

The influence of the *CYP2D6**4 polymorphism on drug response and disease susceptibility

Monique Johanna Bijl

The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

The work described in this thesis was conducted at the Department of Epidemiology in collaboration with the department of hospital pharmacy of the Erasmus Medical Center.

The contributions of the general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are greatly acknowledged.

The publication of this thesis was financially supported by the Department of Hospital Pharmacy, Erasmus Medical Center and the Erasmus University Rotterdam, the Netherlands. Additional support for publication of this thesis was kindly provided by: Dutch Association of pharmacists (KNMP), Astra Zeneca, Roche Diagnostics, GlaxoSmithKline, Lundbeck BV

Lay-out and printed by: Optima Grafische Communicatie, Rotterdam (www.ogc.nl)

Cover photograph and design: Monique Bijl, Optima Grafische Communicatie

ISBN: 978-90-8559-593-9

© Monique Bijl, Rotterdam

No part of this thesis may be produced, stored in a retrieval system or transmitted in any form or means, without permission of the author, or, when appropriate, of the publisher of the publications.

The influence of the *CYP2D6**4 polymorphism on drug response and disease susceptibility

De invloed van het *CYP2D6**4 polymorfisme op geneesmiddel respons en het ontstaan van ziekten

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 20 november 2009 om 13.30 uur

door

Monique Johanna Bijl

geboren te Vlaardingen



Promotiecommissie

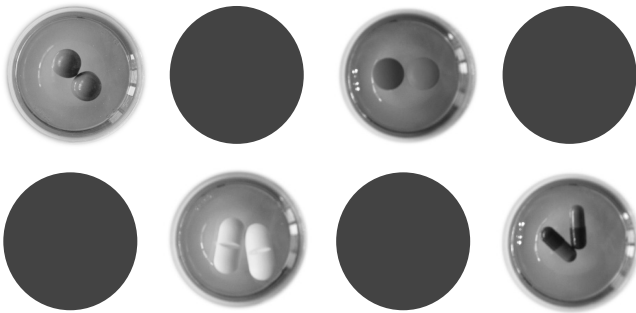
Promotores: Prof. dr. B.H.Ch. Stricker
Prof. dr. A.G. Vulto

Overige leden: Prof. dr. J. Lindemans
Prof. dr. A.G. Uitterlinden
Prof. dr. A.C.G. Egberts

Copromotores: Dr. L.E. Visser
Dr. T. van Gelder

Contents

Chapter	1	Introduction	7
Chapter	2	The CYP2D6*4 polymorphism and depression	21
	2.1	Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants	23
	2.2	Variation in the CYP2D6 gene is associated with a lower serum sodium concentration in patients on antidepressants	37
	2.3	Association between the CYP2D6*4 polymorphism and depression and anxiety in the elderly	45
Chapter	3	The CYP2D6*4 polymorphism and b-blockers	57
	3	Genetic variation in the CYP2D6 gene is associated with a lower heart rate and blood pressure in β -blocker users	59
Chapter	4	The CYP2D6 polymorphism and codeine	73
	4	The influence of CYP2D6, CYP3A4/3A5 and UGT2B7 genetic polymorphisms on prescription of other analgesics in codeine users	75
Chapter	5	The CYP2D6*4 polymorphism and cancer	89
	5.1	The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users	91
	5.2	The influence of CYP2D6 genotype on basal cell carcinoma (BCC) susceptibility and multiple BCCs	103
Chapter	6	Discussion	115
Chapter	7	Summary	131
Chapter	8	Appendices	
		Samenvatting	141
		Dankwoord	145
		List of Publications	149
		Phd Portfolio	151



Introduction

CHAPTER 1

Introduction

This thesis is about the role of CYP2D6, a drug-metabolizing enzyme, in today's pharmacotherapy. Cytochrome P450 2D6 (CYP2D6) is an important member of a large family of enzymes with the name cytochrome P450 which is abundantly present in most non-monocellular living organisms. Its history probably goes back for millions of years, when animals developed detoxifying enzymes as a defence mechanism against plant stress metabolites. Evolutionary pressure led to more and more diversity in these P450 genes. Nowadays, this genetic variation in CYP2D6 plays an important role in explaining variability in drug response. In this introduction, the variability in drug response will be discussed, followed by genetic variation, pharmacokinetics and pharmacodynamics, the cytochrome P450 enzyme family, the possible influence of CYP2D6 on drug response and adverse reactions to drugs, disease susceptibility, and aims and scope of this thesis.

Variability in drug response

Patients differ in their response to drugs. On average only 40% of all patients will benefit from a particular drug [1]. Some patients will experience adverse drug reactions, while others will not. Although variability in drug response can be explained by age, gender, renal and liver function, underlying disease or drug interactions, genetic factors also contribute to differences in drug response [2].

Pharmacogenetics is the study of how genetic differences influence variability in patients' responses to drugs [3]. The history of pharmacogenetics goes back as far as 510 b.c. when Pythagoras noted that ingestion of fava beans resulted in hemolytic anemia in some individuals in southern Italy. This potentially fatal reaction following the intake of fava beans is provoked by an enzyme deficiency, glucose-6-phosphate dehydrogenase (G6PD), an X chromosome linked trait occurring in approximately 30% of the population around the Mediterranean [4]. Nevertheless, the idea that genes explain variability in drug response was suggested as late as in the 1950s [5,6], when a prolonged muscle relaxing effect of succinylcholine during anesthesia was found in patients with a genetic lack of butyrylcholinesterase. One year later, it was described that genetic variation in N-acetyltransferase could lead to peripheral neuropathy in patients using the antituberculosis drug isoniazid [7]. The early studies concentrated mostly on genetic variation in drug metabolism (pharmacokinetics). Nowadays, genetic variation in drug targets (pharmacodynamics) and multifactorial influences on drug response are more and more investigated. Most differences are not predicted by mutations in a single gene, but by the altered function of several genes or by interaction between genes and environment. This has led to the movement of the field of pharmacogenetics to pharmacogenomics, a broader term for the research area studying influence of genetic variation on drug response in the treatment of complex diseases.

Currently, many drugs are still prescribed according to the model that 'one dose fits all', although we know that some patients may not benefit from the drug or experience adverse drug reactions. This thesis focuses on the influence of genetic variation in CYP2D6 on drug response and disease susceptibility.

Genetic variation

Between two unrelated persons, 99.9% of the human genome is identical. Genetic variability exists in only 0.1% of 3.3 billion base pairs (on average one variant per 100-300 base pairs). DNA consists of two long polymers of nucleotides forming a double-helix. The bases in this nucleotide structure of DNA are made up of adenine (A), cytosine (C), guanine (G) and thymine (T), attached to sugar/phosphate to form the complete nucleotide. A gene is a sequence of DNA that contains introns and exons, the latter with genetic information for the synthesis of a particular protein. Transcription of DNA into messenger RNA (mRNA) takes place in the cell nucleus. Subsequently, mRNA is translated into amino acids on the basis of base triplets in cytoplasmic ribosomes where ultimately the protein structure is defined. The process of transcription and translation is depicted in figure 1.

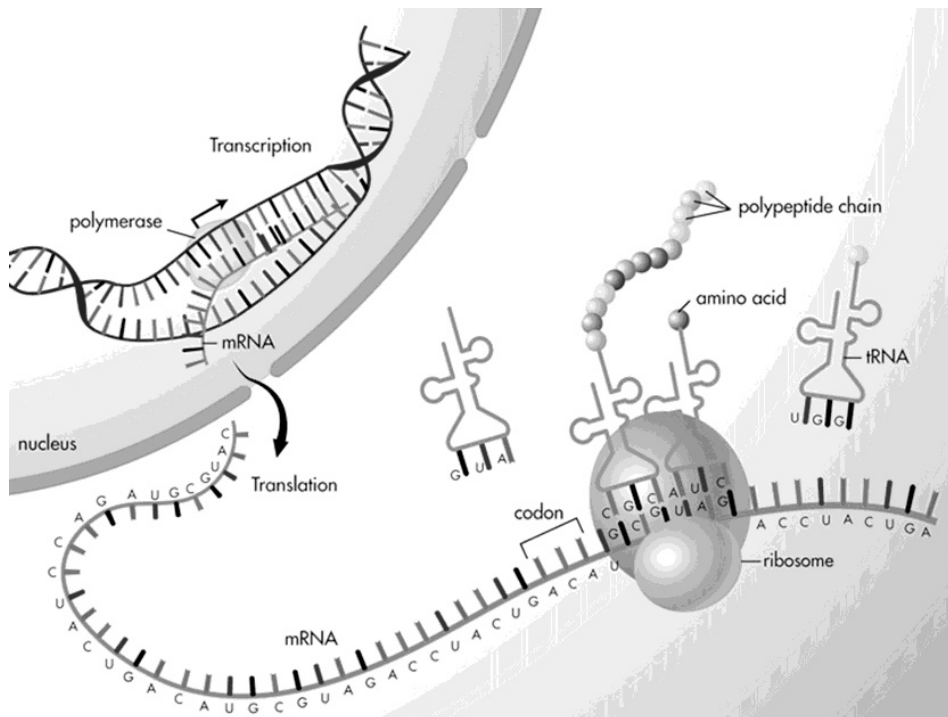


Figure 1. From genome to protein [adapted from www.genome.gov]

Proteins form the basis of almost all enzymes, cell and tissue structures. They also control chemical reactions and carry signals between cells. If there is a mutation in the nucleotide sequence of DNA, an abnormal protein might be produced, potentially leading to a change in biological function and processes.

The human genome is diploid (one paternal and one maternal allele of each chromosome). The most common type of variant is a single nucleotide polymorphism (SNP), a single-base difference in the DNA sequence that can be observed between individuals in the population. A genetic polymorphism has been defined as the minor allele occurring in more than 1% of the population, whereas mutations are those with a lower frequency [8]. Many mutations have been discovered in coding sequences of genes causing rare inherited diseases. When a SNP occurs at a coding region, it may have no influence on the amino acid sequence of the protein that is produced (synonymous SNP or silent mutation) or it may result in the synthesis of a new amino acid sequence leading to an altered protein function (non-synonymous SNP). SNPs that are not in protein-coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding RNA. A SNP can be present on 0, 1 or 2 alleles. If an individual has no polymorphism on both alleles, this individual is called homozygous wild type. One speaks of homozygous variant if both alleles are affected. An individual with one wild type and one variant allele is heterozygous.

Duplication or multiplication (copy number variations) of a DNA sequence also occur and can result in increased or decreased protein product or altered disease susceptibility. Other types of genetic variation are deletion, insertion, translocations and tandem repeats, but are not discussed in detail.

Pharmacokinetics and pharmacodynamics

When a drug is administered, it is absorbed from the gastrointestinal tract into the blood circulation and transported through the liver to the site of action (target, receptor). In the liver, drugs can be further metabolized to water soluble substances that are easily excreted [9]. Drug metabolism can be classified into two pathways. Phase 1 reactions may occur by oxidation, reduction or hydrolysis to convert drugs into more polar water soluble metabolites. Cytochrome P450 enzymes are typically involved in oxidation reactions. In phase 2 reactions, drugs are conjugated (acetylation, glucuronidation, sulfation or methylation) leading to inactivation and increased molecular weight of the drug. In case of so-called prodrugs, an inactive substance is metabolized into an active metabolite. Genetic variation may affect pharmacokinetics as well as pharmacodynamics. In pharmacokinetics, variation in genes encoding drug metabolizing enzymes or drug transporters may have consequences for the absorption, distribution, metabolism and elimination of a drug. In pharmacodynamics, the pharmacological effect of a drug at the site of action (target, receptor) may be modified by genetic variation.

CYP450

The cytochrome P450 enzymes are a family of drug-metabolizing enzymes that catalyze phase 1 drug metabolism (i.e. oxidation, reduction and hydrolysis). Drug-metabolizing enzymes are responsible for the detoxification and excretion of foreign chemicals (xenobiotics) such as drugs, plant metabolites and environmental pollutants. Following the evolution of the CYP450 superfamily, a lot of new genes developed during the past 400 million years, probably as a result of ingestion of plants by animals. As a defence mechanism, plants developed stress metabolites (phytoalexins) to make them less attractive for consumption. Evolutionary pressure favoured the survival of animals with a more elaborate system of detoxifying enzymes, such as the CYP450 family [10]. In this way both plants and animals came to more and more diversity in mutual defence mechanism.

Cytochrome P450 enzymes are classified by their amino acid similarities and are assigned a family number (e.g., CYP1, CYP2), a subfamily letter (e.g., CYP3A, CYP3C) and a number for the individual enzyme within the subfamily. Cytochrome P450 2D6 (CYP2D6) is an important member of the cytochrome P450 family, and is responsible for the metabolism of approximately 25% of all drugs metabolized by CYPs [11]. CYP2D6 is predominately expressed in the liver, but can also be found in the central nervous system, intestines and skin [12-14]. The amount of CYP2D6 enzyme in the liver is relatively small (about 2%) compared to other important CYP enzymes such as CYP3A (about 30%), CYP2C (about 20%) and CYP1A2 (about 13%) [15]. The expression of cytochrome P450 enzymes can be influenced by a large number of inducing and inhibiting factors including genetic variation and drug-drug interactions. CYP2D6 is not inducible, but several drugs are known to inhibit enzyme activity (fluoxetine, paroxetine, bupropion, quinidine, sertraline, duloxetine, cimetidine, terbinafine, amiodaron) [16].

The *CYP2D6* gene is highly polymorphic with more than 70 variant alleles [17]. Each genetic variant allele is defined by an asterisk followed by a number. Several of these variants encode an inactive protein or absence of an enzyme product (e.g., *CYP2D6*3*, **4*, **5*, **6*). Individuals carrying two of these non-functional alleles completely lack CYP2D6 enzyme activity and are classified as 'poor metabolizers' (PMs). This PM phenotype occurs in 5-10% of the Caucasian population. Individuals carrying two functional CYP2D6 alleles (**1*, **2*) have 'normal' enzyme activity and are classified as 'extensive metabolizers' (EMs) [18]. Subjects with one non-functional and one functional allele can be considered as 'intermediate metabolizers' (IMs), although this term also refers to a subject with one non-functional allele and one decreased activity allele or two decreased activity alleles (e.g. **10*, **41*). The clinical impact of the IM phenotype is unclear, probably as a result of diversity in genotypes and may depend on the drug used [19]. Ultrarapid metabolizers have ≥ 2 functional copies of the *CYP2D6* gene – so-called copy number variation - and exhibit extremely high enzyme activity [20]. Individuals carrying this genotype are found in 1-2% of the Caucasian population. The different phenotypes of CYP2D6 in the population are schematically presented in figure 2.

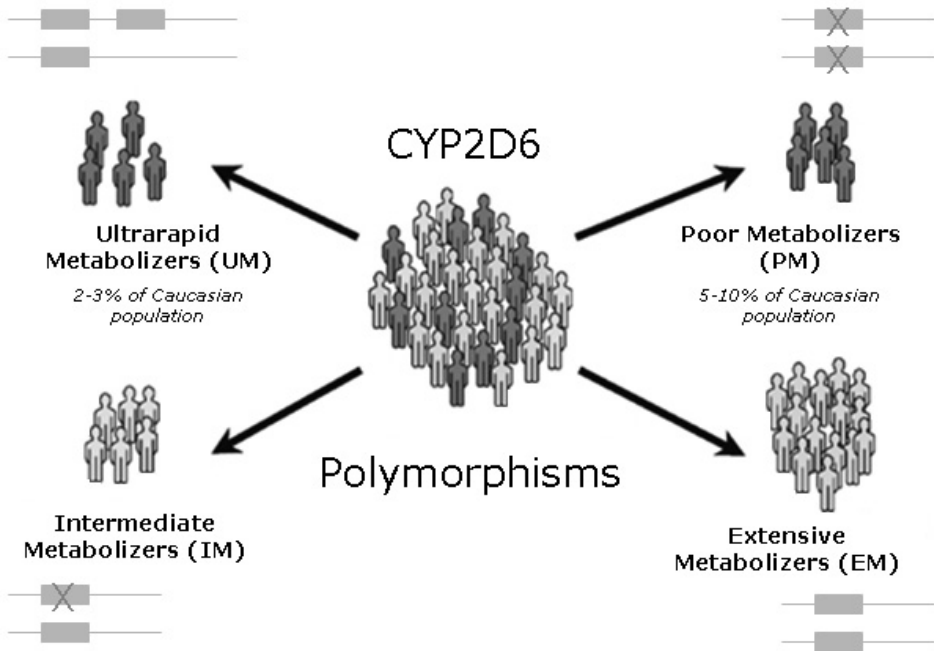


Figure 2. Different phenotypes of CYP2D6

*CYP2D6**4 (1846 G>A) is the most common variant allele (allele frequency of 20%) in Caucasians and is the most frequent nonfunctional allele in the PM phenotype; over 75% of the PMs are carriers of this polymorphism [21]. The frequencies of variant alleles highly depend on ethnicity. Although the frequency of *CYP2D6**4 is high in Caucasians, the *4 allele is rare in Chinese and Japanese people (<1%). On the other hand, *CYP2D6**10 allele has a frequency of <2% in European populations, but in Asians its frequency ranges from 38-71%. The *CYP2D6**17 variant is predominantly found in Africans or African Americans and their descendants [21]. Ethnic differences in allele frequencies may be the result of differences in diet over hundreds of thousand years [10].

The correlation between CYP2D6 genotype and phenotype is high. Genotyping for 12 SNPs in the *CYP2D6* gene could predict phenotype with an accuracy of about 90-95% [22, 23].

Drug response and adverse drug reactions

As already mentioned above, in current pharmacotherapy the 'one size fits all' approach prevails. Patients with a certain disease are more or less treated in the same way: with a standard drug in a fixed dose according to drug label or guidelines. Some patients do not respond to

the drug, while others experience adverse drug reactions. On average only 40% of all patients will benefit from a particular drug [1].

Due to absent enzyme activity in CYP2D6 PMs, plasma concentrations and total exposure to drugs metabolized by CYP2D6 are higher than in EMs (figure 3), and PMs are therefore more likely to suffer from dose-dependent adverse drug reactions (ADRs) requiring dose reductions or discontinuation of the drug.

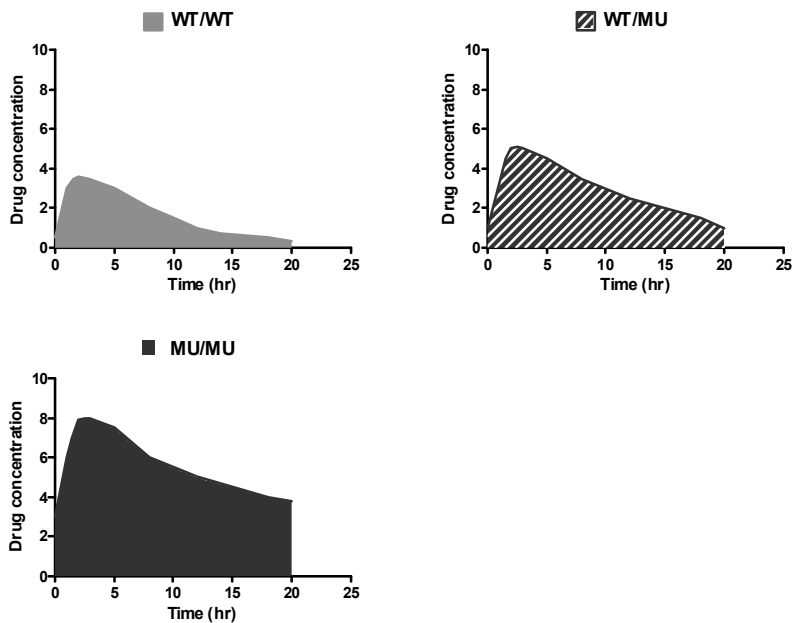


Figure 3. Potential impact of genetic variation in the CYP2D6 gene on plasma concentration and drug exposure after administration of a single dose, adapted from Evans et al. [2] WT= wild type, MU = mutant.

Indeed, in a study of Rau et al. an increased frequency of CYP2D6 poor metabolizers (29%) among patients experiencing adverse effects during treatment with antidepressants was found [24]. Table 1 lists commonly used drugs (partially) metabolized by CYP2D6 [16, 25].

In case of pro-drugs where CYP2D6 is involved in the activation of the drug, CYP2D6 PMs have low plasma concentrations of the active metabolite, leading to impaired treatment response. For example, several studies found that patients with decreased CYP2D6 enzyme activity had a diminished response to tramadol analgesia [26, 27]. CYP2D6 is also involved in the formation of endoxifen, the active metabolite of tamoxifen, one of the most widely used drugs for post-menopausal women with estrogen receptor-positive breast cancer.

Genotyping prior to the start of pharmacotherapy could identify patients who are at increased risk of developing adverse reactions, and are likely better off with a lower dose or another drug. On the other hand, pharmacogenetic testing before therapy with a pro-drug metabo-

lized by CYP2D6 (see table 1) could also identify individuals in whom the drug is less efficacious. These non-responders could still experience adverse reactions from the parent drug or other metabolites, and probably respond better to higher doses or another drug. In this manner, pharmacotherapy could be individualized.

Table 1. Selection of drugs metabolized by CYP2D6 (substrates)

Antidepressants
- Tricyclic antidepressants (TCAs): amitriptyline, clomipramine, doxepine, imipramine, maprotiline and nortriptyline
- SSRIs: fluoxetine, fluvoxamine, paroxetine
- Other: duloxetine, mianserine, mirtazapine, venlafaxine
Antipsychotics
- Aripiprazol, clozapine, haloperidol, perfenazine, risperidon, zuclopentixol
β-Blockers
- Alprenolol, carvedilol, metoprolol, nebivolol, propranolol, timolol
Analgesics
- Codeine, dextromethorphan, tramadol
Other
- Atomoxetine, flecainide, propafenone, metoclopramide, ondansetron, tamoxifen

Disease susceptibility

Since cytochrome P450 enzymes metabolize not only drugs but also endogenous substances such as hormones, environmental chemicals and toxins, one might expect that variability in enzyme activity could result in an altered susceptibility to certain diseases, such as cancer [28]. Although it is believed that CYP2D6 plays a minor role in the metabolism of precarcinogens, several studies found an association between variability in CYP2D6 activity and cancer risk (lung, liver, basal cell carcinoma, bladder, thyroid, prostate) [29-34]. However, up till now no major conclusions can be drawn from these studies, because most studies have a limited sample size or small numbers of cases and yielded contradictory results.

In the past, decreased CYP2D6 enzyme activity has been associated with an increased risk of Parkinson's disease [35]. Parkinson's disease is a common disabling disorder among elderly characterized by a progressive neuronal degeneration in the nigrostriatal system of the brain. CYP2D6 detoxifies MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin known to cause Parkinson's disease-like symptoms. Nowadays, it is suggested that the CYP2D6 PM phenotype interacts with certain environmental factors such as pesticide exposure or cigarette smoking, leading to an increased risk of Parkinson's disease [36].

CYP2D6 main biological function is to metabolize xenobiotics, but it may have some endogenous substrates [11]. 5-methoxytryptamine (5-MT), a precursor of serotonin, is considered to be an endogenous substrate *in vitro* [37]. The neurotransmitter serotonin is involved in mood, aggression, sleep, body temperature, sexuality and vomiting in the human body. Several studies investigated relationships between personality traits and CYP2D6, but an effect on personality has not consistently been found [38-42].

Furthermore, CYP2D6 may be involved in the formation of dopamine from tyramine and seems to be involved in the formation of epinephrine and norepinephrine from octapamine and synephrine [43]. However, the consequences of these *in vitro* findings are still unknown, but polymorphisms in CYP2D6 might affect behaviour and the central nervous system through endogenous compounds.

