CYP2C19 identifies patients with low or high risk for side effects in amitriptyline therapy. *Clin Chem* 2005; 51:376-385.

Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; 73:1162-1169.

Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; 68:978-989.

Swanson JR, Jones GR, Krasselt W, Denmark LN, Ratti F. Death of two subjects due to imipramine and desipramine metabolite accumulation during chronic therapy: a review of the literature and possible mechanisms. *J Forensic Sci* 1997; 42:335-339.

Syvänen AC. Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat Rev Genet* 2001; 2:930-942.

Templeton AR, Crandall KA, Sing CF. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 1992; 132:619-633.

Thomas JH. Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. *PLoS Genet* 2007; 3:e67.

Thummel KE, Kunze KL, Shen DD. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* 1997; 27:99-127.

Tishkoff SA, Verrelli BC. Role of evolutionary history on haplotype block structure in the human genome: implications for disease mapping. *Curr Opin Genet Dev* 2003; 13:569-575.

Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood* 2008; published online June 23, DOI: 10.1182/blood-2008-04-149070.

Wadelius M, Pirmohamed M. Pharmacogenetics of warfarin: current status and future challenges. *Pharmacogenomics J* 2007; 7:99-111.

Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; 32:651-662.

Weinshilboum RM, Wang L. Pharmacogenetics and pharmacogenomics: development, science, and translation. *Annu Rev Genomics Hum Genet* 2006; 7:223-245.

Wennerholm A, Dandara C, Sayi J, Svensson JO, Abdi YA, Ingelman-Sundberg M, et al. The African-specific CYP2D617 allele encodes an enzyme with changed substrate specificity. *Clin Pharmacol Ther* 2002; 71:77-88.

Wennerholm A, Johansson I, Hidestrand M, Bertilsson L, Gustafsson LL, Ingelman-Sundberg M. Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity. *Pharmacogenetics* 2001; 11:417-427.

Verrelli BC, McDonald JH, Argyropoulos G, Destro-Bisol G, Froment A, Drousiotou A, et al. Evidence for balancing selection from nucleotide sequence analyses of human G6PD. *Am J Hum Genet* 2002; 71:1112-1128.

Williams PA, Cosme J, Ward A, Angove HC, Matak Vinkovic D, Jhoti H. Crystal structure of human cytochrome P450 2C9 with bound warfarin. *Nature* 2003; 424:464-468.

Vogel F. Moderne probleme der humangenetik. *Ergebn Inn Med Kinderheilkd* 1959; 12:52-125.

Wong SH, Wagner MA, Jentzen JM, Schur C, Bjerke J, Gock SB, et al. Pharmacogenomics as an aspect of molecular autopsy for forensic pathology/toxicology: does genotyping CYP 2D6 serve as an adjunct for certifying methadone toxicity? *J Forensic Sci* 2003; 48:1406-1415.

Wu AH. Use of genetic and nongenetic factors in warfarin dosing algorithms. *Pharmacogenomics* 2007; 8:851-861.

Vuori E, Ojanpera I, Nokua J, Ojansivu R. Oikeuskemiallisesti todetut myrkytyskuolemat Suomessa vuosina 2002–2004. *Suom Lääkäril* 2006; 61:2339-2344.

Xie HG, Kim RB, Wood AJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. *Annu Rev Pharmacol Toxicol* 2001; 41:815-850.

Yu AM, Idle JR, Byrd LG, Krausz KW, Kupfer A, Gonzalez FJ. Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* 2003a; 13:173-181.

Yu AM, Idle JR, Herraiz T, Kupfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. *Pharmacogenetics* 2003b; 13:307-319.

Zackrisson AL, Holmgren P, Gladh AB, Ahlner J, Lindblom B. Fatal intoxication cases: cytochrome P450 2D6 and 2C19 genotype distributions. *Eur J Clin Pharmacol* 2004; 60:547-552.

Zand N, Tajik N, Moghaddam AS, Milanian I. Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. *Clin Exp Pharmacol Physiol* 2007; 34:102-105.

Zanger UM, Fischer J, Raimundo S, Stuven T, Evert BO, Schwab M, et al. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* 2001; 11:573-585.

Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch Pharmacol* 2004; 369:23-37.