Aim and scopes of this thesis

The aim of this thesis was to study the influence of genetic variation in the *CYP2D6* gene on drug response and disease susceptibility from an epidemiological perspective. All studies in this thesis are embedded in the Rotterdam Study, a population-based cohort study among 7983 inhabitants of Ommoord, a district in Rotterdam, aged 55 years or over. Since the start of the study in 1990, follow-up examinations were conducted periodically. In addition, the total cohort is continuously monitored for major morbidity and mortality through linkage with the records of the patient's general practitioner. Blood samples were obtained from which DNA was isolated and information on medication use for all participants is available. This cohort enables us to investigate the association between *CYP2D6* genotype and drug response and disease susceptibility.

In **chapter 2** the influence of *CYP2D6* variant alleles on the response to antidepressants is described as well as the association between *CYP2D6* genotype and risk of depression. In patients with decreased *CYP2D6* enzyme activity, plasma concentrations of some antidepressants are higher, which could lead to adverse drug reactions requiring dose reduction or discontinuation of the drug. **Chapter 2.1** describes the influence of the *CYP2D6**4 polymorphism on dose, switching and discontinuation of antidepressants. **Chapter 2.2** focuses on the association between *CYP2D6* genotype and serum sodium concentration in antidepressant users. In **chapter 2.3** the association between *CYP2D6* genotype and risk of depression and anxiety is described.

Chapter 3 in this thesis reports the association between the *CYP2D6**4 polymorphism and heart rate and blood pressure in β -blocker users.

In **chapter 4** variation in the *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* gene is studied in relation to co-prescription of other analgesics and switching to opioids in codeine users. The analgesic effect of codeine is mostly dependent on its metabolism to morphine by *CYP2D6*. Apart from *CYP2D6*, codeine is metabolized by *CYP3A4* and *UGT2B7*.

In **chapter 5** we investigated *CYP2D6* genotype in relation to cancer. **Chapter 5.1** describes the impact of impaired *CYP2D6* metabolism on breast cancer survival in tamoxifen users. **Chapter 5.2** focuses on the influence of *CYP2D6* genotype on basal cell carcinoma (BCC) susceptibility and subsequent BCCs.

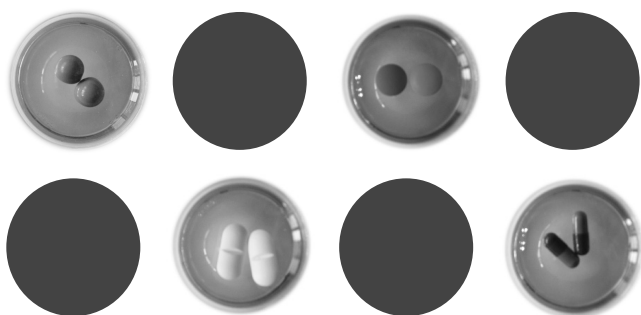
Finally, in **chapter 6** we discuss the main findings of this thesis and speculate on the implementation of pharmacogenetic testing in clinical practice.

References

1. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends in molecular medicine*. 2001;7(5):201-4.
2. Evans WE, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med*. 2003;348(6):538-49.
3. Roses AD. Pharmacogenetics and the practice of medicine. *Nature*. 2000;405(6788):857-65.
4. Nebert DW, Zhang G, Vesell ES. From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. *Drug Metab Rev*. 2008;40(2):187-224.
5. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. *Nature*. 2004;429(6990):464-8.
6. Johnson JA. Pharmacogenetics: potential for individualized drug therapy through genetics. *Trends Genet*. 2003;19(11):660-6.
7. Kalow W. Pharmacogenetics and pharmacogenomics: origin, status, and the hope for personalized medicine. *Pharmacogenomics J*. 2006;6(3):162-5.
8. Human Genome Project Information. http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml 2009 [accessed 05-2009]; Available from: http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml
9. Weinshilboum R. Inheritance and drug response. *N Engl J Med*. 2003;348(6):529-37.
10. 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(6):182-6.
11. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*. 2005;5(1):6-13.
12. Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE, Zeldin DC. The human intestinal cytochrome P450 "pie". *Drug Metab Dispos*. 2006;34(5):880-6.
13. 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(3):237-45.
14. Yengi LG, Xiang Q, Pan J, Scatina J, Kao J, Ball SE, et al. Quantitation of cytochrome P450 mRNA levels in human skin. *Anal Biochem*. 2003;316(1):103-10.
15. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *The Journal of pharmacology and experimental therapeutics*. 1994;270(1):414-23.
16. Flockhart D. Drug Interactions: Cytochrome P450 Drug Interaction Table. <http://medicineiupui.edu/flockhart/table.htm> 2007 [accessed 05-2009]
17. Human Cytochrome P450 (CYP) Allele Nomenclature Committee <http://www.cypalleles.ki.se/> 2009 [accessed 05-2009]
18. 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(2):284-95.
19. 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(2):234-42.
20. 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(24):11825-9.
21. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002;3(2):229-43.

22. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacology & therapeutics*. 2007;116(3):496-526.
23. 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. *Pharmacogenetics and genomics*. 2007;17(2):93-101.
24. Rau T, Wohlleben G, Wuttke H, Thuerauf N, Lunkenheimer J, Lanczik M, et al. CYP2D6 genotype: impact on adverse effects and nonresponse during treatment with antidepressants—a pilot study. *Clin Pharmacol Ther*. 2004;75(5):386-93.
25. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev*. 2002;34(1-2):83-448.
26. Poulsen L, Arendt-Nielsen L, Brosen K, Sindrup SH. The hypoalgesic effect of tramadol in relation to CYP2D6. *Clin Pharmacol Ther*. 1996;60(6):636-44.
27. Stamer UM, Lehnen K, Hothker F, Bayerer B, Wolf S, Hoeft A, et al. Impact of CYP2D6 genotype on postoperative tramadol analgesia. *Pain*. 2003;105(1-2):231-8.
28. Rodriguez-Antona C, Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. *Oncogene*. 2006;25(11):1679-91.
29. Abdel-Rahman SZ, Anwar WA, Abdel-Aal WE, Ghoneim MA, Au WW. The CYP2D6 extensive metabolizer genotype is associated with increased risk for bladder cancer. *Cancer Lett*. 1997;119(1):115-22.
30. Agundez JA, Ledesma MC, Benitez J, Ladero JM, Rodriguez-Lescure A, Diaz-Rubio E, et al. CYP2D6 genes and risk of liver cancer. *Lancet*. 1995;345(8953):830-1.
31. Lemos MC, Carrilho F, Rodrigues F, Coutinho E, Gomes L, Carvalheiro M, et al. Genetic polymorphism of CYP2D6 influences susceptibility to papillary thyroid cancer. *Clin Endocrinol (Oxf)*. 2007;67(2):180-3.
32. Ramachandran S, Lear JT, Ramsay H, Smith AG, Bowers B, Hutchinson PE, et al. Presentation with multiple cutaneous basal cell carcinomas: association of glutathione S-transferase and cytochrome P450 genotypes with clinical phenotype. *Cancer Epidemiol Biomarkers Prev*. 1999;8(1):61-7.
33. Wadelius M, Autrup JL, Stubbins MJ, Andersson SO, Johansson JE, Wadelius C, et al. Polymorphisms in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association with prostate cancer. *Pharmacogenetics*. 1999;9(3):333-40.
34. Watanabe M. Polymorphic CYP genes and disease predisposition—what have the studies shown so far? *Toxicol Lett*. 1998;102-103:167-71.
35. Smith CA, Gough AC, Leigh PN, Summers BA, Harding AE, Maraganore DM, et al. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet*. 1992;339(8806):1375-7.
36. Mellick GD. CYP450, genetics and Parkinson's disease: gene x environment interactions hold the key. *J Neural Transm Suppl*. 2006(70):159-65.
37. Yu AM, Idle JR, Herraiz T, Kupfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. *Pharmacogenetics*. 2003;13(6):307-19.
38. Bertilsson L, Alm C, De Las Carreras C, Widen J, Edman G, Schalling D. Debrisoquine hydroxylation polymorphism and personality. *Lancet*. 1989;1(8637):555.
39. Gan SH, Ismail R, Wan Adnan WA, Zulmi W, Kumaraswamy N, Larmie ET. Relationship between Type A and B personality and debrisoquine hydroxylation capacity. *Br J Clin Pharmacol*. 2004;57(6):785-9.
40. Kirchheiner J, Lang U, Stamm T, Sander T, Gallinat J. Association of CYP2D6 genotypes and personality traits in healthy individuals. *J Clin Psychopharmacol*. 2006;26(4):440-2.

41. Llerena A, Edman G, Cobaleda J, Benitez J, Schalling D, Bertilsson L. Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P4502D6. *Acta Psychiatr Scand.* 1993;87(1):23-8.
42. Roberts RL, Luty SE, Mulder RT, Joyce PR, Kennedy MA. Association between cytochrome P450 2D6 genotype and harm avoidance. *Am J Med Genet B Neuropsychiatr Genet.* 2004;127(1):90-3.
43. Hiroi T, Imaoka S, Funae Y. Dopamine formation from tyramine by CYP2D6. *Biochem Biophys Res Commun.* 1998;249(3):838-43.



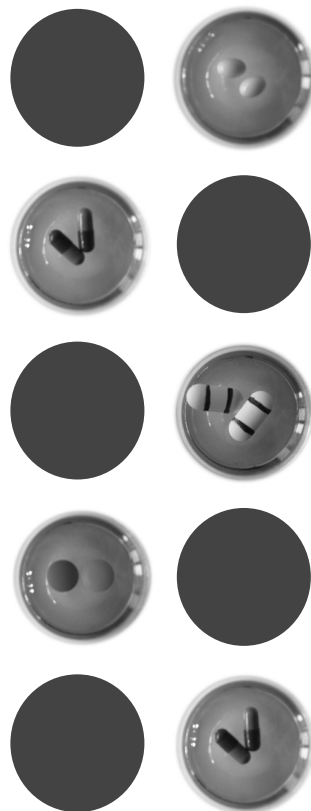
The *CYP2D6**4 polymorphism
and depression

CHAPTER 2

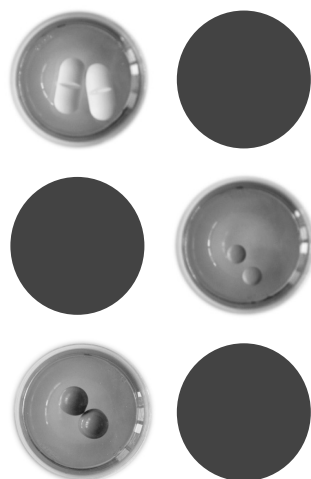
Influence of the *CYP2D6**4 polymorphism on dose, switching and discontinuation of antidepressants

Monique J. Bijl, Loes E. Visser, Albert Hofman, Arnold G. Vulto, Teun van Gelder, Bruno H. Ch. Stricker, Ron H.N. van Schaik

Br J Clin Pharmacol 2008; 65: 558-64



CHAPTER 2.1



Abstract

Aim: To study the effect of *CYP2D6**4 on antidepressant dose, switching and discontinuation of therapy.

Methods: The study consisted of all subjects in the Rotterdam Study, who received a first antidepressant prescription between April 1st 1991 and July 1st 2005 and for whom data on *CYP2D6* genotype were available. Binary logistic regression was performed to study the association between *CYP2D6**4 and switching to any other antidepressant or discontinuation of therapy within 45 days. The difference in mean antidepressant dose was compared between *CYP2D6* genotypes using t-tests and repeated measurements analyses.

Results: In users of tricyclic antidepressants (TCAs) the risk of switching to another antidepressant was significantly higher in poor metabolizers (PMs:*4/*4) compared to extensive metabolizers (EMs:*1/*1), with an adjusted OR of 5.77 (95% CI 1.59-21.03; p=0.01). In SSRI users there was no significant difference (OR 0.91; 95% CI 0.20-4.15; p=0.90). Heterozygous patients did not have an increased risk of switching in both TCA and SSRI users.

The mean TCA dose was significantly lower in PMs than in EMs at the third and fourth prescription (difference 0.11 DDD, p= 0.03). In SSRI users the difference in mean dose between PMs and EMs was significant at the third prescription (0.17 DDD; p=0.02).

Conclusion:The risk of switching to another antidepressant in TCA users is higher in PMs than in EMs. The maintenance doses of antidepressants were significantly lower in PMs. However, the question whether genotyping prior to the start of antidepressant therapy contributes substantially to the optimization of pharmacotherapy, requires further study.

Introduction

Depression constitutes a major health problem in the elderly. For the treatment of major depression tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and other antidepressants are widely prescribed. However, 50% of the patients will not respond adequately to first treatment [1]. This low response rate can be explained by a large interindividual variability in genetic, environmental and pathophysiological factors. In pharmacogenetics, the influence of genes on the pharmacokinetics and pharmacodynamics of drugs is investigated [2].

Most antidepressants are metabolized by cytochrome P450 2D6 (CYP2D6). CYP2D6 accounts for a small percentage of all hepatic CYPs, but is responsible for the metabolism of approximately 25% of all drugs metabolized by CYPs [3]. The *CYP2D6* gene is highly polymorphic with more than 60 variant alleles [<http://www.cypalleles.ki.se>]. Several of these variants encode an inactive protein or no enzyme product (e.g. *3, *4, *5, *6). Subjects with two nonfunctional alleles are classified as 'poor metabolizer' (PM), while carriers of one or two functional alleles (*1, *2) are classified as 'extensive metabolizer' (EM). Approximately 5-10% of the Caucasian population are PM [4-6]. Subjects with one nonfunctional and one functional allele can also be considered as 'intermediate metabolizer' (IM), although this term also refers to a subject with one nonfunctional allele and one decreased activity allele or two decreased activity alleles (e.g. *10, *41). The clinical impact of the IM phenotype is unclear, probably as a result of diversity in genotypes and may depend on the drug used. 'Ultra-rapid metabolizers' (UMs) have >2 functional copies of the *CYP2D6* gene and exhibit extremely high enzyme activity. Many genotyping assays determine the duplication of any *CYP2D6* gene, including nonfunctional genes, leading to false positive UM assignment. In this way, genotyping will only detect 10-30% of CYP2D6 UMs [7].

*CYP2D6**4 is the most common variant allele (allele frequency of 20%) in Caucasians and is the most frequent nonfunctional allele in the PM phenotype; over 75% of the PMs are carriers of this polymorphism [4].

Due to absent CYP2D6-mediated metabolism, poor metabolizers have higher plasma concentrations of antidepressants metabolized by CYP2D6 than extensive metabolizers [8] and are therefore more likely to suffer from dose-dependent adverse drug reactions (ADRs). Especially in patients taking TCAs, PMs may experience cardiotoxicity and other severe ADRs, because TCAs have a narrow therapeutic range. Severe ADRs require dose reductions or discontinuation of antidepressant therapy. In patients receiving a fixed dose of amitriptyline 75 mg twice a day, patients with one nonfunctional *CYP2D6* allele had a higher risk of adverse drug reactions than patients with two functional alleles (76.5% versus 12.1%) [9]. Other studies showed an increased frequency of ADRs in CYP2D6 PMs using antidepressants primarily metabolized by CYP2D6, but these are of limited value due to the small number of patients [10, 11]. In contrast, CYP2D6 genotype had no effect on paroxetine and mirtazapine discontinuations and adverse events in an 8-week, double blind randomised study on antidepressant intolerance [12].

Consequently, the actual influence of CYP2D6 polymorphisms on adverse events and clinical outcomes remains unclear.

Therefore, we performed a population-based cohort study to examine the influence of the *CYP2D6*4* polymorphism on intolerance of antidepressants by studying dose, frequency of switching to another antidepressant or discontinuation of therapy.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly. From 1990–1993, all inhabitants of Ommoord, a district of the city of Rotterdam in the Netherlands, aged 55 years or over, were invited to participate. The rationale and design of this study have been described elsewhere [13]. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. The cohort encompasses 7983 individuals who were all interviewed and investigated at baseline. Since the start of the study, follow-up examinations were conducted periodically. In addition, the total cohort is continuously monitored for major morbidity and mortality through linkage with the records of the patient's general practitioner. More than 99% of the participants have their drug prescriptions filled at seven regional pharmacies, which are all fully computerised. Complete data on drug use are available as of January 1, 1991. The pharmacy data include the Anatomical Therapeutic Chemical (ATC)-code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Cohort definition

The study cohort consisted of all subjects in the Rotterdam Study, who received a first prescription of an antidepressant between April 1st 1991 and July 1st 2005, and for whom there were data on *CYP2D6* genotype available ($n=1198$). The start date of April 1, 1991 was chosen to exclude patients who were treated with antidepressants in the preceding 3 months. Subjects were followed until one of the outcomes, death or the end of study period on July 1st 2005, whichever came first.

Outcome definition

In this study three types of outcomes were used: switching, discontinuation and dose. Switching was defined as a switch to any other antidepressant, irrespective of class, within 45 days after the start of the first prescription.

A switch within 45 days is assumed to occur due to intolerance of the drug, since the efficacy of an antidepressant can only be assessed after at least 6 weeks of therapy. Secondly, we looked at discontinuation of antidepressant therapy within 45 days. Discontinuation of therapy was defined as no further prescriptions for that particular drug after the initial 45 days. Thirdly, the influence of the *CYP2D6* genotype on the mean antidepressant dose was analysed. To compare doses of different antidepressants between genotypes the prescribed daily dose (PDD) was divided by the defined daily dose (DDD), according to the World Health Organization. For each prescription the mean PDD/DDD ratio was calculated for the tricyclic antidepressants (TCAs) amitriptyline, nortriptyline, imipramine, clomipramine, opipramol, doxepin, dosulepin, maprotiline and for the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, paroxetine, fluvoxamine, citalopram, escitalopram and sertraline.

Covariates

The following baseline covariates were considered as potential confounders: age, gender, body mass index (BMI) and renal function. BMI was calculated by dividing weight (kg) by height (m²). Renal function was estimated using the formula of Cockcroft and Gault. In addition, the starting dose of an antidepressant was considered a determinant affecting the risk of switching and discontinuation.

Genotyping

At the baseline examination of the Rotterdam Study, blood was taken from which DNA was isolated. The *CYP2D6**4 (1846G>A) genotyping was done using Taqman allelic discrimination assays on the ABI Prism 9700 HT Sequence detection system. Primers and probes were designed by Applied Biosystems by their Assay-by-Design service. Polymerase chain reactions (PCR) were performed in a reaction volume of 2.0 µl, containing assay-specific primers, allele-specific Taqman MGB probes, Abgene Absolute QPCR Rox Mix and genomic DNA (1 ng). The thermal profile consists of an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute. Genotypes were scored by measuring allele-specific fluorescence using the SDS 2.2.2 software for allelic discrimination (Applied Biosystems).

Subjects were defined as PM if they were homozygous for the *4 allele. In case of heterozygosity the subjects were defined as IM. When the *4 allele is absent, subject were classified as EM. Our method is unable to discriminate between *4/*4 (PMs) and *4/*5 (PMs) individuals, since the gene deletion *5 will result in no PCR product. Likewise, *1/*5 individuals will be genotyped as *1/*1. However, this does not affect the distribution of poor metabolizers and non-poor metabolizers.

Statistical analysis

Genotype frequency was tested for deviations from Hardy-Weinberg equilibrium by using a χ^2 -test. Binary logistic regression analysis was used to analyze the association between *CYP2D6* genotype and switching and the association between genotype and discontinuation of therapy. Confounders were defined as covariates associated with the outcome at a p-value of 0.1 in the univariate analysis and if they changed the point estimate by 10% or more in the multivariate model in addition to age and gender. Analyses were carried out in all antidepressant users, and separately for the different antidepressants classes (TCAs and SSRIs).

To compare the mean PDD/DDD ratio between EM, IM and PM independent sample t-tests were used at consecutive prescriptions. In addition, repeated measurements analysis was performed on the mean PDD/DDD ratio between EM, IM and PM in series of consecutive prescriptions to adjust for dependency of observations within each patient and to adjust for potential confounders. Logistic regression analysis and t-tests were performed with SPSS for Windows, version 11.0. Repeated measurement analysis was performed with SAS, version 8.2, using the Proc Mixed program.

Results

The baseline characteristics of the study population are shown in table 1. The mean age was approximately 69 years and 68% were women. Eight hundred and seven patients used TCAs, 833 used SSRIs and 213 patients used other antidepressants at any time during the study period. Of the TCAs amitriptyline was the most frequently used drug (68.3%), paroxetine was the most frequently used drug in the SSRI-group (46.8%) and mirtazapine was the most frequently used drug in the group of other antidepressants (34.3%). In table 2 the frequencies of antidepressants used and extent of *CYP2D6* metabolism are given.

Table 1. Baseline characteristics of the study population

Variable	Number of patients (%) n=1198
Age, average (SD)	69.4 (8.2) years
Gender	
Male	378 (31.6)
Female	820 (68.4)
BMI (SD) n=1155	26.5 (3.9) kg/m ²
Serum creatinine (SD) n=921	82.2 (20.1) μ mol/l
<i>CYP2D6</i> genotype*	
*1/*1	777 (64.9)
*1/*4	341 (28.5)
*4/*4	80 (6.7)

* Hardy Weinberg equilibrium; $\chi^2 = 23.285$ (p=0.00001)

Genotype distributions of *CYP2D6**4 are given in table 1. The allele frequency of the *CYP2D6**4 polymorphism in our population was 20.8%. We identified 777 patients (64.9%) with the wild type genotype (EM), 341 patients (28.5%) were heterozygous (IM), and 80 patients (6.7%) were homozygous for the *4 allele (PM). Genotype frequencies significantly deviated from Hardy-Weinberg equilibrium.

Table 2. Frequencies and types of antidepressants used during the study period (1991-2005) and the extent of *CYP2D6* metabolism for each antidepressant.

Antidepressant	Number of patients (%) n=1198	Extent of <i>CYP2D6</i> metabolism*
TCA use†		
Amitriptyline	551 (45.9)	++
Maprotiline	99 (8.2)	+++
Clomipramine	79 (6.6)	++
Nortriptyline	35 (2.9)	+++
Imipramine	29 (2.4)	++
Dosulepin	8 (0.7)	-
Doxepin	4 (0.3)	++
Opipramol	2 (0.2)	-
SSRI use†		
Paroxetine	390 (32.5)	+++
Fluvoxamine	161 (13.4)	++
Fluoxetine	139 (11.6)	+++
Sertraline	87 (7.3)	-
Citalopram	55 (4.6)	-/+
Escitalopram	1 (0.1)	-
Other†		
Mirtazapine	73 (6.1)	++
Mianserine	70 (5.8)	++
Venlafaxine	41 (3.4)	++
Trazodon	29 (2.4)	-
Moclobemide	9 (0.8)	-
Nefazodon	1 (0.1)	-

† Some patients used more than 1 antidepressant during the study period.

* Extent of *CYP2D6* metabolism [20, 22]. + minor metabolism route; ++ partly metabolized by *CYP2D6*; +++ major metabolism route; - no *CYP2D6* metabolism

Only ~4% of the antidepressant users switched to another antidepressant within 45 days. Table 3 shows the association between *CYP2D6* genotype and switching. Overall PMs had a higher risk of switching to another antidepressant than EMs, although this difference was not statistically significant. In TCA users the risk of switching was significantly higher in PMs compared to EMs (OR= 5.77; 95% CI 1.59-21.03; p=0.01). This effect was not seen in SSRI users. An increased risk of discontinuation of therapy was seen in PMs compared to EMs (OR=1.45; 95% CI 0.91-2.32; p=0.12), but this difference was not statistically significant (table 4). This association was somewhat more explicit in TCA users (OR=1.62; 95% CI 0.84-3.12; p=0.15) than in SSRI users (OR = 1.20; 95% CI 0.56-2.57; p=0.65).

Table 3. Association between CYP2D6 genotype and switching to any other antidepressant < 45 days

All antidepressants			
CYP2D6 genotype	Number of switchers	OR _{adj} * switching (CI 95%)	p-value
*1/*1	33	ref	
*1/*4	13	0.90 (0.47-1.73)	0.74
*4/*4	6	1.80 (0.72-4.48)	0.20
TCA			
*1/*1	11	ref	
*1/*4	4	0.84 (0.26-2.70)	0.77
*4/*4	4	5.77 (1.59-21.03)	0.01
SSRIs			
*1/*1	19	ref	
*1/*4	9	1.09 (0.48-2.49)	0.84
*4/*4	2	0.91 (0.20-4.15)	0.90

* Adjusted for age and gender.

Table 4. Association between CYP2D6 genotype and discontinuation of antidepressive therapy < 45 days

All antidepressants			
CYP2D6 genotype	Number of discontinuations	OR _{adj} * discontinuation (CI 95%)	p-value
*1/*1	285	ref	
*1/*4	135	1.13 (0.87-1.47)	0.36
*4/*4	36	1.45 (0.91-2.32)	0.12
TCA			
*1/*1	144	ref	
*1/*4	74	1.33 (0.92-1.90)	0.13
*4/*4	19	1.62 (0.84-3.12)	0.15
SSRIs			
*1/*1	118	ref	
*1/*4	51	0.96 (0.63-1.45)	0.83
*4/*4	13	1.20 (0.56-2.57)	0.65

* Adjusted for age and gender.

The change in mean dose (PDD/DDD ratio) over time for TCAs is given in figure 1. The average TCA starting dose was 0.36 DDD. At the first prescription the mean doses did not significantly differ between CYP2D6 genotypes. For all genotypes the prescribed daily dose increased over the first four prescriptions although this increase was smaller in PMs and IMs. At the third and

fourth prescription the mean dose was significantly lower in PMs than in EMs (difference 0.11 DDD, $p=0.03$). Additionally, we analysed the difference in TCA doses between genotypes with a repeated measurements analysis. In PMs the adjusted difference in PDD/DDD ratio was 0.05 DDD compared to EMs (95% CI -0.07-0.16, $p=0.46$). The mean dose of the intermediate metabolizers was lower compared to EMs (difference 0.02 DDD, 95% CI -0.03- 0.08, $p=0.43$). In the repeated measurements analyses we adjusted for age, gender and starting dose. Adjusting for renal function and BMI did not significantly affect the results.

For SSRIs the effect of CYP2D6 genotype on mean dose (PDD/DDD ratio) over time is given in figure 2. The average starting dose was 0.84 DDD. The starting doses of EM, IM or PM patients did not significantly differ. In SSRI users the difference in mean dose between PMs and EMs was significant at the third prescription (difference 0.17 DDD; $p=0.02$), but not significant for the following prescriptions. The curve of the intermediate metabolizers overlapped the EM mean dose to a large extent. With repeated measurements analysis the adjusted difference was 0.08 DDD (95% CI -0.02-0.20; $p=0.11$) between PMs and EMs and 0.13 DDD (95% CI -0.01-0.24; $p=0.04$) between PMs and IMs.

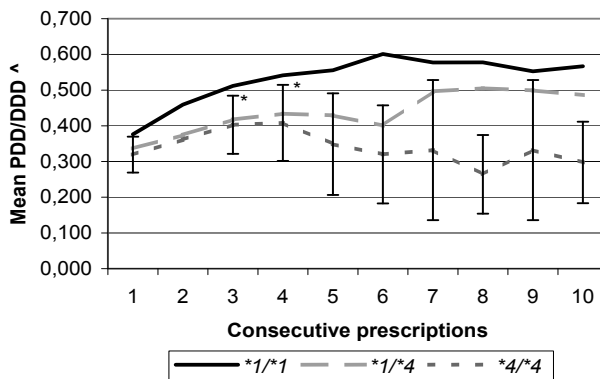


Figure 1. Change in mean tricyclic antidepressant dose over time per genotype.

^ For each prescription the mean prescribed daily dose (PDD) / defined daily dose (DDD) ratio was calculated.

⊥ 95% confidence interval of the mean PDD/DDD ratio of CYP2D6 PMs.

* p -value < 0.05

Discussion

This study demonstrated that in users of TCA the risk of switching to any other antidepressant within 45 days is significantly higher in PMs than in EMs. These findings are in concordance with Mulder et al, who found a HR of 3.50 (1.52-8.10) for switching to another drug in the same therapeutic class in PMs versus EMs [15]. In their study switching could be seen as an overall expression of unsatisfactory response to treatment including ineffectiveness and

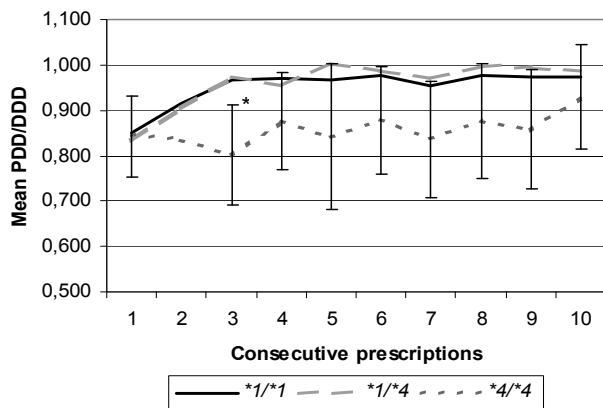


Figure 2. Change in mean SSRI dose over time per genotype.

^ For each prescription the mean prescribed daily dose (PDD) / defined daily dose (DDD) ratio was calculated.

⊥ 95% confidence interval of the mean PDD/DDD ratio of CYP2D6 PMs.

* p-value < 0.05

adverse drug reactions. We studied switching within 45 days of antidepressant use, since after a period of 6 weeks ineffectiveness could be the reason for switching instead of adverse drug reactions. The increased risk of switching to another antidepressant was not seen in SSRI users. Selection of SSRIs primarily metabolized by CYP2D6 (fluoxetine, paroxetine, fluvoxamine) did not alter these results. In contrast to TCAs, for which a narrow therapeutic range exists, no clear relationship between clinical effect and plasma concentration has been found for SSRIs. ADRs seemed not to be associated with high plasma concentrations of SSRIs [16]. Therefore, higher plasma concentrations of SSRIs in poor metabolizers would not lead to an increased frequency of switching. In intermediate metabolizers the risk of switching to any other antidepressant was not increased. Unlike PMs, in whom CYP2D6 enzyme activity is absent, patients heterozygous for the *4 allele have decreased enzyme activity. Plasma concentrations of antidepressants in these IMs would be slightly higher compared to EMs, but apparently did not lead to more switching. The clinical relevance of this genotype was less important than PMs. The low frequency of switching in our study (~4%) may be the result of a carefully chosen low starting dose by general practitioners and psychiatrists diminishing the occurrence of ADRs or adjusting the initial dose. In our study a large proportion of amitriptyline users started on a low dose, probably also because this drug is not only used as antidepressant, but is also prescribed for the treatment of neuropathic pain [17]. As information on the exact indication was not available we have included all amitriptyline users in the study. We have repeated the analysis after exclusion of low dose amitriptyline users (< 25 mg amitriptyline), but this did not alter our results. Due to the low frequency of switching in our cohort genotyping patients prior to the start of antidepressive therapy could only prevent a few patients from switching.

Discontinuation of antidepressant therapy was also studied, but was not associated with CYP2D6 genotype. Over thirty percent of our patients stopped their antidepressive medication within 45 days. Reasons for discontinuation of initial therapy are ADRs, non-compliance, lack of improvement and patient's belief about depression and antidepressants, with non-compliance being the most frequent reason [18].

After titrating the dose of an antidepressant to an optimal level of effectiveness with minimum ADRs, the mean antidepressant dose was significantly lower in PMs than in EMs. The absolute dose difference between PMs and EMs was smaller than 0.10 DDD, corresponding with 2-15 mg depending on the drug. This small difference in mean dose has limited impact on clinical outcome.

In TCA users with genotype *1/*4 (IM) the optimum dose lay between EMs and PMs, while the mean SSRI dose of heterozygous patients overlapped with EMs. This difference could be explained by the amount of drug metabolized by CYP2D6. CYP2D6 is not the only cytochrome P450 enzyme involved in the metabolism of antidepressants. CYP2C19 plays a role in the demethylation of amitriptyline, imipramine, clomipramine, sertraline and citalopram, CYP1A2 and CYP3A4 contribute to a lesser extent [19, 20]. But, CYP2D6 exerts a higher influence on antidepressants metabolism than CYP2C19 [21]. Most tricyclic antidepressants are (partly) metabolized by CYP2D6, whereas some SSRIs are not metabolized by CYP2D6 (citalopram, escitalopram and sertraline). Our results indicate that the metabolism of TCAs depend more on CYP2D6 than SSRIs. Due to the low number of prescriptions of the newer SSRIs (non CYP2D6 substrates) we did not separate the SSRI group in substrates and non-substrates in our analysis.

Some potential limitations of our cohort study should be considered. Selection bias was unlikely because all antidepressant users were identified in a population based cohort study and prescribing doctors were not aware of CYP2D6 status of their patients. Missing blood samples and difficulties with genotyping (due to the suboptimal quality of long-term storage of DNA or a homozygous *5 subject) were probably not related to CYP2D6 genotype. The frequency of the CYP2D6*4 allele in our study (20.8%) was in accordance with the literature [4], but interestingly no Hardy-Weinberg equilibrium (HWE) was observed. The assay was validated by DNA sequencing, and thus seems not to be responsible for this discrepancy. However, our genotyping assay discriminated between the presence and absence of the CYP2D6*4 allele, but was unable to distinguish *4/*5 from *4/*4 individuals, who will be classified as CYP2D6*4/*4. This led to an overestimation of *4/*4 individuals in HWE. However, this does not affect phenotype classification since both *4/*4 and *4/*5 are PMs.

In this study we only determined CYP2D6*4, because this polymorphism is the most cost-effective in this large number of subjects while cost-effectiveness is a consideration of increasing importance in healthcare. High throughput assays are very expensive. Moreover determination of CYP2D6*4 in our population should predict >75% of PMs [4]. Taking into account other, less frequent genetic variants (*3, *5, *6, *7, *8, *10, *41), it can be calculated that

we missed approximately 209 IMs (17%), 10 PMs (0.8%) and 24 UMs (2%), which are now all included in the EM group. Nevertheless, we believe that these misclassifications would have led to an underestimation of our association.

Information bias is not likely, since data on genotype and prescription data were collected prospectively without prior knowledge of the study hypothesis. We assessed potential confounding factors such as age, gender, BMI, renal function and starting dose in the multivariate analyses, but no association was found between CYP2D6 and BMI, renal function and starting antidepressant dose. Elderly subjects frequently use multiple drugs, due to comorbidity. Potentially, this could lead to ADRs due to drug-drug interactions. However, we assumed that comedication of CYP2D6 inhibitors did not confound our results, because comedication is prescribed independent of CYP2D6 genotype.

Our observational study demonstrates that CYP2D6 PM genotype is associated with an increased risk of switching to another antidepressant within the first 6 weeks of TCA pharmacotherapy and showed that PMs required a lower maintenance dose compared to EMs. Our data show that starting doses of antidepressants prescribed to the elderly general population are carefully low and are titrated to the optimum dose, reducing the risk of adverse drug reactions. Therefore, although this study demonstrated that the CYP2D6 polymorphism is associated with antidepressant use, the question remains whether genotyping prior to the start of antidepressant therapy contributes substantially to the optimization of pharmacotherapy.

References

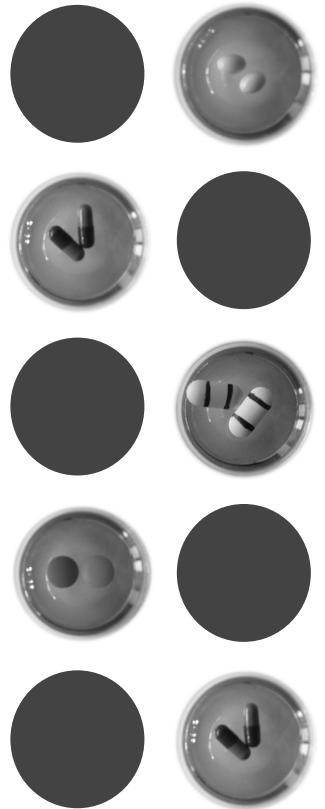
1. Roose SP, Schatzberg AF. The efficacy of antidepressants in the treatment of late-life depression. *J Clin Psychopharmacol* 2005;25(4 Suppl 1):S1-7.
2. Evans WE, McLeod HL. Pharmacogenomics-drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348(6):538-49.
3. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5(1):6-13.
4. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3(2):229-43.
5. Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. *Am J Hum Genet* 1991;48(5):943-50.
6. 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(2):284-95.
7. Lovlie R, Daly AK, Matre GE, Molven A, Steen VM. Polymorphisms in CYP2D6 duplication-negative individuals with the ultrarapid metabolizer phenotype: a role for the CYP2D6*35 allele in ultrarapid metabolism? *Pharmacogenetics* 2001;11(1):45-55.
8. Mulder H, Herder A, Wilmink FW, Tamminga WJ, Belitser SV, Egberts AC. The impact of cytochrome P450-2D6 genotype on the use and interpretation of therapeutic drug monitoring in long-stay patients treated with antidepressant and antipsychotic drugs in daily psychiatric practice. *Pharmacoepidemiol Drug Saf* 2006;15(2):107-14.
9. Steimer W, Zopf K, von Amelunxen S, Pfeiffer H, Bachofer J, Popp J, Messner B, Kissling W, Leucht S. 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(2):376-85.
10. Chou WH, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, Pho M, Xiao V, Ryder TB, Liu WW, Teiling C, Wedlund PJ. 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(2):246-51.
11. Rau T, Wohlleben G, Wuttke H, Thuerauf N, Lunkenheimer J, Lanczik M, Eschenhagen T. CYP2D6 genotype: impact on adverse effects and nonresponse during treatment with antidepressants—a pilot study. *Clin Pharmacol Ther* 2004;75(5):386-93.
12. Murphy GM, Jr., Kremer C, Rodrigues HE, Schatzberg AF. Pharmacogenetics of antidepressant medication intolerance. *Am J Psychiatry* 2003;160(10):1830-5.
13. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7(4):403-22.
14. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16(1):31-41.
15. Mulder H, Wilmink FW, Beumer TL, Tamminga WJ, Jedema JN, Egberts AC. The association between cytochrome P450 2D6 genotype and prescription patterns of antipsychotic and antidepressant drugs in hospitalized psychiatric patients: a retrospective follow-up study. *J Clin Psychopharmacol* 2005;25(2):188-91.
16. Rasmussen BB, Brosen K. Is therapeutic drug monitoring a case for optimizing clinical outcome and avoiding interactions of the selective serotonin reuptake inhibitors? *Ther Drug Monit* 2000;22(2):143-54.
17. Max MB, Culnane M, Schafer SC, Gracely RH, Walther DJ, Smoller B, Dubner R. Amitriptyline relieves diabetic neuropathy pain in patients with normal or depressed mood. *Neurology* 1987;37(4):589-96.

18. Hansen HV, Kessing LV. Adherence to antidepressant treatment. *Expert Rev Neurother* 2007;7(1):57-62.
19. Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, Soqvist F, Spina E, Brockmoller J. CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. *Acta Psychiatr Scand* 2001;104(3):173-92.
20. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev* 2006;58(3):521-90.
21. Grasmader K, Verwohlt PL, Rietschel M, Dragicevic A, Muller M, Hiemke C, Freymann N, Zobel A, Maier W, Rao ML. Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. *Eur J Clin Pharmacol* 2004;60(5):329-36.
22. Kirchheiner J, Nickchen K, Bauer M, Wong M-L, Licinio J, Roots I, Brockmoller J. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol Psychiatry* 2004;9:442-473.

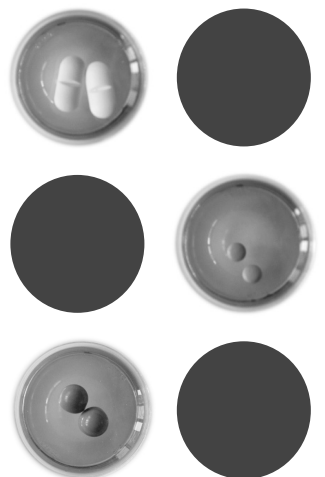
Variation in the CYP2D6 gene is associated with a lower serum sodium concentration in patients on antidepressants

Sonja Kwadijk-de Gijssel, Monique J. Bijl,
Loes E. Visser, Ron H.N. van Schaik, Albert
Hofman, Arnold G. Vulto, Teun van Gelder,
Bruno H.Ch. Stricker

Br J Clin Pharmacol 2009; 68: 221-5



CHAPTER 2.2



Abstract

Background: Several antidepressants are metabolized by the polymorphic enzyme cytochrome P450 2D6 (CYP2D6). The variant allele *CYP2D6*4* is the main polymorphism resulting in decreased enzyme activity in Caucasians. Decreased CYP2D6 enzyme activity potentially leads to higher plasma concentrations of antidepressants. Consequently, patients carrying the **4* allele are more likely to suffer from adverse drug reactions such as hyponatremia.

Aim: To study the effect of the *CYP2D6*4* polymorphism on serum sodium concentration in users of antidepressants (SSRIs and TCAs).

Methods: In this population-based cohort study, all subjects in the Rotterdam Study were included, who used an antidepressant at baseline and from whom a blood sample was available in which *CYP2D6* genotype and serum sodium concentration could be determined (n=76). Multivariate linear regression was used to study the association between *CYP2D6*4* and serum sodium concentration.

Results: CYP2D6 PMs (**4/*4*) had a significantly lower mean serum sodium concentration in comparison to CYP2D6 EMs (**1/*1*) (difference -3.9 mmol/l; CI 95% -0.86;-7.03; p = 0.013). In *CYP2D6*4* heterozygotes (**1/*4*) serum sodium concentration was 1.7 mmol/l (CI 95% -3.48; 0.18) lower compared to CYP2D6 EMs, but this difference was not statistically significant (p=0.077)

Conclusion: The serum sodium concentration in PMs was lower in users of an antidepressant, especially in TCA users. Therefore, CYP2D6 PMs might be at increased risk of developing symptoms of hyponatremia.

Introduction

Hyponatremia is the most common electrolyte disorder in ambulatory out patients, especially in the elderly [1]. Hyponatremia can be defined as a serum sodium concentration of less than 136 mmol/l and the prevalence is estimated to vary between 5-10% in a healthy elderly population to 30% in patients admitted to a hospital [1,2]. Predisposing factors for hyponatremia are an increasing age, female gender, usage of diuretics (especially thiazides), recent history of pneumonia, low Body Mass Index (BMI) and impaired renal function [3].

The use of tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) has also been reported as a cause of hyponatremia [3-10]. Although the precise mechanism is not known, antidepressants are thought to provoke the syndrome of inappropriate antidiuretic hormone release (SIADH) by direct or indirect stimulation of vasopressin release from the posterior pituitary gland. SIADH can lead to retention of water and to hyponatremia [4]. The occurrence of SIADH in patients using antidepressants (TCAs and SSRIs) was previously described in several case reports and a case series and is estimated to occur in 5 on every 1000 patients treated per year [1,5,7,11,12].

Most antidepressants are metabolized by the hepatic enzyme cytochrome P450 2D6 (CYP2D6), which is highly polymorphic with more than 60 variant alleles [<http://www.cypalleles.ki.se>]. Individuals carrying two functional CYP2D6 alleles (*1,*2) have 'normal' enzyme activity and are classified as extensive metabolizers (EMs). However, 5-10% of the population lack enzyme activity due to inheritance of two non-functional alleles (*3,*4,*5,*6) and form the so-called poor metabolizers (PMs). Heterozygous carriers of non-functional alleles exhibit decreased enzyme activity and are usually classified as intermediate metabolizers (IMs). *CYP2D6*4* is the most common variant allele in Caucasians (allele frequency of 20%) [13].

PMs have higher plasma concentrations of antidepressants metabolized by CYP2D6 and are therefore more likely to suffer from adverse drug events [14]. Hyponatremia or low serum sodium concentration may be one of these adverse events. In one study, it was found that SSRI-related hyponatremia is not related to *CYP2D6* genotype, or excessive drug concentrations, but the study population was small (n=20) and concerned only severe cases of hyponatremia (<130 mmol/l) [15]. It is unknown whether *CYP2D6* genotype influences serum sodium concentration in users of antidepressants, especially in TCA users.

Therefore, the objective of this population-based cohort study was to examine the influence of the *CYP2D6*4* polymorphism on serum sodium concentration in patients treated with a TCA or SSRI.

Methods

Setting

This study is part of the Rotterdam Study, a prospective population based cohort study among inhabitants of Ommoord, a suburb of Rotterdam. Between 1990 and 1993, all 10,275 persons aged ≥ 55 years were invited to participate. The aims of the Rotterdam Study are to investigate incidence of, and risk factors for cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly [16,17]. The Medical Ethics Committee of the Erasmus Medical Centre approved the study and written informed consent was obtained from all participants. All drug prescriptions dispensed to participants were made available in computerized form as of January 1991.

The study population consisted of all subjects in the Rotterdam Study who used an antidepressant (TCA or SSRI) at baseline ($n=139$) and from whom a blood sample was available in which *CYP2D6* genotype and serum sodium concentration could be determined ($n=76$).

Genotyping

At the baseline examination of the Rotterdam Study, blood was obtained from which DNA was isolated. Genotyping for the *CYP2D6**4 polymorphism (1846G>A) was performed using Taqman allelic discrimination assays as described earlier [14].

Exposure and outcome definition

Use of antidepressants was defined as current use of an antidepressant (N06AA/AC or N06AB/AE) at the time of blood sampling for DNA genotyping and determination of the serum sodium levels. Serum sodium concentration in mmol/l was considered as the outcome of interest.

Statistical analysis

Genotype frequency was tested for deviation from Hardy-Weinberg equilibrium using a chi-square test. A multivariate linear regression model was used to assess the effect of the *CYP2D6**4 polymorphism on serum sodium concentration. The model was adjusted for age and gender and additionally for covariates that changed the point estimate by more than 10%. The following covariates were considered as potential confounders: age, gender, use of diuretics, Body Mass Index (BMI) in kg/m^2 , antidepressant dose and renal function [14].

Analyses were performed in the total cohort of antidepressant users and separately in TCA and SSRI users. All analyses were performed with SPSS software (version 15.0).

Results

The study population characteristics are shown in table 1. Information on *CYP2D6* genotype and serum sodium concentration was available in 76 antidepressant users. The allele frequency of the *CYP2D6**4 allele in our study population was 18.4%. The genotype distribution was in Hardy-Weinberg equilibrium.

Table 1. Baseline characteristics of the study population

Variable	Number of patients (%)
Mean age, years (SD) (n=76)	69.7 (8.5)
Gender	
Female	57 (75%)
Male	19 (25%)
<i>CYP2D6</i> genotype*	
*1/*1 (EM)	53 (69.7%)
*1/*4 (IM)	18 (23.7%)
*4/*4 (PM)	5 (6.6%)
Antidepressant Use	
SSRI	12 (15.8%)
TCA	64 (84.2%)
Mean dose in DDD (SD)	
AD	0.8 (0.5)
SSRI	1.1 (0.7)
TCA	0.5 (0.4)
Co-administration	
Diuretic Use	14 (18.4%)
Mean level of albumine (g/l)	42.8 (2.4)
Body Mass Index kg/m ² (SD)	26.6 (3.6)
Glomerular filtration rate ml/min (SD)	69 (18.6)

* Hardy-Weinberg equilibrium, $\chi^2 = 3.42$ (p=0.06)

EM = extensive metabolizer, PM = poor metabolizer, IM= intermediate metabolizer

AD= antidepressant, TCA= tricyclic antidepressant, SSRI= selective serotonin reuptake inhibitor

DDD= defined daily dose equivalents

No association was demonstrated between *CYP2D6* genotype and serum sodium concentration among non-antidepressant users in the Rotterdam Study (p=0.146). The association between *CYP2D6* genotype and serum sodium concentration in antidepressant users is given in table 2. *CYP2D6* PMs (*4/*4) had a significantly lower mean serum sodium concentration in comparison to *CYP2D6* EMs (*1/*1) (difference -3.9 mmol/l; CI 95% -0.86;-7.03; p = 0.013). In *CYP2D6**4 heterozygotes (*1/*4) serum sodium concentration was 1.7 mmol/l (CI 95% -3.48; 0.18) lower compared to *CYP2D6* EMs, but this difference was not statistically significant (p=0.077).

Table 2. Association between CYP2D6 genotype and serum sodium concentration in users of antidepressants

CYP2D6 genotype	Patients (n=76)	Mean serum sodium conc in mmol/l*	Mean dif serum sodium conc (95% CI) in mmol/l*
All antidepressants (TCA or SSRI)			
*1/*1 (EM)	53	140.3	Ref
*1/*4 (IM)	18	138.6	-1.65 (-3.48; 0.18)
*4/*4 (PM)	5	136.4	-3.94 (-0.86;-7.03)
TCA			
*1/*1 (EM)	44	140.4	Ref
*1/*4 (IM)	16	138.9	-1.37 (-3.39; 0.64)
*4/*4 (PM)	4	136.0	-4.32 (-7.85; -0.79)
SSRI			
*1/*1 (EM)	9	140.0	ref
*1/*4 (IM)	2	136.0	5.38 (-11.76; 1.00)
*4/*4 (PM)	1	138.0	1.07 (-9.57; 7.42)

Ref = reference group

* Adjusted for age and gender

The analysis was repeated separately for participants using a TCA or SSRI. The association between CYP2D6 and serum sodium concentration in the subgroup analysis of TCA users was similar to the difference found in all antidepressant users and is given in table 2. The results of the analysis for SSRIs showed no statistically significant difference in serum sodium concentration between the different CYP2D6 genotypes (table 2).

Discussion

The main finding in this study is that antidepressant users homozygous for the CYP2D6*4 allele had a significantly lower serum sodium concentration than antidepressant users with the wildtype genotype. When the analyses were performed separately for TCAs and SSRIs, this association was still observed in users of TCAs but not in SSRI users. The last observation may be explained by the small number of SSRI users (n=12) and the observation that there was only one PM in this group. In addition, the amount of drug metabolized by CYP2D6 might explain the absence of association in SSRI users, since TCA metabolism seems more dependent on CYP2D6 enzyme activity than that of SSRIs [14,18].

Although the clinical relevance of severe hyponatremia (Na <118 mmol/L) is well recognized and leads to serious symptoms such as confusion, unconsciousness, grand mal seizures and even death, mild hyponatremia may cause important symptoms, with instability, delayed reaction time and more mental errors in patients with a normal serum sodium concentration [19].

In this study, serum sodium concentration was examined as a continuous variable. The number of patients with a serum sodium concentration \leq 136 mmol/l in our cohort was too small to assess the effect of CYP2D6 genotype, on hyponatremia.

Besides SIADH as a cause of hyponatremia, excess of water intake or dietary sodium deficiency could also result in hyponatremia. The lower serum sodium concentration of the PMs was probably not caused by excess water intake due to a dry mouth, compulsive water drinking or primary polydipsia because albumin concentrations in these subjects were within the normal range, which suggest, that the lower serum sodium is not a dilution of body water.

Observational studies are prone to selection, information and confounding bias. Selection bias is unlikely since missing blood samples and or genotypes were not expected to be specifically related to *CYP2D6* genotype. Information bias seems also unlikely as both *CYP2D6* genotype and serum sodium concentration measurements were performed prior to formulation of the research question. In this study, only the *CYP2D6**4 polymorphism was determined, since this is the most common polymorphism among PMs. The frequency of the *4 allele (18.5%) corresponded with the literature and the population was in Hardy-Weinberg equilibrium though borderline significant ($p=0.06$) [13].

The genotyping assay was validated by DNA sequencing, but the assay was unable to deal with the gene deletion (*5). *4/*5 were classified as *CYP2D6* *4/*4 which may have lead to an overestimation of *4/*4 individuals in HWE. This did not affect a subjects phenotype, because both *4/*4 and *4/*5 were classified as *CYP2D6* PM. However, some misclassification of PMs, IMs and ultra-rapid metabolizers in the reference group (EM) might have occurred. These misclassifications however, will lead to an underestimation of the association rather than an overestimation.

Potential confounding by age, gender, BMI, glomerular filtration rate (GFR), antidepressant dose and use of diuretics was dealt with in the multivariate analyses. Bijl et al found an association between *CYP2D6* genotype and TCA maintenance dose [14]. In our study, antidepressant dose was not of any influence (data not shown).

In conclusion, the data in this study show that among users of antidepressants *CYP2D6* PMs have a lower serum sodium concentration than EMs.

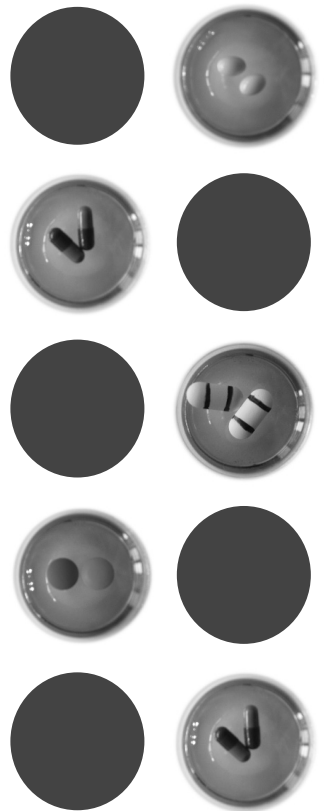
References

1. Movig KL, Leufkens HG, Lenderink AW, Egberts AC. Serotonergic antidepressants associated with an increased risk for hyponatraemia in the elderly. *Eur J Clin Pharmacol* 2002;58:143-8.
2. Hoorn EJ, Lindemans J, Zietse R. Development of severe hyponatraemia in hospitalized patients: treatment-related risk factors and inadequate management. *Nephrol Dial Transplant* 2006; 21:70-6.
3. Jacob S, Spinler SA. Hyponatremia associated with selective serotonin-reuptake inhibitors in older adults. *Ann Pharmacother* 2006;40:1618-22.
4. Adroque HJ, Madias NE. Hyponatremia. *N Engl J Med* 2000;342:1581-9.
5. Bavbek N, Kargili A, Akcay A, Kaya A. Recurrent hyponatremia associated with citalopram and mirtazapine. *Am J Kidney Dis* 2006; 48:e61-e62.
6. Bouman WP, Johnson H, Trescoli-Serrano C, Jones RG. Recurrent hyponatremia associated with sertraline and lofepramine. *Am J Psychiatry* 1997;154:580.
7. Fitzgerald MA. Hyponatremia associated with SSRI use in a 65-year-old woman. *Nurse Pract* 2008; 33:11-2.
8. Ladino M, Guardiola VD, Paniagua M. Mirtazapine-induced hyponatremia in an elderly hospice patient. *J Palliat Med* 2006; 9:258-60.
9. Liskin B, Walsh BT, Roose SP, Jackson W. Imipramine-induced inappropriate ADH secretion. *J Clin Psychopharmacol* 1984; 4:146-7.
10. Miller MG. Tricyclics as a possible cause of hyponatremia in psychiatric patients. *Am J Psychiatry* 1989;146:807.
11. Twardowschy CA, Bertolucci CB, Gracia CM, Brandao MA. Severe hyponatremia and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) associated with fluoxetine: case report. *Arq Neuropsiquiatr* 2006;64:142-5.
12. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005;352:2211-21.
13. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3:229-43.
14. Bijl MJ, Visser LE, Hofman A, Vulto AG, van Gelder T, Stricker BH, van Schaik RH. Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *Br J Clin Pharmacol* 2008;65:558-64.
15. Stedman CA, Begg EJ, Kennedy MA, Roberts R, Wilkinson TJ. Cytochrome P450 2D6 genotype does not predict SSRI (fluoxetine or paroxetine) induced hyponatraemia. *Hum Psychopharmacol* 2002;17:187-90.
16. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22:819-29.
17. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7:403-22.
18. Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, Brockmüller J. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol Psychiatry* 2004;9:442-73.
19. Renneboog B, Musch W, Vandemergel X, Manto MU, Decaux G. Mild chronic hyponatremia is associated with falls, unsteadiness, and attention deficits. *Am J Med* 2006;119:71-8.

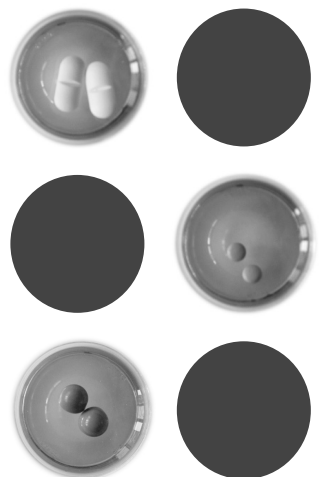
Association between the *CYP2D6**4 polymorphism and depression and anxiety in the elderly

Monique J. Bijl, Hendrika J. Luijendijk, Julia F. van den Berg, Loes E. Visser, Ron H.N. van Schaik, Albert Hofman, Arnold G. Vulto, Teun van Gelder, Henning W. Tiemeier, Bruno H.Ch. Stricker

Pharmacogenomics 2009;10:541-7



CHAPTER 2.3



Abstract

Background: 5-methoxytryptamine (5-MT), a precursor of serotonin, is considered to be an endogenous substrate of cytochrome P450 2D6 (CYP2D6). Homozygous carriers of the variant allele *CYP2D6**4 lack CYP2D6 enzyme activity. Relative to extensive metabolizers (EMs), these poor metabolizers (PMs) may have lower baseline serotonin concentrations in various brain regions, and may be more prone to depression or anxiety.

Aim: To test whether the *CYP2D6* *4/*4 genotype is associated with a predisposition to depression or anxiety disorders in the elderly.

Methods: We conducted a cross-sectional study within the Rotterdam Study, a population-based cohort study, among persons, aged 55 years or over, who were screened for depression and anxiety disorders at two consecutive examination rounds. Logistic regression was used to analyze the association between the *CYP2D6**4 polymorphism and the risk of depression or anxiety disorders.

Results: The risk of major depression in *CYP2D6* *4/*4 was not significantly different from extensive metabolizers (OR=0.85; 95%CI 0.36-2.00; p=0.72). Neither did we find an association between CYP2D6 genotype and minor depression (OR=1.56; 95%CI 0.69-3.52; p=0.28). No increased risk of anxiety disorders was found (OR=1.19; 95% CI 0.68-2.09; p=0.55).

Conclusion: Variation in the *CYP2D6* gene is not related to a predisposition to depression or anxiety disorders in the elderly.

Introduction

Cytochrome P450 2D6 (CYP2D6) is predominantly expressed in the liver and is one of the most important enzymes in the metabolism of therapeutic drugs, including antidepressants, β -blockers, antiarrhythmics and antipsychotics [1]. CYP2D6 shows a large phenotypical variability, largely due to genetic polymorphisms. In Caucasians, 5-10% of the population lack CYP2D6 enzyme activity resulting from two non-functional alleles of the *CYP2D6* gene. *CYP2D6*4* is the most common variant allele (allele frequency of 20%) which leads to the poor metabolizer (PM) phenotype in Caucasians [2]. Subjects with two functional alleles have normal enzyme activity and are classified as extensive metabolizer (EM). Individuals heterozygous for the *CYP2D6*4* allele have slower rates of metabolism than EMs and are usually classified as intermediate metabolizer, although translation from this genotype to phenotype is rather complex [3]. In addition, ultrarapid metabolizers (UMs) have multiple functional copies of the CYP2D6 gene and exhibit extremely high enzyme activity. This UM genotype was discovered in depressed patients who did not respond to normal doses of tricyclic antidepressants [4,5].

Besides the liver, CYP2D6 can also be found in small amounts in the brain [6]. It has been suggested that a precursor of serotonin is one of the CYP2D6 endogenous substrates [7]. The first observations came from studies on CYP2D6 enzyme activity and variation in human personality [8,9]. Poor metabolizers of CYP2D6 had a higher frequency of extreme responses, including high vitality, alertness, lack of hesitation, efficiency and ease of decision making than EMs in one study [8]. Conflicting results were reported from another study in which PMs were more anxiety prone and less successfully socialized than EMs [9]. Since these two studies, numerous studies on the relationship between personality and CYP2D6 have been performed, but an effect on personality has not consistently been found [10-12]. This inconsistency may be the result of small sample sizes, different personality questionnaires and interethnic differences. Anxiety and impulsive aggressive behaviour are both related to low levels of serotonin [13]. Several personality traits are associated with depression, but the etiological role in depression is unclear [14].

In the search for possible endogenous substrates Yu et al. found that CYP2D6 was involved in the conversion of 5-methoxytryptamine (5-MT) to serotonin (figure 1). Serum serotonin level was about three-fold higher in transgenic mice, expressing CYP2D6, compared to wild type mice [15]. In another study significantly higher baseline serotonin concentrations in platelets were detected in CYP2D6 UMs compared to EMs and PMs [16]. In one study it was hypothesized that CYP2D6 PMs have lower baseline serotonin concentration in various brain regions and are therefore more prone to depression or anxiety [13].

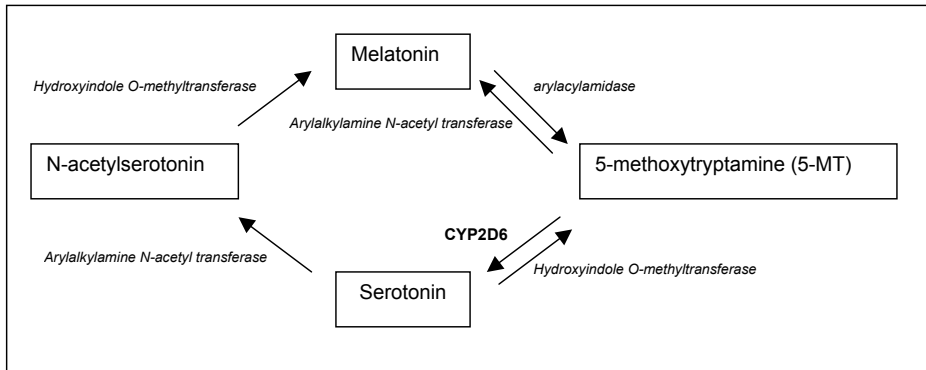


Figure 1. The Serotonin – Melatonin cycle

5-Methoxytryptamine (5-MT) is metabolized to serotonin by cytochrome P450 2D6 (CYP2D6). Serotonin can be N-acetylated to form N-acetylserotonin, which is converted into melatonin. The major metabolic pathway for melatonin is 6-hydroxylation (not shown), but deacetylation completes the cycle by the formation of 5-MT [15].

Subsequently, several studies found an increased frequency of CYP2D6 PMs among psychiatric inpatients. In one study, the proportion of people with CYP2D6 deficiency was two-fold higher (14%) than in the normal population [17]. This observation could be due to an association between *CYP2D6* genotype and mental disease, or alternatively to selection bias, because the higher proportion of CYP2D6 PMs among patients admitted to psychiatric hospitals might be caused by higher failure rates of previous antidepressive pharmacotherapy in PMs. It has been shown that PMs are overrepresented in patients in whom antidepressive therapy is discontinued as a result of adverse events [18].

As stated above the association between *CYP2D6* genotype and mental disease is inconsistent. The relation between the *CYP2D6* gene and vulnerability to depression or anxiety disorders is still unclear. To test whether *CYP2D6* *4/*4 individuals have a predisposition to depression or anxiety disorders, we performed a cross-sectional study to investigate the association between the *CYP2D6**4 polymorphism and the risk of depression or anxiety in the elderly.

Methods

Setting

This study was part of the Rotterdam Study, a prospective population-based cohort study on neurologic, cardiovascular, locomotor, ophthalmologic and psychiatric diseases in the elderly [19,20]. Between 1990 and 1993, all inhabitants of a particular district of Rotterdam, aged 55 years or over, were invited to participate. Eventually, 7983 (78%) took part in the baseline examination, which consisted of an extensive home interview followed by two visits to the

research center. The Medical Ethics Committee of the Erasmus Medical Center approved the study. Follow-up examinations were conducted in 1993-1995, 1997-1999 and 2002-2004.

Depressive symptoms and disorders were assessed at the third and fourth examination (1997-1999 and 2002-2004), and anxiety disorders at the fourth examination. As of January 1, 1991, all drug prescriptions dispensed to participants have been stored in a computer network.

We conducted a cross-sectional study among persons who had been screened for depressive symptoms at the third (n=4601) and fourth (n=3437) survey, and among persons in whom anxiety disorders had been assessed during the fourth examination round.

Assessment of depression and anxiety

All participants were invited to fill out the Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D), a 20-item questionnaire that measures depressive symptoms on a scale from 0-60 [21, 22]. A value of ≥ 16 is used as a cut-off value for depressive symptoms.

Persons with a positive value (≥ 16) underwent a psychiatric interview including the Dutch version of the Schedules for Clinical Assessment in Neuropsychiatry (formerly known as Present State Examination). The diagnoses were assigned according to the DSM-IV criteria to the following categories: 1) major depression and dysthymia, 2) minor depression and 3) no depression (all others, including negative CES-D score). Since impaired cognitive function can influence the diagnosis of depression, all subjects with dementia were excluded from the analyses. The diagnosis of dementia was made by a panel of neurologists in accordance with internationally accepted guidelines (DSM-III-R, NINCDS-ADRA and NINDS-AIREN).

Anxiety disorders were assessed at the fourth examination round using the Munich Composite International Diagnostic Interview (M-CIDI), including generalized anxiety disorder, specific and social phobia, panic disorder and agoraphobia without panic disorder [23].

Genotyping

At the baseline examination of the Rotterdam Study, blood was taken from which DNA was isolated. Genotyping for the *CYP2D6**4 polymorphism (1846G>A) was performed by using Taqman allelic discrimination assays as described previously [24]. In brief, polymerase chain reactions (PCR) were performed in a reaction volume of 2.0 μ l containing 1 ng genomic DNA. The thermal profile consists of an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds and by annealing and extension at 60°C for 1 minute.

Homozygous subjects for the *4 allele were classified as PM. Subjects were classified as IM if they were heterozygous. Subjects in whom *CYP2D6**4 was not detected were assumed to be extensive metabolizers (*1).

Statistical analysis

Hardy-Weinberg equilibrium was determined by using a χ^2 -test to compare genotype frequencies with the expected values. Binary logistic regression analysis was used to study the association between *CYP2D6* genotype and depression or anxiety disorders, separate analyses being performed for major and minor depression. In the model we adjusted for age and gender. Antidepressant use at time of the interview was considered as potential confounder and/or effect modifier. The study had 93% power to detect a relative difference of 1.5 in occurrence of depression between PMs and EMs. All analyses were performed with SPSS software (version 11.0, Chicago, USA).

Results

Of the 4601 participants in the third examination round, 436 patients with a diagnosis of dementia were excluded. No blood sample was available from 283 subjects; in 68 subjects sub-optimal blood samples (due to excessive storage times) had led to difficulties with *CYP2D6**4 genotyping. Of the remaining 3814 subjects, 265 had a positive CES-D value (>16). The fourth examination round included 3437 subjects, of whom 447 participants were excluded (184 due to dementia, 211 due to lack of blood sample, and 52 participants due to genotyping difficulties). Three hundred and seventy-five subjects had a CES-D value ≥ 16 .

Table 1 shows the characteristics of the study population. In this study there were in total 122 patients with major depression or dysthymia and 94 patients with a minor depression at the third or fourth examination round. The allele frequency of the *CYP2D6**4 allele in this population was 20.8%. Genotype frequencies deviated significantly from Hardy-Weinberg equilibrium ($p < 0.01$). There was no statistically significant difference in the percentage of *CYP2D6* *4/*4 between subjects with a depressive disorder (6.6%) and subjects without depressive disorders (5.7%).

Table 2 shows the risk of minor and major depression by *CYP2D6* genotype. The risk of major depression in *CYP2D6* *4/*4 PMs was not significantly different from EMs (OR=0.85; 95%CI 0.36-2.00; $p=0.72$). There was no association between *CYP2D6* *4/*4 genotype and minor depression (OR=1.56; 95%CI 0.69-3.52; $p=0.28$). Stratification on antidepressant use did not influence the results.

At the fourth examination round, 225 patients had one or more anxiety disorders. Table 3 shows the overall risk of an anxiety disorder. This risk was not higher in *CYP2D6* PMs than in EMs.

Table 1. Characteristics of the study population

	3 rd examination round	4 th examination round
n	3814	2990
Mean age, years (SD)	72.4 (7.1)	75.7 (6.3)
Gender		
female	2196 (57.6%)	1759 (58.8%)
CYP2D6 genotype		
*1/*1	2451 (64.3%)	1921 (64.2%)
*1/*4	1138 (29.8%)	896 (30.0%)
*4/*4	225 (5.9%)	173 (5.8%)
CES-D positive value (≥ 16)	265	375
Depression*		
major depression and dysthemia	54	78
minor depression	48	50
Anxiety		
1 or more anxiety disorder	not assessed	225
generalized anxiety disorder		63
specific phobia		47
social phobia		21
panic disorder		9
agoraphobia without panic disorder		112
Antidepressant use	100	118

*Some patients had depressive disorders at both examination rounds.

CYP2D6: Cytochrome P450 2D6; CES-D: Center for Epidemiological Studies Depression Scale

Table 2. Relationship between CYP2D6 genotype and depression in the Rotterdam Study

CYP2D6 genotype	Major depression			Minor depression		
	Cases	OR [^] (95% CI)	p-value	Cases	OR [^] (95% CI)	p-value
*1/*1	79	1.00 (ref)	-	55	1.00 (ref)	-
*1/*4	37	1.04 (0.70-1.56)	0.84	32	1.34 (0.85-2.11)	0.21
*4/*4	6	0.85 (0.36-2.00)	0.72	7	1.56 (0.69-3.52)	0.28

[^] Odds ratios calculated with logistic regression adjusted for age and gender

Table 3. Relationship between CYP2D6 genotype and anxiety disorder in the Rotterdam Study

CYP2D6 genotype	Anxiety disorder		
	Cases	OR [^] (95% CI)	p-value
*1/*1	140	1.00 (ref)	-
*1/*4	70	1.04 (0.77-1.41)	0.78
*4/*4	15	1.19 (0.68-2.09)	0.55

[^] Odds ratios calculated with logistic regression adjusted for age and gender

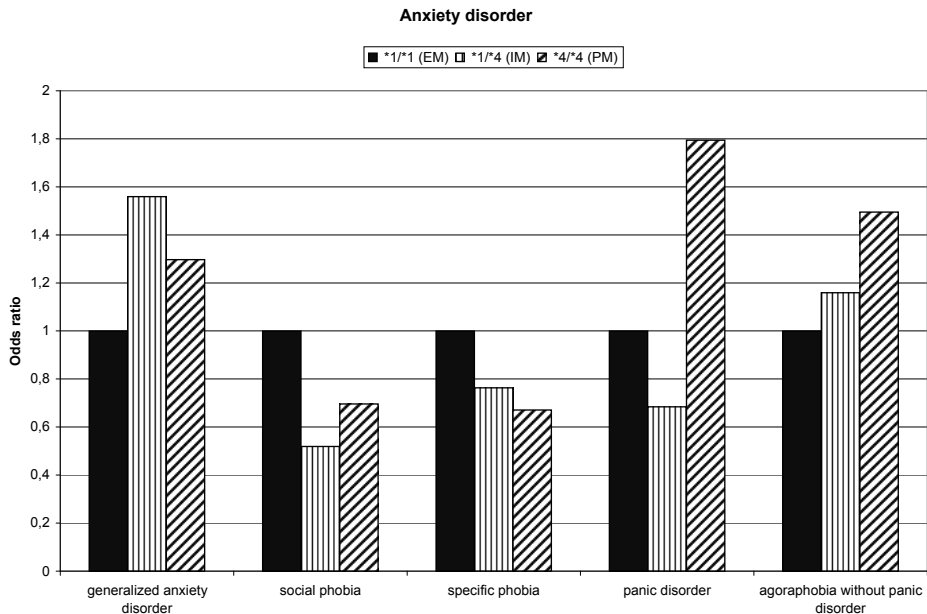


Figure 2. Relationship between CYP2D6 genotype and anxiety disorders in the Rotterdam Study

In CYP2D6 PMs the risk of generalized anxiety disorder, specific and social phobia, panic disorder or agoraphobia without panic disorder was not significantly different from EMs or IMs.

In CYP2D6 PMs the risk of generalized anxiety disorder, specific and social phobia, panic disorder or agoraphobia without panic disorder was not significantly different from EMs or IMs (figure 2).

Discussion

The present study showed that *CYP2D6* *4/*4 individuals did not have a predisposition towards developing depression or anxiety disorders compared to EMs. The impaired metabolism of 5-methoxytryptamine to serotonin in PMs did not increase the risk of depression or anxiety disorders. Several explanations for these findings are conceivable. The first explanation is that *CYP2D6* polymorphisms may affect serotonin concentrations in the brain to such a small degree that it has no clinical consequences. Yu et al. suggested that, in tissues where monoamine oxidase type A (MAO-A) is also expressed, deamination of 5-MT, a precursor of serotonin, by monoamine oxidase type A rather than demethylation by *CYP2D6* probably dominates [15], meaning that the influence of *CYP2D6* polymorphisms on serotonin concentrations in these tissues is only small. The second possible explanation is, that 5-MT itself has a high affinity for serotonin receptors similar to that of serotonin [25]. The third possible explanation is that other neurochemical pathways (e.g. dopamine) could interact with low serotonin levels. Stimulation

of the serotonin receptors on the nigrostriatal pathway reduces dopamine release. Low levels of serotonin in CYP2D6 PMs may lead to increased dopamine levels in these individuals [13]. Although CYP2D6 is also involved in the conversion of tyrosine to dopamine, this has no large physiological impact [25, 26]. Homeostatic reactions could maintain a certain balance between serotonin and dopamine in the brain.

Some potential limitations of our study should be mentioned. In our study, we determined only the *CYP2D6**4 polymorphism. In a Caucasian population *CYP2D6**4 is the most frequent non-functional allele leading to the PM phenotype (>75% of the PMs are carriers of this polymorphism [3]). Other less frequent genetic variants (*3, *5, *6, *8, *10, and *41) were not assessed, which led to the inclusion of some IMs, PMs, and UMs in the reference group (EMs). However, we believe that these misclassifications would have a minor influence on our risk estimates. The allele frequency of 20.8% was in accordance with the literature [3], but no Hardy-Weinberg equilibrium was observed. Our genotyping assay was validated by DNA sequencing, but the assay identified individuals heterozygous for the gene deletion *CYP2D6**5 as homozygous: *4/*5 individuals (PMs) will be identified as *4/*4 (PMs) and *1/*5 individuals as *1/*1 (EMs). Although this phenomenon led to an overestimation of the number of *4 homozygous individuals in our population, this will have only minor effects on the outcome of the study, because these patients will be classified PM in either way.

This study determined only the participants' genotype. The consequences for the expression of CYP2D6 in the human brain are questionable.

Cross-sectional studies lack any information on timing of relationships between exposure and outcome. Since genotypes do not change over time, a cross-sectional design is sufficient to examine the association between *CYP2D6* genotype and predisposition to depression or anxiety. In a way we combined the data of the third and fourth examination round as a measure for cumulative depression. Depressive episodes often recur throughout a person's life. If only incident depression data were taken into account in this elderly population, we would probably exclude all subjects most vulnerable to depression, since they are likely to have experienced an earlier depressive episode [22]. Depressive disorders that occurred between the third and fourth examination round and were of short duration were missed in this study.

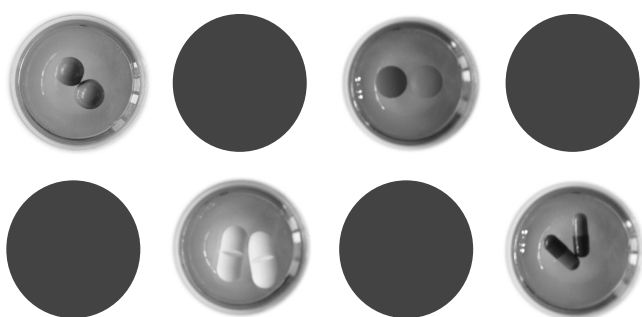
It should be mentioned that the number of poor metabolizers that were diagnosed with depression or an anxiety disorder was small. The study had enough power (93%) to detect a relative difference of 1.5 in occurrence of depression between PMs and EMs, but the power was insufficient for the small differences we found.

In conclusion, our study suggests that there is no clinically relevant association between *CYP2D6* genotype and depression or anxiety disorders in the elderly. Further research on endogenous substrates and their impact on serotonergic neurophysiology is necessary as the exact mechanism of serotonin regeneration by CYP2D6 in human brain is unknown.

References

1. Ingelman-Sundberg M: Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5, 6-13.
2. Bradford LD: CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3,229-43.
3. 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;83,234-42.
4. Bertilsson L, Dahl ML, Sjoqvist F, Aberg-Wistedt A, Humble M, Johansson I, Lundqvist E, Ingelman-Sundberg M: Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. *Lancet* 193;341,63.
5. 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 USA* 1993;90,11825-9.
6. 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;11,237-45.
7. Gervasini G, Carrillo JA, Benitez J: Potential role of cerebral cytochrome P450 in clinical pharmacokinetics: modulation by endogenous compounds. *Clin Pharmacokinet* 2004;43,693-706.
8. Bertilsson L, Alm C, De Las Carreras C, Widen J, Edman G, Schalling D: Debrisoquine hydroxylation polymorphism and personality. *Lancet* 1989;1,555.
9. Llerena A, Edman G, Cobaleda J, Benitez J, Schalling D, Bertilsson L: Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P4502D6. *Acta Psychiatr Scand* 1993;87,23-8.
10. Roberts RL, Luty SE, Mulder RT, Joyce PR, Kennedy MA: Association between cytochrome P450 2D6 genotype and harm avoidance. *Am J Med Genet B Neuropsychiatr Genet* 2004;127,90-3.
11. Gan SH, Ismail R, Wan Adnan WA, Zulmi W, Kumaraswamy N, Larmie ET: Relationship between Type A and B personality and debrisoquine hydroxylation capacity. *Br J Clin Pharmacol* 2004;57, 785-9.
12. Kirchheiner J, Lang U, Stamm T, Sander T, Gallinat J: Association of CYP2D6 genotypes and personality traits in healthy individuals. *J Clin Psychopharmacol* 2006;26,440-2.
13. Ozdemir V, Gunes A, Dahl ML, Scordo MG, Williams-Jones B, Someya T: Could endogenous substrates of drug-metabolizing enzymes influence constitutive physiology and drug target responsiveness? *Pharmacogenomics* 2006;7,1199-210.
14. Enns MW, Cox BJ: Personality dimensions and depression: review and commentary. *Can J Psychiatry* 1997; 42,274-84.
15. Yu AM, Idle JR, Byrd LG, Krausz KW, Kupfer A, Gonzalez FJ: Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* 2003;13,173-81.
16. Kirchheiner J, Henckel HB, Franke L et al.: Impact of the CYP2D6 ultra-rapid metabolizer genotype on doxepin pharmacokinetics and serotonin in platelets. *Pharmacogenet Genomics* 2005;15,579-87.
17. de Leon J, Barnhill J, Rogers T, Boyle J, Chou WH, Wedlund PJ: Pilot study of the cytochrome P450-2D6 genotype in a psychiatric state hospital. *Am J Psychiatry* 1998;155,1278-80.
18. Rau T, Wohlleben G, Wuttke H et al.: CYP2D6 genotype: impact on adverse effects and nonresponse during treatment with antidepressants-a pilot study. *Clin Pharmacol Ther* 2004;75, 386-93.
19. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA: Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7,403-22.
20. Hofman A, Breteler MM, van Duijn CM et al.: The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22,819-29.

21. Radloff L: The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1977;1,385-401.
22. Luijendijk HJ, van den Berg J, Dekker MJHJ et al.: Incidence and recurrence of late-life depression. *Archives of General Psychiatry* 2008 (in press).
23. Wittchen HU, Lachner G, Wunderlich U, Pfister H: Test-retest reliability of the computerized DSM-IV version of the Munich-Composite International Diagnostic Interview (M-CIDI). *Soc Psychiatry Psychiatr Epidemiol* 1998;33,568-78.
24. Bijl MJ, Visser LE, Hofman A et al.: Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *Br J Clin Pharmacol* 2008;65,558-64.
25. Yu AM, Idle JR, Gonzalez FJ: Polymorphic cytochrome P450 2D6: humanized mouse model and endogenous substrates. *Drug Metab Rev* 2004;36,243-77.
26. Hiroi T, Imaoka S, Funae Y: Dopamine formation from tyramine by CYP2D6. *Biochem Biophys Res Commun* 1998;249,838-43.



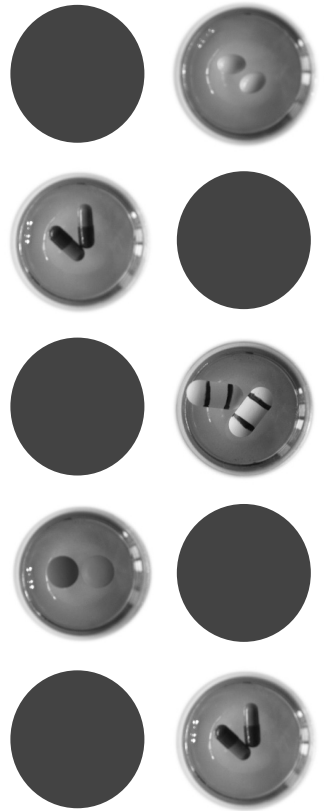
The *CYP2D6**4 polymorphism
and b-blockers

CHAPTER 3

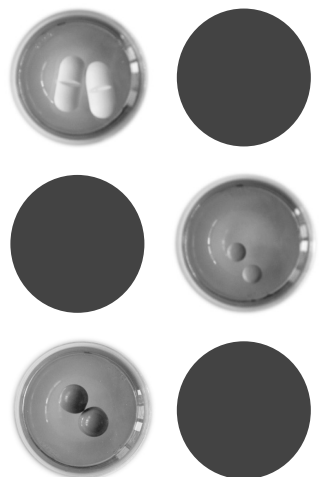
**Genetic variation in the
CYP2D6 gene is associated
with a lower heart rate and
blood pressure in b-blocker
users**

Monique J. Bijl, Loes E. Visser, Ron H.N.
van Schaik, Jan A. Kors, Jacqueline C.M.
Witteaman, Albert Hofman, Arnold G. Vulto,
Teun van Gelder, Bruno H.Ch. Stricker

Clin Pharmacol Ther 2009;85:45-50



CHAPTER 3



Abstract

Background: Several β -blockers are metabolized by the polymorphic enzyme cytochrome P450 2D6 (CYP2D6). *CYP2D6**4 is the main polymorphism leading to decreased enzyme activity. The clinical significance of impaired elimination of β -blockers is controversial and most studies suffer from small numbers of CYP2D6 poor metabolizers (PMs). The aim of this population-based cohort study was to examine the influence of the *CYP2D6**4 polymorphism on heart rate and blood pressure in patients treated with β -blockers.

Methods: The study cohort consisted of all subjects in the Rotterdam Study, in whom blood pressure and ECG was recorded and for whom data on genotype were available (n=1533). Repeated measurement analysis was used to analyze the association between CYP2D6 genotype and blood pressure or heart rate in β -blocker users.

Results: In *CYP2D6* *4/*4 PMs the adjusted heart rate in metoprolol users was 8.5 beats/min lower compared to *1/*1 extensive metabolizers (EMs) ($p < 0.001$), leading to an increased risk of bradycardia in PMs (OR= 3.86; 95% CI 1.68-8.86; $p = 0.0014$). The diastolic blood pressure in PMs was 5.4 mmHg lower in users of β -blockers metabolized by CYP2D6 ($p = 0.017$) and 4.8 mmHg lower in metoprolol users ($p = 0.045$) compared to EMs.

Conclusion: In users of β -blockers selectively metabolized by CYP2D6, including the widely prescribed β -blocker metoprolol, CYP2D6 PMs have a lower heart rate and diastolic blood pressure than EMs.

Introduction

β - Adrenoreceptor blockers are widely prescribed for the treatment of cardiovascular diseases, including hypertension, coronary heart disease and heart failure. Insight into the variability in pharmacokinetics can contribute to the understanding of interindividual variability in drug response and tolerability. Cytochrome P450 2D6 (CYP2D6) is involved in the hepatic elimination of several β -blockers. Among β -blockers metoprolol is most extensively metabolized by CYP2D6, accounting for 70-80% of its metabolism [1,2]. Carvedilol, nebivolol, propranolol, alprenolol are other substrates of the enzyme, but the contribution of CYP2D6 to their metabolism is lower than for metoprolol [3,4]. Other β -blockers, like atenolol, are eliminated predominately unchanged by glomerular filtration and elimination is therefore independent of CYP2D6 activity [5].

In Caucasians, 5-10% of the population lack CYP2D6 enzyme activity due to inheritance of two non-functional alleles of the *CYP2D6* gene (e.g. *CYP2D6**3, *4, *5, *6). These individuals are classified as poor metabolizers (PM) [6,7]. *CYP2D6**4 is the most common variant allele (allele frequency of 20%) leading to the PM phenotype in Caucasians: >75% of PMs carry this polymorphism [7]. Other genetic variants occur less frequently. Subjects with two functional alleles (e.g. *1, *2) are classified as extensive metabolizers (EMs), while heterozygous carriers of CYP2D6 polymorphisms show large variability in their phenotype [6]. Ultrarapid metabolizers (UMs) have more than 2 functional copies of the *CYP2D6* gene and exhibit extremely high enzyme activity.

The poor metabolizer phenotype is associated with an increased metoprolol plasma concentration and more intense and sustained receptor blockade [2,8]. If standard doses of metoprolol are used in poor metabolizers, these subjects may be susceptible to dose-dependent adverse drug reactions. Wuttke et al. found an increased frequency of CYP2D6 PMs in patients on metoprolol treatment with adverse drug reactions (ADRs): 9 out of 24 subjects with ADRs were PM (38%). In that study, the risk of bradycardia in PMs tended to be higher than in non-poor metabolizers [9]. However, the number of subjects in that study was small. Other studies showed no increased risk of adverse drug reactions or increased antihypertensive response to metoprolol [10-12]. Consequently, the clinical significance of impaired elimination of β -blockers is controversial and most studies suffer from small number of CYP2D6 PMs.

Therefore, the objective of this population-based cohort study was to examine the influence of the *CYP2D6**4 polymorphism on heart rate and blood pressure in patients treated with β -blockers.

Methods

Setting

This study was embedded in The Rotterdam Study, a prospective population-based cohort study among inhabitants of Ommoord, a suburb of Rotterdam [13,14]. Between 1990 and 1993, all 10275 persons aged 55 years or over were invited to participate. Of them, 7983 took part in the baseline examination, consisting of an extensive home interview followed by two visits to the research center. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993-1996, 1997-1999 and 2002-2004. In addition, the total cohort is continuously monitored for major morbidity and mortality through linkage with the records of the patient's general practitioner. All drug prescriptions dispensed to participants are available in computerized form as of January 1, 1991. The pharmacy data include the Anatomical Therapeutic Chemical (ATC)-code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Cohort definition

The study population consisted of all subjects in the Rotterdam Study for whom a blood sample was available and for whom the CYP2D6 genotype could be determined, and who had used a β -blocker on at least one out of 4 cross-sectional assessments: at baseline and three subsequent follow-up assessments. For every subject up to 4 ECG measurements and blood pressure measurements were available. Due to the suboptimal quality of long-term stored DNA samples, CYP2D6 genotype could not be assessed in a small number of patients.

Outcome assessment

At every visit to the research center, systolic and diastolic blood pressure were measured twice with a random-zero sphygmomanometer after a 5-min rest in a sitting position, and these values were averaged. A 12-lead resting electrocardiogram (ECG) with an ACTA electrocardiograph (Eseaeote, Florence, Italy) was also recorded. Heart rate was calculated from the mean RR-interval in the electrocardiogram, the time interval between consecutive heartbeats (calculated on an average of 10 consecutive heart beats). Bradycardia was defined as a heart rate lower than 60 beats/minute.

Exposure definition

At the baseline examination of the Rotterdam Study, blood was taken from which DNA was isolated. Genotyping for the CYP2D6*4 polymorphism (1846G>A) was performed by using

Tagman allelic discrimination assays on the ABI Prism 9700 HT Sequence detection system, as described earlier [15]. In short, 1 ng of genomic DNA was amplified in 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute.

Individuals were classified as PM if they were homozygous for the *4 allele. Heterozygous individuals were referred as *4 heterozygotes or *1/*4. All individuals without a *4 allele were considered to have the wild type allele (*1) and were subsequently classified as extensive metabolizers (EMs).

Current use of β -blockers was defined as use at the time of ECG or blood pressure measurement.

Covariates

The following covariates were considered as potential confounders: age, gender and β -blocker dose. β -blocker dose was calculated as the prescribed daily dose divided by the defined daily dose, according to the World Health Organization. Because *CYP2D6* genotype may be associated with the use of other antihypertensive drugs, we adjusted for the current use of miscellaneous antihypertensive drugs (ATC C02), diuretics (ATC C03), calcium channel blockers (ATC C08), ACE inhibitors and angiotensin II receptor antagonists (ATC C09). Co-administration of strong *CYP2D6* inhibitors (fluoxetine, paroxetine, bupropion, quinidine) or weak inhibitors (sertraline, duloxetine, terbinafine, cimetidine, amiodaron) at the time of ECG or blood pressure measurement was considered as potential confounder or effect modifier [3].

Statistical analysis

Genotype frequency was tested for deviations from Hardy-Weinberg equilibrium by using a χ^2 -test. We used repeated measurements analysis (linear mixed model) to analyze the association between *CYP2D6* genotype and heart rate, or blood pressure in β -blocker users. This model can adjust for the correlation between observations within the same person in addition to potential confounders. Bradycardia was analyzed likewise using the generalized estimating equation (GEE) method for discrete outcomes. Analyses were performed for β -blockers selectively metabolized by *CYP2D6*, β -blockers not selectively metabolized by *CYP2D6*, and separately for metoprolol and atenolol.

The analyses mentioned above were repeated in a subgroup of patients in whom ECGs with atrial fibrillation were excluded, since this condition could influence heart rate.

We performed a second longitudinal analysis by examining the within person change in heart rate or blood pressure between two consecutive measurements (t_{x-1} , t_x) with repeated measurements analysis. The difference in heart rate or blood pressure between two subsequent measurements was calculated for each person and compared between persons who started a β -blocker ($t_{x-1} = 0$, $t_x = 1$) or discontinued a β -blocker ($t_{x-1} = 1$, $t_x = 0$) and those who were non-users at both measurements ($t_{x-1} = 0$, $t_x = 0$).

Repeated measurements analysis was performed with SAS, version 8.2, using the Proc Mixed and Proc Genmod program.

Results

Characteristics of the study population are given in table 1. The percentage of β -blocker use at the time of blood pressure or ECG measurement changed over time from 10.5% at baseline to 23.6% in the last examination round. *CYP2D6*4* genotype in combination with information on heart rate and blood pressure was available in 1430 and 1533 subjects on β -blockers respectively. The most frequently used β -blockers were atenolol (n=625), metoprolol (n=513) and bisoprolol (n=156) during the total study period. The allele frequency of the *CYP2D6*4* allele was 20.7%. Genotype distributions significantly deviated from Hardy-Weinberg equilibrium ($p < 0.01$).

Table 1. Characteristics of the study population

	Baseline (1990-1993)	1st follow-up (1993-1996)	2nd follow-up (1997-1999)	3rd follow-up (2002-2004)
n	6348	5431	3874	2878
Mean age, years (SD)	69.5 (9.1)	70.4 (8.6)	72.8 (7.4)	75.6 (6.3)
Gender				
female	3763 (59.3%)	3190 (58.7%)	2241 (57.8%)	1670 (58.0%)
CYP2D6 genotype				
*1/*1	4082 (64.3%)	3504 (64.5%)	2499 (64.5%)	1840 (63.9%)
*1/*4	1897 (29.9%)	1610 (29.6%)	1152 (29.7%)	869 (30.2%)
*4/*4	369 (5.9%)	317 (5.8%)	223 (5.8%)	169 (5.9%)
β -blocker use (total) ^{a,b}	667 (10.5%)	735 (13.5%)	661 (17.1%)	680 (23.6%)
atenolol	307 (4.8%)	319 (5.9%)	252 (6.5%)	223 (7.7%)
metoprolol	199 (3.1%)	243 (4.5%)	205 (5.3%)	196 (6.8%)
bisoprolol	7 (0.1%)	16 (0.3%)	56 (1.4%)	116 (4.0%)
Mean β -blocker dose, DDD (SD)	0.70 (0.4)	0.67 (0.4)	0.61 (0.4)	0.60 (0.4)
Co-administration of CYP2D6 inhibitors ^d				
strong	1 (0.1%)	5 (0.7%)	4 (0.6%)	8 (1.1%)
weak	10 (1.5%)	19 (2.6%)	10 (1.5%)	8 (1.1%)
Co-administration ^c				
diuretics	169 (25.3%)	191 (26.0%)	161 (24.4%)	200 (29.4%)
calcium channel blockers	93 (13.9%)	126 (17.1%)	118 (17.9%)	141 (20.7%)
ACE inhibitors/ AT-II	54 (8.1%)	93 (12.7%)	133 (20.1%)	202 (29.7%)
other antihypertensives	13 (1.9%)	15 (2.0%)	8 (1.2%)	13 (1.9%)
Mean blood pressure (SD)	(n=6225)	(n=5037)	(n=3833)	(n=2864)
Diastolic BP (mmHg)	73.7 (11.5)	77.2 (11.8)	75.3 (11.3)	79.5 (11.5)
Systolic BP (mmHg)	139.3 (22.3)	141.7 (22.2)	143.5 (21.2)	153.0 (21.8)
Mean heart rate (SD) (beats/min)	(n=4716)	(n=4496)	(n=3491)	(n=2809)
	71.1 (12.6)	69.5 (12.1)	68.3 (11.8)	68.4 (11.7)

The characteristics of all individuals with information on CYP2D6 genotype available. ACE, angiotensin-converting enzyme; AT-II, angiotensin II receptor antagonist; DDD, defined daily dose

^a β -blocker use at the time of ECG or blood pressure measurement. In total 1533 subjects used a β -blocker at any time during the study period (1991-2004). ^b The absolute numbers of PMs using the frequently prescribed β -blockers atenolol and metoprolol at baseline ECG were 19 and 11, respectively; at 1st follow-up ECG 20 and 10, respectively; at 2nd follow-up ECG 12 and 10, respectively; at 3rd follow-up ECG 9 and 9, respectively. ^c during β -blocker use

Heart rate

There was no association between *CYP2D6* genotype and heart rate among non-users ($p=0.73$). The association between *CYP2D6* genotype and heart rate was most obvious in users of metoprolol and is given in figure 1a. *CYP2D6* poor metabolizers (*4/*4) had a significant lower heart rate than in EMs (*1/*1). In contrast, *CYP2D6* genotype had no effect on heart rate in atenolol users (figure 1b).

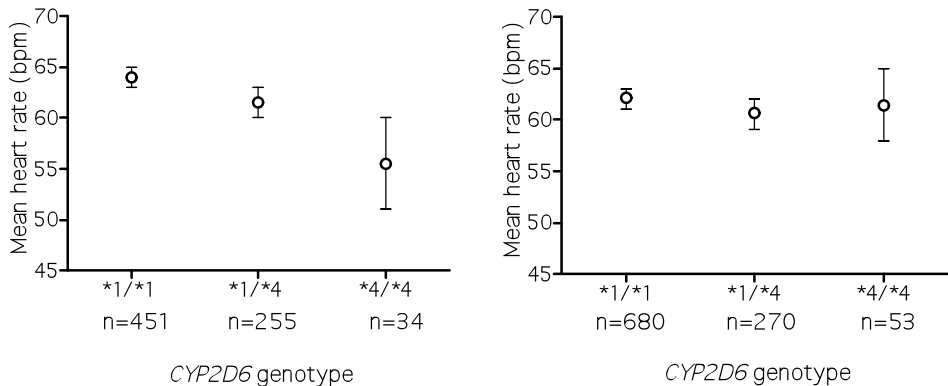


Figure 1. Association between *CYP2D6* genotype and heart rate in users of (a) metoprolol and (b) atenolol. The data are adjusted for age, gender, b-blocker dose and use of other antihypertensives

Using repeated measurements analysis, the adjusted heart rate in metoprolol users was 8.5 beats/min lower in PMs than in EMs ($p<0.0001$). In *4 heterozygotes the heart rate was 2.5 beats/min lower ($p=0.013$). The adjusted difference between PMs and EMs in atenolol users was 0.7 beats/min, but this difference was not statistically significant ($p=0.67$). These results are given in table 2.

The mean β -blocker dose was significantly lower in *CYP2D6* PMs (0.38 DDD) than in EMs (0.48 DDD) in users of β -blockers selectively metabolized by *CYP2D6* ($p=0.03$). The risk of bradycardia in metoprolol users was significantly higher in PMs than in EMs (OR= 3.86; 95% CI 1.68-8.86; $p=0.0014$). An effect of *CYP2D6* genotype in users of β -blockers selectively metabolized by *CYP2D6* (metoprolol, carvedilol, nebivolol, propranolol and alprenolol) was also observed, but the difference of -6.7 beats/min in PMs compared to EMs can largely be explained by the large amount of metoprolol users. No effect of *CYP2D6* genotype on heart rate was seen in users of β -blockers not selectively metabolized by *CYP2D6* (oxprenolol, pindolol, sotalol, penbutolol, atenolol, acebutolol, bevantolol, bisoprolol, celiprolol and labetalol). Within person changes in heart rate between two consecutive ECGs are given in table 3. The within person change in heart rate was significantly larger (7.3 beats/min; 95% CI 1.2 - 13.4; $p=0.019$) in *CYP2D6* PMs compared to EMs in persons starting metoprolol. In individuals with the *1/*4 genotype starting metoprolol, the within person change was 2.8 beats/min (CI 95% 5.8 - 0.11; $p=0.06$) lower than in EMs. No significant increase in heart rate was observed in persons discontinuing metoprolol between the different *CYP2D6* genotypes.

Table 2. Effect of CYP2D6 genotype on heart rate or blood pressure in users of b-blockers

Current use	n	Mean difference in heart rate ^a (95% CI)	p-value	n	Mean difference in DBP ^a	p-value
β-blockers selectively metabolized by CYP2D6 ^b						
*1/*1	515	ref		578		
*1/*4	282	-2.40 (-4.17;-0.58)	0.009	305	-0.27 (-2.29;1.76)	0.796
*4/*4	43	-6.72 (-10.50;-2.94)	0.001	45	-5.39 (-9.90;-0.99)	0.017
Metoprolol						
*1/*1	451	ref		496		
*1/*4	255	-2.49 (-4.43;-0.55)	0.012	276	-0.37 (-2.50;1.75)	0.730
*4/*4	34	-8.53 (-12.77;-4.29)	<0.001	37	-4.77 (-9.43;-0.11)	0.045
β-blockers not metabolized by CYP2D6 ^c						
*1/*1	982	ref		1048	ref	
*1/*4	384	-1.39 (-2.94;0.16)	0.079	422	0.11 (-1.32;1.55)	0.880
*4/*4	79	-0.82 (-3.83;2.19)	0.594	85	0.52 (-2.28;3.32)	0.716
Atenolol						
*1/*1	680	ref		716		
*1/*4	270	-1.50 (-3.17;0.18)	0.080	295	-0.49 (-2.18;1.20)	0.567
*4/*4	53	-0.71 (-3.98;2.57)	0.672	57	0.92 (-2.39;4.23)	0.586

The mean difference in heart rate (beats/min) or blood pressure (mmHg) is calculated by repeated measurements analysis.

DBP, diastolic blood pressure; ref, reference group

^a adjusted for age, gender, β-blocker dose, use of diuretics, calcium channel blockers, ACE inhibitors and angiotensin II receptor antagonists and other antihypertensive drugs. ^b β-blockers selectively metabolized by CYP2D6 are metoprolol, carvedilol, nebivolol, propranolol and alprenolol. ^c β-blockers not metabolized by CYP2D6 are oxprenolol, pindolol, sotalol, penbutolol, atenolol, acebutolol, bevantolol, bisoprolol, celiprolol and labetalol

Table 3. Effect of CYP2D6 genotype on within person change in heart rate in persons starting or discontinuing metoprolol

CYP2D6 genotype	Start metoprolol therapy ($t_{x-1}=0$ and $t_x=1$)			Discontinuation of metoprolol therapy ($t_{x-1}=1$ and $t_x=0$)		
	n	Difference in heart rate ^a (beats/min)	p-value	n	Difference in heart rate ^a (beats/min)	p-value
*1/*1	172	ref		91		
*1/*4	72	-2.84 (-5.80;0.12)	0.06	49	1.71 (-6.35;9.78)	0.68
*4/*4	12	-7.32 (-13.43;-1.21)	0.02	7	-3.08 (-6.74;0.57)	0.10

^a adjusted for age and gender

Subgroup analyses

We repeated the analyses after exclusion of subjects with atrial fibrillation. The exclusion of, in total 113 subjects using β -blockers, had only a minimal effect on the outcomes. After exclusion of the atrial fibrillation patients there were 406 EMs, 249 heterozygous patients and 29 PMs using metoprolol left. The adjusted heart rate in metoprolol users was 7.1 beats/min lower in PMs than in EMs ($p < 0.0014$). In *4 heterozygotes the heart rate was 2.3 beats/min lower ($p = 0.017$). PMs had an increased risk of bradycardia (OR = 2.94; $p = 0.013$) compared to EMs. In users of β -blockers selectively metabolized by CYP2D6 the effect of CYP2D6 genotype on heart rate was also minimally influenced by the exclusion of atrial fibrillation (data not shown).

Blood pressure

There was no association between CYP2D6 genotype and systolic or diastolic blood pressure among non-users ($p = 0.73$, and $p = 0.67$ respectively). There was no influence of the CYP2D6*4 polymorphism on systolic blood pressure, but the diastolic blood pressure in PMs was 4.8 mmHg lower in metoprolol users ($p = 0.045$) and 5.4 mmHg lower in users of β -blockers selectively metabolized by CYP2D6 ($p = 0.017$) than in EMs (table 2). The diastolic blood pressure in individuals with the *1/*4 genotype did not differ significantly from EMs. No association between CYP2D6 genotype and blood pressure was observed in β -blockers not selectively metabolized by CYP2D6 including atenolol.

In table 4 the within person change of diastolic blood pressure is presented. The within person change was 4.9 mmHg lower (CI 95% 10.7-0.9; $p = 0.10$) in CYP2D6 PMs than EMs in persons starting metoprolol.

Table 4. Effect of CYP2D6 genotype on within person change in diastolic blood pressure (DBP) in persons starting or discontinuing metoprolol

	Start metoprolol therapy ($t_{x-1}=0$ and $t_x=1$)			Discontinuation of metoprolol therapy ($t_{x-1}=1$ and $t_x=0$)		
	<i>n</i>	Difference in DBP ^a (mmHg)	<i>p</i> -value	<i>n</i>	Difference in DBP ^a (mmHg)	<i>p</i> -value
CYP2D6 genotype						
*1/*1	211	ref		102		
*1/*4	92	-0.91 (-3.60;1.79)	0.51	52	4.52 (-3.85;12.89)	0.29
*4/*4	14	-4.87 (-10.68;0.95)	0.10	7	-1.39 (-5.09;2.31)	0.46

^a adjusted for age and gender

Discussion

The main finding in this cohort study is that metoprolol users homozygous for the CYP2D6*4 allele had a significantly lower heart rate and diastolic blood pressure than users with the wild type genotype. These effects were also observed for β -blockers selectively metabolized

by CYP2D6 (metoprolol, carvedilol, nebivolol, propranolol and alprenolol), but the differences can largely be explained by the large proportion of metoprolol users in this group. In users of atenolol and other β -blockers not metabolized by CYP2D6 no association between CYP2D6 genotype and heart rate or blood pressure was observed. This was expected since atenolol is predominantly excreted by the kidneys. Our results correspond with those of Kirchheiner et al., in which a significant effect on resting heart rate was found. However, no association of CYP2D6 genotype with systolic and diastolic blood pressure was found in that study [16].

In our study, the diastolic blood pressure in PMs was approximately 5 mmHg lower than in EMs using β -blockers selectively metabolized by CYP2D6. This difference in blood pressure was adjusted for β -blocker dose and use of other antihypertensive drugs. The clinical relevance of this result is unclear. In the estimation of the 10-year risk of fatal cardiovascular disease, the systolic blood pressure rather than the diastolic blood pressure is taken into account in addition to other predictors (cholesterol, age, gender) [17]. In a recent Cochrane review, β -blockers were less efficacious in lowering blood pressure than other blood pressure lowering drugs [18]. Reduction in heart rate is associated with a lower risk on cardiovascular morbidity and mortality [19]. Beta blockers reduce the risk on mortality partly due to their effect on heart rate. On the other hand, heart rate is strongly related to sudden cardiac death due to arrhythmias. In our study the risk of bradycardia in metoprolol users is almost 4-times higher in PMs than in EMs. Bradycardia is a known adverse drug reaction of β -blockers and is dose-dependent. Bradycardia could be life-threatening and could be avoided in poor metabolizers by reducing the dose. Patients with the *1/*4 genotype did have a lower heart rate, but no increased risk of bradycardia. Heterozygous *4 individuals have in contrast to PMs, in which CYP2D6 enzyme activity is absent, decreased enzyme activity. The literature describes that plasma concentrations of metoprolol are somewhat higher in heterozygous persons than in EMs, suggesting an allele-dose effect [11,20]. In contrast to PMs, ultrarapid metabolizers may show limited response to β -blockers metabolized by CYP2D6. The clearance of metoprolol is increased, leading to lower plasma concentrations of metoprolol [16]. However, we did not determine multiple copies of functional alleles in our study, since the frequency of CYP2D6 ultrarapid metabolizers is expected to be low (1-2%) in the Dutch population resulting in limited statistical power [7].

A PM genotype increases the possibility to develop adverse drug reactions. In our study subjects who experienced severe adverse drug reactions and therefore immediately discontinued their pharmacotherapy were probably missed. Thus, our results are likely to be an underestimation of the real effect.

In population-based studies, bias may affect the results. Selection bias was probably negligible, because all β -blocker users at the time of ECG or blood pressure measurements were selected independently of CYP2D6 metabolizer status in a large cohort study. Information bias was unlikely, since data on genotype, ECG, blood pressure and prescription data were gathered pro-

spectively. We adjusted for the possible confounders age, gender, β -blocker dose, use of other antihypertensive drugs, diuretics, calcium channel blockers, ACE inhibitors and angiotensin II receptor antagonists in the repeated measurements analyses. The dose is titrated on antihypertensive effect and is dependent on *CYP2D6* genotype (PMs require lower doses). Apparently, in practice titrating is not performed properly, since PMs had a lower heart rate and an increased risk of bradycardia after adjusting for β -blocker dose. Use of other antihypertensive drugs due to a suboptimal antihypertensive effect is probably more likely in EMs. Co-administration of strong and weak *CYP2D6* inhibitors did not influence our results, but concurrent use occurred sporadically. Compliance of β -blockers was assessed in the Rotterdam study. Cardiovascular drug use, as presented during patient interview, was compared with the computerized pharmacy medication history. The highest agreement was observed in β -blockers (94%), especially for atenolol and metoprolol [21].

In our study, we only determined the *CYP2D6**4 polymorphism, since this polymorphism should predict >75% of PMs in a Caucasian population [7]. Other less frequent genetic variants (*3, *5, *6, *8, *10, *41) were not assessed, which led to the inclusion of some intermediate metabolizers, poor metabolizers and ultrarapid metabolizers in the reference group (EM). However, we believe that these misclassifications would tend to underestimate, rather than inflate our risk estimates. The frequency of the *4 allele (20.7%) corresponded with the literature [7], but our population was not in Hardy-Weinberg equilibrium. The genotyping assay was validated by DNA sequencing, but was unable to deal with the gene deletion *CYP2D6**5: *4/*5 subjects were also classified as *CYP2D6**4/*4. Within a random sample of 500 participants *CYP2D6**5 was determined in the *4/*4 individuals. When the results of *4 and *5 were combined, the genotype frequencies were in HWE. However, this did not affect a subject's phenotype since both *4/*5 as *4/*4 are classified as PMs.

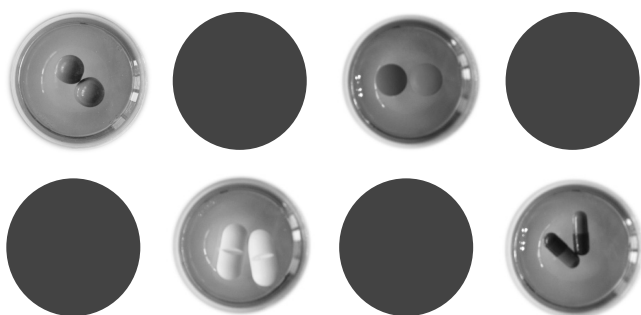
The strengths of this study are the high number of *CYP2D6* PMs and the availability of up to four measurements per subject, allowing us to analyze between and within person changes in efficacy. In cross-sectional studies the relationship between determinant and outcome are measured at the same point in time, which makes it difficult to distinguish whether the determinant preceded or followed the outcome difficult. To exclude this phenomenon we also conducted an analysis based on within person change.

In conclusion, this study is the first large population-based study assessing the influence of the *CYP2D6**4 polymorphism on heart rate and blood pressure in users of β -blockers. Our data demonstrate that *CYP2D6* PMs have a lower heart rate and diastolic blood pressure when using metoprolol. These patients should be carefully monitored in clinical practice, since they have an increased risk of bradycardia.

References

1. Regardh CG, Johnsson G. Clinical pharmacokinetics of metoprolol. *Clin Pharmacokinet* 1980;5(6):557-69.
2. Lennard MS, Silas JH, Freestone S, Ramsay LE, Tucker GT, Woods HF. Oxidation phenotype--a major determinant of metoprolol metabolism and response. *N Engl J Med* 1982;307(25):1558-60.
3. Flockhart D. Drug Interactions: Cytochrome P450 Drug Interaction Table. <http://medicineiupui.edu/flockhart/table.htm> 2007
4. Wuttke H, Rau T, Eschenhagen T. Genetic polymorphisms in drug metabolizing enzymes--impact on treatment with beta-blockers. *Genpolymorphismen in Arzneimittel-abbauenden Enzymen. Bedeutung fur die Therapie mit beta-Blockern. Dtsch Med Wochenschr* 2004;129(15):831-5.
5. Lennard MS, Tucker GT, Silas JH, Woods HF. Debrisoquine polymorphism and the metabolism and action of metoprolol, timolol, propranolol and atenolol. *Xenobiotica* 1986;16(5):435-47.
6. Gaedigk A, Simon S, Pearce R, Bradford L, Kennedy M, Leeder J. The CYP2D6 Activity Score: Translating Genotype Information into a Qualitative Measure of Phenotype. *Mol Ther* 2008;83(2):234-42.
7. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3(2):229-43.
8. Lennard MS, Tucker GT, Woods HF. The polymorphic oxidation of beta-adrenoceptor antagonists. Clinical pharmacokinetic considerations. *Clin Pharmacokinet* 1986;11(1):1-17.
9. Wuttke H, Rau T, Heide R, Bergmann K, Bohm M, Weil J, et al. Increased frequency of cytochrome P450 2D6 poor metabolizers among patients with metoprolol-associated adverse effects. *Clin Pharmacol Ther* 2002;72(4):429-37.
10. Clark DW, Morgan AK, Waal-Manning H. Adverse effects from metoprolol are not generally associated with oxidation status. *Br J Clin Pharmacol* 1984;18(6):965-7.
11. Zineh I, Beitelshes AL, Gaedigk A, Walker JR, Pauly DF, Eberst K, et al. Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. *Clin Pharmacol Ther* 2004;76(6):536-44.
12. Fux R, Morike K, Prohmer AM, Delabar U, Schwab M, Schaeffeler E, et al. Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. *Clin Pharmacol Ther* 2005;78(4):378-87.
13. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7(4):403-22.
14. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, Stricker BH, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
15. Bijl MJ, Visser LE, Hofman A, Vulto AG, van Gelder T, Stricker BH, et al. Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *Br J Clin Pharmacol* 2008;65(4):558-64.
16. Kirchheiner J, Heesch C, Bauer S, Meisel C, Seringer A, Goldammer M, et al. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2004;76(4):302-12.
17. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;24(11):987-1003.
18. Wiysonge CS, Bradley H, Mayosi BM, Maroney R, Mbewu A, Opie LH, et al. Beta-blockers for hypertension. *Cochrane Database Syst Rev* 2007(1):CD002003.
19. Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, et al. Resting heart rate in cardiovascular disease. *J Am Coll Cardiol* 2007;50(9):823-30.
20. Rau T, Heide R, Bergmann K, Wuttke H, Werner U, Feifel N, et al. Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. *Pharmacogenetics* 2002;12(6):465-72.

21. Sjahid SI, van der Linden PD, Stricker BH. Agreement between the pharmacy medication history and patient interview for cardiovascular drugs: the Rotterdam elderly study. *Br J Clin Pharmacol* 1998;45: 591-595.



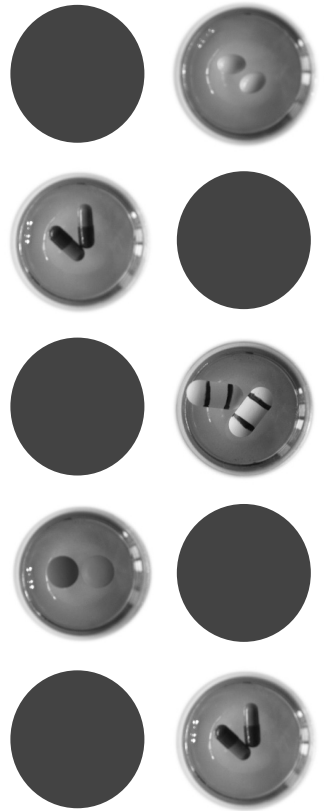
The *CYP2D6**4 polymorphism
and codeine

CHAPTER 4

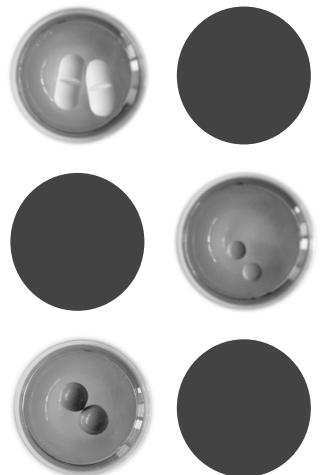
**The influence of CYP2D6,
CYP3A4/3A5 and UGT2B7
genetic polymorphisms on
prescription of other analgesics
in codeine users**

Monique J. Bijl, Loes E. Visser, Ron H.N.
van Schaik, Mark Eijgelsheim, Albert
Hofman, Andre G. Uitterlinden, Arnold G.
Vulto, Teun van Gelder, Bruno H.Ch. Stricker

Submitted



CHAPTER 4



Abstract

Background: It is widely accepted that the analgesic effect of codeine is mostly dependent on its metabolism to morphine by cytochrome P450 2D6 (CYP2D6). Besides metabolism by CYP2D6, codeine is metabolized by CYP3A4 to norcodeine or glucuronidated by UGT2B7. We studied the influence of the most common variant alleles in the *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* gene on codeine effectiveness.

Methods: The study cohort consisted of all incident codeine users in the Rotterdam Study, a population-based cohort study. Logistic regression analysis was performed to study the association between the most common variant alleles in Caucasians *CYP2D6*4*, *CYP3A4*1B*, *CYP3A5*3C*, *UGT2B7*2* and switching to an opioid within 90 days after start of codeine. We compared the number of prescriptions of other analgesics during this period per genotype using multivariate linear regression analysis.

Results: There was no significantly increased risk of switching to an opioid in individuals with the *CYP2D6 *4/*4* genotype compared with the wild type (OR=1.44; CI 95% 0.53-3.90;p=0.48). In individuals carrying a *CYP3A4*1B* allele the risk of switching was increased (OR=3.08; CI 95% 1.47-6.42;p=0.003), and these individuals required more co-prescription of other analgesics after start of codeine use than non-carriers.

Conclusion: Patients carrying a *CYP3A4*1B* allele more often switch to an opioid, and have more co-prescription of other analgesics than non-carriers. Interestingly, a decreased effectiveness of codeine was not observed in patients with the *CYP2D6*4/*4* genotype, while morphine concentrations are expected to be low in these subjects.

Introduction

Codeine is a weak analgesic which is structurally related to morphine and is often used in the treatment of mild and moderate pain. It is widely accepted that the analgesic effect of codeine is mostly dependent on its metabolism to morphine, which has an affinity to the μ -opioid receptor that is approximately 200 times greater than the parent compound [1,2]. However, some researchers argue that other metabolites may contribute to the analgesic properties of codeine as well [3,4], since only 5% of codeine is O-demethylated to morphine by CYP2D6 [5]. Codeine is mainly metabolized (~70-80%) to codeine-6-glucuronide by UGT2B7 [6], whereas 15% is metabolized to norcodeine by CYP3A4 [7]. The metabolism of codeine is schematically represented in figure 1.

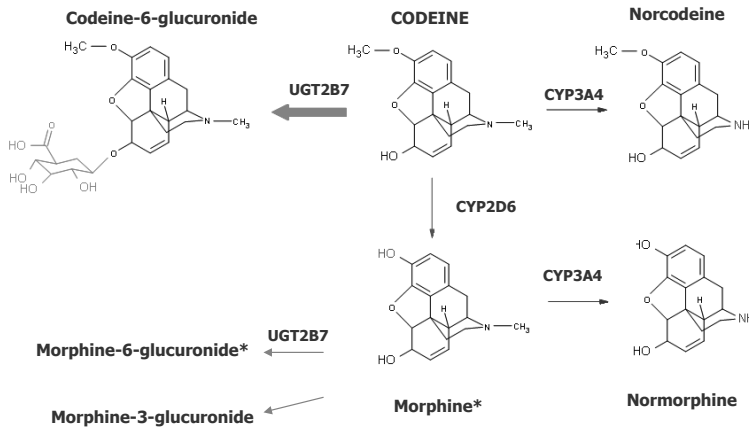


Figure 1. The metabolism of codeine.

Codeine is mainly metabolized to codeine-6-glucuronide by glucuronidation (70-80%) (UGT2B7) and 15% is metabolized by CYP3A4 to norcodeine. Only 5-10% of codeine is converted to morphine by CYP2D6. Norcodeine is further glucuronidated to norcodeine-6-glucuronide. Morphine is further metabolized into morphine-6-glucuronide and morphine-3-glucuronide. *Metabolites considered to possess analgesic potency are morphine and morphine-6-glucuronide.

In CYP2D6 poor metabolizers, patients lacking CYP2D6 enzyme activity, serum concentrations of morphine and morphine-6-glucuronide were extremely low or below the limit of detection [8,9], leading to decreased efficacy of codeine in experimental pain models [9,10]. Several case reports show severe opioid intoxication in ultra-rapid metabolizers of CYP2D6 after administration of codeine. In a 62-year-old man, coma and respiratory depression was seen after repeated small doses of codeine for the treatment of a cough. This patient was an ultra-rapid metabolizer of CYP2D6 and additionally had decreased CYP3A4 activity due to co-medication of a CYP3A4 inhibitor, which augmented the morphine accumulation [11]. Ultra-rapid metabolizers have >2 functional copies of the *CYP2D6* gene and exhibit extremely high enzyme activity. Individuals car-

rying this genotype are found in 1-2% of the northern European population. In contrast, 5-10% of Caucasians lack CYP2D6 enzyme activity due to inheritance of two nonfunctional alleles of the *CYP2D6* gene (e.g. *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*). These individuals are classified as poor metabolizers (PMs). *CYP2D6*4* is the most common variant allele (allele frequency of 20%) leading to the PM phenotype in Caucasians; over 75% of the PMs are carriers of this polymorphism [12]. The phenotype of heterozygous carriers of *CYP2D6* polymorphisms shows large variability and can be classified as intermediate metabolizer (IM) or heterozygous EM [13].

CYP3A4 is responsible for most CYP3A-mediated drug metabolism, but CYP3A5, CYP3A7 and CYP3A43 also contribute to the total CYP3A activity [14,15]. CYP3A4 and CYP3A5 have overlapping substrate specificity. It is suggested that the metabolic activity of CYP3A5, when optimally expressed, is similar to that of CYP3A4 for many drugs [15]. High interindividual variability in CYP3A activity exists and can be the result of both genetic and environmental factors. Many polymorphisms in the *CYP3A4* gene have been identified, but the clinical impact on drug metabolism is unclear, mainly because of the very low frequency of these variant alleles. The *CYP3A4*1B* variant allele has been associated with a moderately increased CYP3A4 expression [16]. *CYP3A5*3* is the most common nonfunctional allele in Caucasians (allele frequency of 90%) leading to decreased CYP3A5 activity [17]. In livers expressing CYP3A5, this isoform contributes at least 20% of the total CYP3A activity [14]. The CYP3A7 isoform is predominately found in fetal livers. The expression of CYP3A7 in adult livers is thought to be negligible in comparison to CYP3A4 and CYP3A5 content [15]. CYP3A43 expression in liver is also low. Both CYP3A7 and CYP3A43 are probably more interesting from the physiological point of view than for their influence on drug metabolism [14].

In the *UGT2B7* gene a cytosine to thymine polymorphism (*UGT2B7*2*) at base pair 802 (802 C>T) has been identified [18]. This polymorphism leads to a histidine to tyrosine amino-acid substitution, but does not seem to influence UGT2B7 enzyme activity in vitro [19]. The clinical impact of the *UGT2B7*2* polymorphism is still unclear. In a study of Sawyer et al. morphine-6-glucuronide and morphine-3-glucuronide concentrations were significantly lower in C/C patients compared with C/T and T/T patients combined [20]. In another study no variation in morphine-3-glucuronide/morphine-6-glucuronide, morphine-3-glucuronide/ morphine and morphine-6-glucuronide/ morphine ratios was found [21].

Most studies concentrated on CYP2D6 in relation to codeine efficacy and adverse drug reactions [10,22,23], but these studies were based on a small number of CYP2D6 PMs. To our knowledge, no previous study examined genetic variation in the *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* gene in relation to codeine effectiveness in a large population-based cohort study.

Therefore, we investigated the influence of common genetic variation in these genes on codeine effectiveness by studying co-prescription and switching to other analgesics in a population-based cohort study.

Methods

Setting

We conducted this study within the Rotterdam Study, a population-based cohort study among 7,983 persons aged ≥ 55 years in Ommoord, a district of Rotterdam [24,25]. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. All participants were interviewed and investigated at baseline. Blood samples were obtained from which DNA was isolated. Since the start of the study, participants have been re-examined periodically. In addition, the cohort is continuously monitored for major morbidity and mortality through linkage with the medical records from general practitioners. Information on medication use for all participants is available since January 1991. The seven computerized pharmacies cover the research area and provide information on the drug dispensed (Anatomical Therapeutic Chemical (ATC)-code), dispensing date, the total amount of drug units per prescription and the prescribed daily number of units of the drugs.

Cohort and outcome definition

The study cohort consisted of all subjects in the Rotterdam Study, who received a first prescription of codeine as analgesic formulation 'paracetamol/codeine' (N02AA59) between April 1st 1991 and December 31st 2007 and for whom a DNA sample for genotyping was available. Subjects who used codeine as antitussive (R05DA04, i.e. prescriptions without paracetamol) were not included. No other codeine-containing products were filled in the Rotterdam Study cohort during the study period.

In this study two different outcomes were studied. The first outcome was switching, defined as a switch to an opioid (N02A), irrespective of class, within 90 days after the start of the first prescription of codeine. A switch within 90 days is assumed to occur due to incomplete response to the drug, since prescription of a strong opioid is the next step on the pain relief ladder according to the World Health Organization. A 90-day period was chosen, since it is the maximum duration of a prescription in the Netherlands.

As a second outcome, we studied the number of prescriptions of other analgesics: NSAIDs (M01A), opioids (N02A) and other (N02B) in the 90-days period before and after the first codeine prescription. Additionally, the influence on mean codeine dose was analyzed.

Covariates

As potential confounders we considered: age, gender and codeine dose. Codeine dose was calculated as the prescribed daily dose divided by the defined daily dose (DDD) representing the recommended daily dose for an adult of 70 kg for the main indication according to the World Health Organization [26]. Coadministration of strong CYP3A4 inhibitors (claritromycine, indinavir, itraconazol, nelfinavir, ritonavir, saquinavir), weak inhibitors (aprepitant, cimetidine, diltia-

zem, erythromycine, fluconazol and verapamil) or CYP3A4 inducers (rifampicine, carbamazepine) at the time of codeine use was considered as potential confounder or effect modifier [27].

Genotyping

Genotyping of the *CYP2D6*4*, *CYP3A4*1B* and *CYP3A5*3C* polymorphisms was performed on an ABI Prism 9700 HT sequence detection system using Taqman allelic discrimination assays. Primers and probes were designed by Applied Biosystems by their Assay-by-Design service. Polymerase chain reactions (PCR) were performed in a reaction volume of 2.0 μ l, containing assay-specific primers, allele-specific Taqman MGB probes, Abgene Absolute QPCR Rox Mix and genomic DNA (1 ng). The thermal profile consists of an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute. Genotypes were scored by measuring allele-specific fluorescence using the SDS 2.2.2 software for allelic discrimination (Applied Biosystems). Patients in whom *CYP2D6*4*, *CYP3A4*1B* or *CYP3A5*3* were absent, were regarded as wild type (**1/*1*).

For determination of *UGT2B7*2*, microarray genotyping was performed in the entire original Rotterdam Study cohort with proper quality DNA samples using the Infinium II HumanHap550K Genotyping BeadChip® version 3 (Illumina, Inc, San Diego, CA). Genotyping procedures were followed according to Illumina manufacturer's protocols. Microarray genotyping procedures in the Rotterdam Study haven been described previously [28]. All SNPs present within the UGT2B7 gene area (Chromosome 4; base pairs 69,997 to 70,013; +/- 20kb) were extracted (n=6). Markers were excluded if they deviated significantly from Hardy – Weinberg equilibrium ($p < 1 \times 10^{-4}$), if they had a minor allele frequency <5%, or if they had a SNP call rate <95% within the samples. SNPs rs 7662029 and rs 4274916 were in complete linkage disequilibrium with *UGT2B7*2* ($r^2=1.00$, $D'=1.00$) in the HapMap central European reference population (CEU-CEPH) [29]. For further analyses rs 4274916 was used as a proxy for *UGT2B7*2*.

Statistical analysis

Genotype frequency was tested for deviations from Hardy-Weinberg equilibrium by using a χ^2 –test. Binary logistic regression analysis was used to analyze the association between genotype and switching to an opioid. A multivariate linear regression model was used to analyze the association between the mean number of prescriptions of other analgesics besides codeine and genetic variation in the *CYP2D6*, *CYP3A4*, *CYP3A5* and *UGT2B7* gene, and to analyze the difference in the number of prescriptions in the period of 90 days before and after codeine start between the different genotypes. To adjust for potential confounding, covariates were included in the model, in addition to age and gender, if they changed the point estimate by more than 5% upon inclusion in the model. To compare the mean codeine dose between the different genotypes, independent sample t-test were used at consecutive prescriptions. In case of missing genotypes due to genotyping difficulties, analyses were run as complete case

analyses. We assessed the effect per genotype in the model. Effect modification by genotype or cytochrome P450 3A4 enzyme inhibitors or inducers was studied with interaction terms and stratification. Logistic regression analyses, multivariate regression analyses, χ^2 -test and t-test were performed with SPSS for Windows, version 15.0.

Results

The baseline characteristics of the study population are shown in table 1, including genotype distributions of *CYP2D6*4*, *CYP3A4*1B*, *CYP3A5*3* and rs 4274916 (as a proxy for *UGT2B7*2*). The allele frequencies of *CYP2D6*4*, *CYP3A4*1B*, *CYP3A5*3* and *UGT2B7*2* were 20.1%, 3.9%, 92.0% and 45.1% respectively. Genotype frequencies did not significantly deviate from Hardy-Weinberg equilibrium, except for *CYP2D6*4* ($p=0.03$). There were 432 patients (65.2%) with the *CYP2D6 *1/*1* genotype (EM), 195 patients (28.5%) were heterozygous (IM), and 36 patients (5.4%) were homozygous for the *4 allele (PM).

Table 1. Characteristics of the study population

Variable	
n	675
Age at start codeine use, mean (SD)	75.8 (7.8) years
Gender	
Male	223 (33.0 %)
Female	452 (67.0 %)
Codeine starting dose, mean (SD)	27.0 (14.9) mg
CYP2D6 genotype*	
*1/*1	432 (65.2 %)
*1/*4	195 (29.4 %)
*4/*4	36 (5.4 %)
CYP3A4 genotype*	
*1A/*1A	599 (92.2 %)
*1A/*1B	51 (7.8 %)
*1B/*1B	0
CYP3A5 genotype*	
*1/*1	2 (0.3 %)
*1/*3C	99 (15.4 %)
*3C/*3C	543 (84.3 %)
UGT2B7 genotype*	
(rs 4274916)	
CC	192 (30.4 %)
CT	308 (48.8 %)
TT	131 (20.8 %)

* Hardy Weinberg equilibrium for *CYP2D6*4*, *CYP3A4*1B*, *CYP3A5*3* and *UGT2B7*2* respectively; $\chi^2 = 4.85$ ($p=0.028$), $\chi^2 = 1.10$ ($p=0.30$), $\chi^2 = 1.26$ ($p=0.26$) and $\chi^2 = 0.08$ ($p=0.78$)

There was no significant association between codeine mean dose and *CYP2D6*, *CYP3A4*, *CYP3A5* and *UGT2B7* genotype at the first ten consecutive prescriptions of codeine. Table 2

shows the association between *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* genotype and switching to an opioid. There was no increased risk of switching to an opioid within 90 days in *CYP2D6* PMs compared with EMs (OR=1.44; CI 95% 0.53-3.90). In heterozygous individuals carrying the *CYP3A4*1B* allele there was an increased risk with an odds ratio of 3.08 of switching to an opioid (CI 95% 1.47-6.42), while there were no homozygous study participants. *CYP3A5*3* had no influence on the risk of switching to an opioid in codeine users ($p=0.22$). There was also no association between rs 4274916 (*UGT2B7*2*) and switching to an opioid.

The influence of genetic variation on co-prescription of other analgesics is given in table 3. The mean number of prescriptions of other analgesics in the period of 90 days after starting codeine in *CYP2D6* PMs was not significantly different from EMs (1.54 versus 1.42; $p=0.740$). There was no difference in the number of prescriptions in the period of 90 days before and after codeine start between the different *CYP2D6* genotypes.

In contrast, codeine users with the *CYP3A4*1B* allele had more prescriptions of other analgesics than non-carriers (2.32 versus 1.31; $p=0.001$) and the difference in the number of prescriptions in the period of 90 days before and after codeine start was significantly higher in *CYP3A4*1B* carriers ($p=0.004$). In users with active *CYP3A5* (*CYP3A5*1/*3* plus **1/*1*) the mean number of prescriptions was higher than in codeine users homozygous for the **3* allele (1.76 versus 1.32; $p=0.051$), but this difference was borderline significant. The difference in prescriptions before and after the start of codeine was not statistically significant between the different genotypes ($p=0.12$). No association was found between rs 4274916 (*UGT2B7*2*) and the number of prescriptions of other analgesics.

There was no significant interaction between the *CYP2D6*, *CYP3A4*, *CYP3A5* and *UGT2B7* genotypes. Co-administration of *CYP3A4* inhibitors occurred infrequently: 14 patients used a strong *CYP3A4* inhibitor (claritromycine) and 20 patients used a weak inhibitor (verapamil, diltiazem, cimetidine, fluconazol and erytromycine). Only 7 patients used carbamazepine, an inducer of *CYP3A4* activity. Neither use of *CYP3A4* inhibitors nor inducers modified the results, but the number of patients was small.

Discussion

The main finding of this population-based cohort study is that in patients carrying a *CYP3A4*1B* allele the risk of switching to an opioid is increased and *CYP3A4*1B* carriers required more co-prescription of other analgesics after start of codeine use. Interestingly, this decreased effectiveness of codeine was not observed in patients with the *CYP2D6*4/*4* genotype, while morphine and morphine-6-glucuronide concentrations are expected to be low in these subjects. The finding that codeine is less active in patients with enhanced *CYP3A4* activity is supported by other studies. Rifampicin, a *CYP3A4* inducer, enhanced N-demethylation in users of codeine,

Table 2. The association between CYP2D6, CYP3A4/3A5 and UGT2B7 genotype and switching to an opioid.

Genotype	Number of switchers	ORadjusted (95% CI)	Sign. (p)
<i>CYP2D6</i>			
*1/*1	44	Reference	
*1/*4	16	0.80 (0.44-1.48)	0.47
*4/*4	5	1.44 (0.53-3.90)	0.48
<i>CYP3A4</i>			
*1A/*1A	51	Reference	
*1A/*1B	11	3.08 (1.47-6.42)	0.003
*1B/*1B	-	-	-
<i>CYP3A5</i>			
*3/*3	48	Reference	
*1/*3 + *1/*1	13	1.51 (0.79-2.92)	0.22
<i>UGT2B7</i> rs4274916			
CC	18	Reference	
CT	33	1.16 (0.63-2.13)	0.63
TT	13	1.04 (0.49-2.21)	0.92

* Adjusted for age and gender.

Table 3. The association between CYP2D6, CYP3A4/3A5 and UGT2B7 genotype and co-prescription of other analgesics

Genotype	Mean number of prescriptions*	Sign. (p)	Difference in number of prescriptions [^]	Sign. (p)
<i>CYP2D6</i>				
*1/*1	1.42	ref	ref	
*1/*4	1.35	0.703	-0.125	0.516
*4/*4	1.54	0.740	0.429	0.266
<i>CYP3A4</i>				
*1A/*1A	1.31	ref	ref	
*1A/*1B	2.32	0.001	0.933	0.004
*1B/*1B	-	-	-	-
<i>CYP3A5</i>				
*3/*3	1.32	ref	ref	
*1/*3 + *1/*1	1.76	0.051	0.377	0.119
<i>UGT2B7</i> rs4274916				
CC	1.28	ref	ref	
CT	1.45	0.401	0.169	0.406
TT	1.55	0.275	0.206	0.412

* Mean number of prescriptions of analgesics in the period of 90 days after codeine start calculated with multivariate linear regression; adjusted for age and gender.

[^] Difference in the number of prescriptions in the period of 90 days before and after codeine start between the different genotypes; adjusted for age and gender.

leading to lower plasma concentrations of codeine, codeine-6-glucuronide, morphine, morphine-3-glucuronide and morphine-6-glucuronide [30]. In another study, coadministration of rifampicin led to a decreased analgesic effect of morphine [31].

The effect of the *CYP3A4*1B* polymorphism on CYP3A activity is controversial. Amirimani et al. found a 1.2 to 1.9-fold higher luciferase activity for the *CYP3A4*1B* construct in different cell lines than the *CYP3A4* wild type promoter, suggesting that *CYP3A4*1B* is associated with enhanced *CYP3A4* expression [16]. However, other studies claimed no or decreased *CYP3A4* expression in relation to this polymorphism [32,33]. Furthermore, no influence of *CYP3A4*1B* on the pharmacokinetics of dextromethorphan N-demethylation was found [32]. Although no clear effect on *CYP3A4* expression exists, clinical data indicate that there is an association between the *CYP3A4*1B* allele and treatment tolerability [15]. In contrast to *CYP3A4*, *CYP3A5* is known to be expressed in only a small percentage of Caucasians due to the common *CYP3A5*3* polymorphism, which leads to deficient enzyme activity. The frequency of the *CYP3A5*3* allele in our study (92.0%) was in accordance with the literature [17]. *CYP3A4* is responsible for most CYP3A mediated drug metabolism, but the contribution of *CYP3A5* to CYP3A activity is estimated to be at least 20% [14]. The influence of *CYP3A5* genotype on the number of prescriptions of other analgesics was almost significant. This could be due to a contribution of *CYP3A5* to the metabolism of codeine or due to a linkage disequilibrium between *CYP3A4*1B* and *CYP3A5*1*. Although a high linkage between *CYP3A4*1B* and *CYP3A5*1* has been described in the literature [34], our data did not reflect this ($r^2=0.30$, $D'=0.80$). CYP3A enzyme activity can be influenced by a large number of inducing and inhibiting factors beside genetic variation. In our study, the use of *CYP3A4* inhibitors and inducers was limited to approximately 5% and 1% of the patients respectively, and did not influence our results.

In this study, we did not find an association between rs 4274916 as a proxy for *UGT2B7*2* and codeine effectiveness. We were not surprised by this finding, since as mentioned in the introduction, *UGT2B7*2* does not seem to influence enzyme activity in vitro, and moreover the clinical relevance is still unclear [18].

Experimental pain models (like the cold pressor test, heat pain thresholds and pressure pain thresholds) pain scores, and pupil diameter are often used in the assessment of opioid efficacy. Unfortunately, we had no access to this kind of data. Alternatively, we studied switching to an opioid, the next step on the WHO pain ladder, within 90 days after codeine start. In addition, we looked at co-prescription of other analgesics. Although these outcomes have their limitations, they reflect the reality of daily practice and can be seen as proxy for codeine effectiveness. No association between codeine dose and genotype was found, probably as a result of varying and imprecise dose regimen, since analgesics are usually dosed 'as-needed'.

In the Netherlands, codeine is available as antitussive to reduce cough (codeine 10 or 20 mg) or in a combination product with paracetamol (paracetamol/codeine 500/10 mg, 500/20 mg or 500/50 mg) as analgesic. To exclude patients with a cough we selected patients who were prescribed paracetamol/codeine. As a result we probably miss patients with pain who were only prescribed codeine. However, selection bias is unlikely, since codeine users were

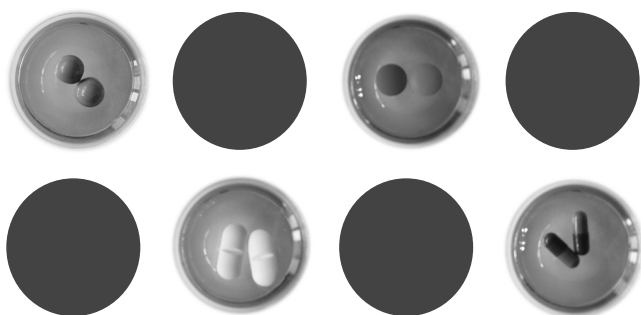
selected independently of CYP2D6 metabolizer status in a large population-based cohort study. Since we used pharmacy data registered prospectively without prior knowledge of the study hypothesis, information bias is improbable. Some random non-differential misclassification may be present, because we limited ourselves to the most common polymorphisms in the *CYP2D6*, *CYP3A4* and *CYP3A5* genes. Nevertheless, we believe that these misclassifications will lead to a conservative estimate of the association. The frequency of the *CYP2D6**4 allele in our study (20.8%) was in concordance with the literature [12], but appeared to be outside Hardy-Weinberg equilibrium (HWE). All genotyping assays were validated by DNA sequencing, and seem not to be responsible for this discrepancy. However, the CYP2D6 genotyping assay will overestimate the frequency of the *CYP2D6**4 allele, because of the existence of the CYP2D6 gene deletion (*5): *CYP2D6**4/*5 individuals will appear as *CYP2D6**4/*4, who will be classified as *CYP2D6**4/*4 (PMs). This may lead to an overestimation of *4/*4 individuals in HWE. However, this does not affect phenotype classification since both *4/*4 and *4/*5 are PM.

In conclusion, our study indicates that the analgesic effect of codeine is decreased in patients carrying a *CYP3A4**1B or *CYP3A5**1 allele by means of an increased risk of switching to an opioid and increased number of co-prescriptions of other analgesics. Interestingly, this decreased efficacy of codeine was not observed in patients with the *CYP2D6**4/*4 genotype, while morphine concentrations are expected to be low in these subjects.

References

1. Chen ZR, Irvine RJ, Somogyi AA, Bochner F. Mu receptor binding of some commonly used opioids and their metabolites. *Life Sci* 1991;48:2165-2171.
2. Sindrup SH, Brosen K. The pharmacogenetics of codeine hypoalgesia. *Pharmacogenetics* 1995; 5:335-346.
3. Lotsch J, Skarke C, Schmidt H, Rohrbacher M, Hofmann U, Schwab M, et al. Evidence for morphine-independent central nervous opioid effects after administration of codeine: contribution of other codeine metabolites. *Clin Pharmacol Ther* 2006;79:35-48.
4. Vree TB, van Dongen RT, Koopman-Kimenai PM. Codeine analgesia is due to codeine-6-glucuronide, not morphine. *Int J Clin Pract* 2000;54:395-398.
5. Chen ZR, Somogyi AA, Reynolds G, Bochner F. Disposition and metabolism of codeine after single and chronic doses in one poor and seven extensive metabolisers. *Br J Clin Pharmacol* 1991;31:381-390.
6. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos* 1997;25:1-4.
7. Caraco Y, Tateishi T, Guengerich FP, Wood AJ. Microsomal codeine N-demethylation: cosegregation with cytochrome P4503A4 activity. *Drug Metab Dispos* 1996;24:761-764.
8. Poulsen L, Riishede L, Brosen K, Clemensen S, Sindrup SH. Codeine in post-operative pain. Study of the influence of sparteine phenotype and serum concentrations of morphine and morphine-6-glucuronide. *Eur J Clin Pharmacol* 1998;54:451-454.
9. Sindrup SH, Brosen K, Bjerring P, Arendt-Nielsen L, Larsen U, Angelo HR, Gram LF. Codeine increases pain thresholds to copper vapor laser stimuli in extensive but not poor metabolizers of sparteine. *Clin Pharmacol Ther* 1990;48:686-693.
10. Poulsen L, Brosen K, Arendt-Nielsen L, Gram LF, Elbaek K, Sindrup SH. Codeine and morphine in extensive and poor metabolizers of sparteine: pharmacokinetics, analgesic effect and side effects. *Eur J Clin Pharmacol* 1996;51:289-295.
11. 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.
12. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3:229-243.
13. 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.
14. Daly AK. Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* 2006;45: 13-31.
15. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002;54:1271-1294.
16. Amirimani B, Ning B, Deitz AC, Weber BL, Kadlubar FF, Rebbeck TR. Increased transcriptional activity of the CYP3A4*1B promoter variant. *Environ Mol Mutagen* 2003;42:299-305.
17. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 2002;48:1668-1671.
18. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 2003;3:136-158.
19. Coffman BL, King CD, Rios GR, Tephly TR. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab Dispos* 1998;26:73-77.
20. Sawyer MB, Innocenti F, Das S, Cheng C, Ramirez J, Pantle-Fisher FH, et al. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. *Clin Pharmacol Ther* 2003;73: 566-574.

21. Holthe M, Klepstad P, Zahlsen K, Borchgrevink PC, Hagen L, Dale O, et al. Morphine glucuronide-to-morphine plasma ratios are unaffected by the UGT2B7 H268Y and UGT1A1*28 polymorphisms in cancer patients on chronic morphine therapy. *Eur J Clin Pharmacol* 2002;58: 353-356.
22. Eckhardt K, Li S, Ammon S, Schanzle G, Mikus G, Eichelbaum M. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. *Pain* 1998;76:27-33.
23. Williams DG, Patel A, Howard RF. Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic reliability. *Br J Anaesth* 2002;89:839-845.
24. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, Stricker BH, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007; 220:819-829.
25. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7:403-422.
26. WHO Collaborating Centre for Drug Statistics Methodology Website. Complete ATC/DDD Index 2009 <http://www.whocc.no/atcddd/indexdatabase/>. Accessed 02-2009.
27. Flockhart D. Drug Interactions: Cytochrome P450 Drug Interaction Table. <http://medicine.iupui.edu/flockhart/table.htm>. Accessed 02-2009.
28. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008; 371:1505-1512.
29. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21: 263-265.
30. Caraco Y, Sheller J, Wood AJ. Pharmacogenetic determinants of codeine induction by rifampin: the impact on codeine's respiratory, psychomotor and miotic effects. *J Pharmacol Exp Ther* 1997; 281:330-336.
31. Fromm MF, Eckhardt K, Li S, Schanzle G, Hofmann U, Mikus G, et al. Loss of analgesic effect of morphine due to coadministration of rifampin. *Pain* 1997;72:261-267.
32. Garcia-Martin E, Martinez C, Pizarro RM, Garcia-Gamito FJ, Gullsten H, Raunio H, et al. CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. *Clin Pharmacol Ther* 2002; 71:196-204.
33. Rodriguez-Antona C, Sayi JG, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M. Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. *Biochem Biophys Res Commun* 2005;338:299-305.
34. Dally H, Bartsch H, Jager B, Edler L, Schmezer P, Spiegelhalder B, et al. Genotype relationships in the CYP3A locus in Caucasians. *Cancer Lett* 2004;207:95-99.



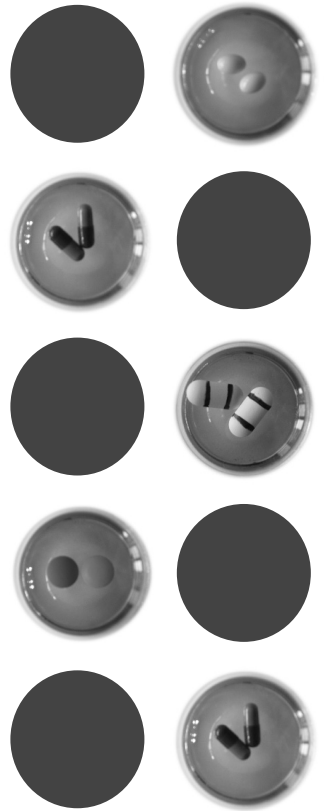
The *CYP2D6**4 polymorphism
and cancer

CHAPTER 5

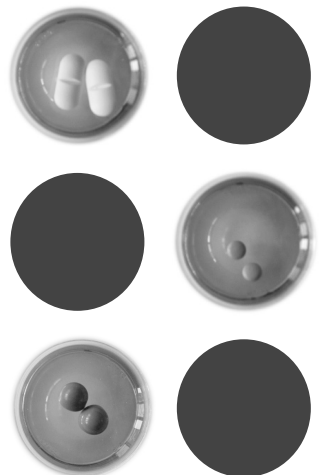
The *CYP2D6**4 polymorphism affects breast cancer survival in tamoxifen users

Monique J. Bijl , Ron H.N. van Schaik,
Laureen A. Lammers, Albert Hofman,
Arnold G. Vulto, Teun van Gelder, Bruno H.
Ch. Stricker, Loes E. Visser

Breast Cancer Res Treat 2009; Feb 3 [Epub ahead of print]



CHAPTER 5.1



Abstract

Background: Tamoxifen is one of the most widely used drugs for the treatment of estrogen receptor-positive breast cancer in post-menopausal women. Cytochrome P450 2D6 (CYP2D6) plays an important role in the formation of endoxifen, the active metabolite of tamoxifen. Genetic variation in the *CYP2D6* gene may lead to a decreased efficacy of tamoxifen therapy. In this study the association between the most prevalent CYP2D6 null-allele in Caucasians (*CYP2D6*4*) and breast cancer mortality was examined among users of tamoxifen in a population-based cohort study.

Methods: In the Rotterdam Study all incident tamoxifen users with *CYP2D6* genotype available (n=85) were followed until death. The association between *CYP2D6* genotype and all-cause mortality, cancer mortality and breast cancer mortality was examined using Cox proportional hazard models with drug use as time-dependent variable.

Results: Breast cancer mortality was significantly increased in patients with the **4/*4* genotype (HR=4.1, CI 95% 1.1-15.9, p=0.041) compared to wild type patients. The breast cancer mortality increased with a hazard ratio of 2.0 (CI 95% 1.1-3.4, p=0.015) with each additional variant allele.

No increased risk of all-cause mortality or all-cancer mortality was found in tamoxifen users carrying a *CYP2D6*4* allele.

Conclusion: The risk of breast cancer mortality is increased in tamoxifen users with decreased CYP2D6 activity, consistent with the model in which endoxifen formation is dependent on CYP2D6 activity.

Introduction

Breast cancer is a major public health problem. In the Netherlands the incidence of breast cancer increased from 85 per 100 000 person years in 1974 to almost 130 per 100 000 person years in 2004 [1]. Tamoxifen is one of the most widely used drugs for post-menopausal women with estrogen receptor-positive breast cancer. In metastatic breast cancer, about 30% of the women respond to tamoxifen therapy [2,3]. Women with estrogen receptor-positive breast cancer taking adjuvant tamoxifen for 5 years have a decreased risk of breast cancer recurrence and significantly lower mortality rates compared to women not using tamoxifen [2,3].

Tamoxifen undergoes extensive hepatic metabolism to more potent metabolites, including 4-hydroxy-tamoxifen and endoxifen (4-hydroxy-N-desmethyl-tamoxifen). Endoxifen and 4-hydroxy-tamoxifen have a 50-fold higher affinity for the estrogen receptor than tamoxifen. Plasma concentrations of endoxifen are on average 5-10 times higher than those of 4-hydroxy-tamoxifen, making endoxifen the most active substance [4,5]. Cytochrome P450 2D6 (CYP2D6) plays an important role in the formation of endoxifen from tamoxifen [6]. The activity of CYP2D6 is mainly determined by the presence of genetic polymorphisms, rather than by induction or inhibition of expression, giving rise to a 1,000-fold difference in CYP2D6 metabolic capacity [7]. Individuals carrying two non-functional alleles of the *CYP2D6* gene lack CYP2D6 enzyme activity and are therefore classified as poor metabolizer (PM), whereas extensive metabolizers (EMs) have 2 functional alleles and exhibit normal enzyme activity. Carriers of one functional and one non-functional allele are usually classified as intermediate metabolizers (IMs) [8,9]. For CYP2D6, the *CYP2D6*4* allele is the most common variant allele in Caucasians leading to the PM phenotype [9,10].

Homozygosity for CYP2D6 non-functional alleles has been associated with lower plasma concentrations of endoxifen [11,12]. Goetz et al. were the first to describe that women using adjuvant tamoxifen had a higher risk of breast cancer recurrence and a lower incidence of hot flashes when they had the *CYP2D6*4/*4* genotype [13]. In another study patients with decreased CYP2D6 activity due to co-administration of CYP2D6 inhibitors or **4* carriership had a significantly shorter time to breast cancer recurrence and shorter disease free survival, again illustrating the important role of CYP2D6 in tamoxifen therapy [14]. Recently, Schroth et al. confirmed that patients with impaired CYP2D6 metabolism had an increased risk of breast cancer recurrence and worse event free survival rates [15]. In contrast, two other studies showed no association or a tendency towards a decreased recurrence rate in users of tamoxifen with the *CYP2D6*4* variant allele [16-18]. In fact, Wegman et al. surprisingly found that patients carrying a *CYP2D6*4* allele surprisingly had a significantly better prognosis than wild type individuals [17,18]. A comparable, but not statistically significant, result was found by Nowell et al. in which a hazard ratio of 0.67 (95% CI 0.33-1.35) on progression free survival was found in *CYP2D6*4* carriers [16].

The differences in the effect of the *CYP2D6**4 polymorphism on breast cancer survival found among the different studies are as yet unresolved, and therefore additional studies are needed, as indicated by Lash et al [19].

The current study investigated the association between the *CYP2D6**4 polymorphism and breast cancer mortality in incident tamoxifen users in a population-based cohort study.

Methods

Setting

Data were obtained from the Rotterdam Study, a population-based cohort study among 7983 persons aged 55 years and older [20,21]. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Baseline examination took place between 1990 and 1993 and consisted of a home interview followed by two visits to the research center. Blood samples were obtained from which DNA was isolated. Since the start of the study, participants have been re-examined periodically. To identify all mortality cases, the vital status of the participants was obtained regularly from the municipal population registry. The cause of death was established by information from the general practitioner, including medical history, and in case of hospitalization, discharge reports from medical specialists were obtained. Two research physicians coded all events independently according to the International Classification of Diseases-10th edition. Information on medication use for all participants was available since January 1991. The seven computerized pharmacies cover the research area and provide information on the drug dispensed (Anatomical Therapeutical Chemical (ATC)-code), dispensing date, the total amount of drug units per prescription and the prescribed daily number of units of the drugs.

Study design and outcomes

The study cohort consisted of all women in the Rotterdam Study, who received a first prescription of tamoxifen between April 1st 1991 and July 1st 2005. Subjects in whom no *CYP2D6* genotype was available and who received tamoxifen in the first 3 months of available pharmacy data were excluded from the analysis in order to have a complete medication survey and to include only incident users. Participants should at least have a follow-up of 180 days. Subjects were followed from their first tamoxifen prescription until death or the end of the study period whichever came first. Cancer mortality and breast cancer mortality were independently assessed by two medical doctors on the basis of the medical record and pathology data according to the International Classification of Diseases (ICD-10). In case of discrepancy, a cancer epidemiologist decided.

Genotyping

All participants in the Rotterdam Study were genotyped for the *CYP2D6**4 polymorphism (1846G>A) as described earlier [22]. Briefly, 1 ng of genomic DNA was amplified in 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute using Taqman assays (Applied Biosystems, Foster City, USA).

Individuals were classified as homozygous *4/*4 (PM), heterozygous *1/*4 (IM) or, in the absence of the 1846G>A SNP, as *1/*1 (EM).

Statistical Analysis

The association between *CYP2D6* genotype and mortality due to any cause, cancer mortality, or mortality due to breast cancer was examined using Cox proportional hazard models with drug exposure as time-dependent variable. In this model the mortality date was taken as the index date. To each mortality case, all persons using tamoxifen who were still alive on the index date of the case were matched on duration of use of tamoxifen. Analyses were adjusted for age at the index date, total tamoxifen duration, average tamoxifen dose and calendar time. Co-administration of strong *CYP2D6* inhibitors (fluoxetine, paroxetine, bupropion, quinidine) or weak inhibitors (sertraline, duloxetine, cimetidine, terbinafine, amiodaron) was considered as potential confounder or effect modifier. Confounders were adjusted for in the analyses if they caused a change in the point estimate of more than 10 percent. The association between *CYP2D6* and breast cancer mortality was studied with an allele-effect model (gene-dose effect), with a genotype-effect model (*CYP2D6**1/*1, *1/*4, *4/*4 separately), and with a dominant/recessive model (*1/*4 and *4/*4 versus *1/*1 or *4/*4 versus *1/*4 and *1/*1).

In addition, we used all women in the Rotterdam Study, in whom information on *CYP2D6* genotype was available, to examine the association between *CYP2D6* and breast cancer mortality in the whole population including non-users. Genotype frequencies were tested for Hardy Weinberg equilibrium using a Chi-square test. All analyses were performed using SPSS software (version 11.0, Chicago, USA).

Results

Of the 4878 women in the Rotterdam Study 108 patients used tamoxifen at any time during the study period. *CYP2D6* genotype was known in 85 of these patients. In the other 23 patients there was no DNA sample available for genotyping. The characteristics of the study population are given in table 1. The allele frequency of the *CYP2D6**4 allele was 21.8%. Genotype frequencies were in Hardy-Weinberg Equilibrium ($\chi^2=0.0003$; $p=0.987$).

The association between *CYP2D6* genotype and mortality is shown in table 2. The risk of all-cause mortality did not significantly differ between the different *CYP2D6* genotypes in

tamoxifen users. There was also no increased risk of cancer mortality in PMs compared to EMs. However, in an allele-effect model there was a significantly increased breast cancer mortality risk with a hazard ratio of 2.0 per additional variant allele (CI 95% 1.1-3.4, $p=0.015$). In a genotype-effect analysis, the risk of death due to breast cancer was significantly increased in tamoxifen users with the *CYP2D6* *4/*4 genotype (HR=4.1, CI 95% 1.1-15.9, $p=0.041$) compared to EMs. In IMs using tamoxifen there was a non-significantly increased risk of breast cancer mortality (HR=1.9, CI 95% 0.9-3.9, $p=0.075$).

Table 1. Characteristics of the study population

	Tamoxifen users
n	85
Mean age first tamoxifen use, years (SD)	75.5 (8.8)
<i>CYP2D6</i> genotype	
*1/*1	52 (61.2%)
*1/*4	29 (34.1%)
*4/*4	4 (4.7%)
<i>CYP2D6</i> inhibitors	
strong (duration of use)	6 (20 – 608 days)
weak (duration of use)	5 (15 – 235 days)
Mean tamoxifen duration, years (SD)	2.13 (1.8)
Average tamoxifen dose, mg (SD)	33.7 (8.7)

Table 2. Association between *CYP2D6**4 genotype and mortality risk.

	All-cause mortality			Cancer mortality			Breast cancer mortality		
	cases	HR* (95% CI)	p-value	cases	HR* (95% CI)	p-value	cases	HR* (95% CI)	p-value
<i>CYP2D6</i> genotype									
*1/*1 (EM)	30	1.00 (ref)		20	1.00 (ref)		17	1.00 (ref)	
*1/*4 (IM)	19	1.5 (0.8-2.8)	0.164	15	1.6 (0.8-3.2)	0.183	15	1.9 (0.9-3.9)	0.075
*4/*4 (PM)	4	1.9 (0.6-5.6)	0.261	3	3.1 (0.8-11.4)	0.097	3	4.1 (1.1-15.9)	0.041

* Hazard ratios (HR) were calculated using cox-proportional hazard models with time-dependent variables and were adjusted for age at the index date, total tamoxifen duration, average tamoxifen dose and calendar time

Taking homozygous and heterozygous *4 individuals together in a dominant model, the breast cancer mortality risk was 2.1 (CI 95% 1.1-4.2, $p=0.031$). These results are graphically represented in figure 1. There was no increased mortality due to breast cancer among women with the *CYP2D6* *4/*4 genotype in the whole population (HR=1.1; CI 95% 0.3-3.6, $p=0.88$).

Co-administration of *CYP2D6* inhibitors (fluoxetine, paroxetine, sertraline, cimetidine, amiodaron) occurred in 11 subjects (13%) taking tamoxifen. The duration of use varied from 15 days – 1.7 year. Co-administration of any *CYP2D6* inhibitor did not influence our model as a confounder or effect modifier. In order to assess the potential effect of *CYP2D6* co-medication,

we analyzed a *CYP2D6**1/*1 person concurrently prescribed a CYP2D6 inhibitor as an intermediate metabolizer. Likewise, a heterozygous *CYP2D6**1/*4 was analyzed as a poor metabolizer when taking CYP2D6 co-medication. In this way, the risk of breast cancer mortality in poor metabolizers was 4.0 instead of 4.1 ($p=0.025$).

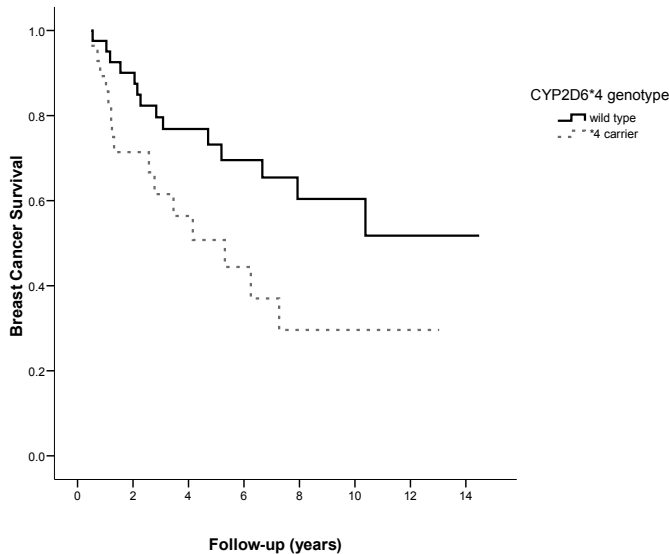


Figure 1. Kaplan Meier breast cancer survival curve for *CYP2D6**4 genotype. Heterozygous and homozygous *4 allele carriers were combined. This figure was unadjusted for age, total tamoxifen duration, average tamoxifen dose and calendar time.

Discussion

Our population-based study showed that tamoxifen users with a decreased CYP2D6 enzyme activity are at increased risk of breast cancer mortality. Per additional variant allele the hazard ratio of breast cancer mortality increased with a factor 2. These results support previous findings of Goetz et al. and Schroth et al [13-15], and are consistent with the model in which endoxifen formation, the most active substance of tamoxifen therapy, is dependent on CYP2D6 activity. Interestingly, we found a statistical significant effect despite the small group ($n=4$) of CYP2D6 PMs, indicating that the impact of this phenotype on breast cancer survival can be impressive. In our study intermediate metabolizers tended to have an increased risk of breast cancer mortality, suggesting an allele dose effect rather than a recessive model where the risk increase would be limited to *4/*4 homozygous individuals. Goetz et al. included the heterozygous *4 carriers (with or without potent CYP2D6 inhibitors) in the decreased enzyme activity group [14]. They found an increased risk of breast cancer recurrence in tamoxifen users with decreased CYP2D6 activity (HR=1.91) and worse relapse free survival (HR=1.74). IMs in

that study did not have a shorter time to breast cancer recurrence, but tended to have worse relapse-free survival, albeit marginally non-significant ($p=0.07$).

Co-administration of CYP2D6 inhibitors leads to lower endoxifen plasma concentrations [11, 12] and Goetz et al. showed that in subjects taking CYP2D6 inhibitors tamoxifen presumably was less effective. In our study, the use of CYP2D6 co-medication was limited to approximately 10% of the patients. When we took CYP2D6 co-medication into account, we found a comparable risk of breast cancer mortality in CYP2D6 PMs as to analysis with genotype alone, but concurrent use of CYP2D6 inhibitors was scarce and differed in duration of use.

Potential biases of population-based studies are selection bias, information bias and confounding. In our study selection bias probably did not occur, since all tamoxifen users were selected independently of CYP2D6 metabolizer status in a large cohort study. Patients for whom no blood sample was available were slightly younger (mean age of 74.7) and probably more diseased, but missing blood samples were not likely to be related to *CYP2D6* genotype. Information bias is unlikely as both information on exposure and disease were gathered prospectively and without knowledge of the research hypothesis and genotype status. Some random misclassification may have occurred, because only the *4 variant allele of the *CYP2D6* gene was determined in our study. This variant is by far the most common polymorphism in Caucasians and >75% of PMs can be identified by genotyping this polymorphism [9,10]. Other less frequent CYP2D6 non-functional alleles like *3 and *6 were not determined, based on their low allele frequencies in Caucasians [10]. The *CYP2D6**5 allele (gene deletion), which has an allele frequency of 5% was incorporated in our assay, because *4/*5 individuals will be scored as *4/*4, with the correct PM phenotype. In the heterozygous *CYP2D6**1/*4 patients, no *5 allele can be present. Only in the 52 individuals of the *CYP2D6**1/*1 group, 5% (2-3 individuals) may have had a *1/*5 genotype which was missed, misclassifying these patients as EM instead of IM. However, this misclassification would lead to a conservative estimation of the currently described association. We adjusted for age, total tamoxifen use, average tamoxifen dose and calendar time. Although we had no complete information on breast cancer stage, tumor size, nodal stage or estrogen receptor (ER) status, *CYP2D6* genotype is not known as being associated with these parameters. Schroth et al. did not find a correlation between genotype and tumor size, nodal stage, histologic grade or ER status [15]. From the information available, we deduced that approximately 25% of the women in our study received tamoxifen for metastatic breast cancer and in 56% of the patients tamoxifen was prescribed as adjuvant.

Improved efficacy of the aromatase inhibitor anastrozole compared to tamoxifen in metastatic breast cancer and as adjuvant treatment for breast cancer was reported in other studies [23, 24]. The difference could be explained by a worse outcome in CYP2D6 PMs using tamoxifen. Modeling suggested that breast cancer survival outcomes in tamoxifen EMs are indeed similar or even superior to those in aromatase inhibitors [25].

Our study demonstrated that the risk of breast cancer mortality is increased in patients carrying the *CYP2D6**4 allele. These patients probably benefit more from aromatase inhibitors, which activity is independent of CYP2D6 enzyme activity. Another option for these patients is to increase the dose of tamoxifen. In our study all *4/*4 individuals received tamoxifen 40 mg once daily and still had an increased risk of breast cancer mortality (after adjustment of dose).

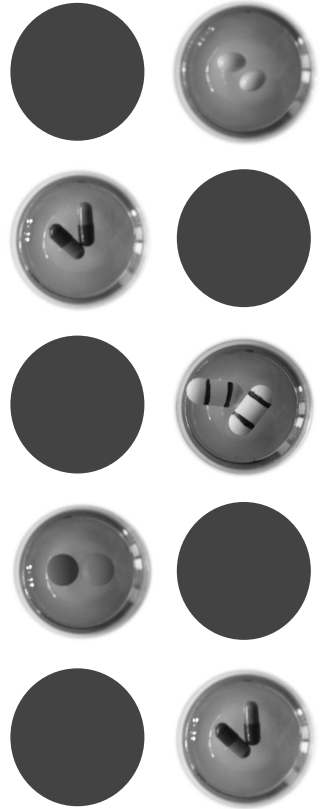
Genotyping of the CYP2D6 gene before start of endocrine treatment in breast cancer could identify PMs who will better respond to aromatase inhibitors than to tamoxifen therapy. CYP2D6 EMs could be prescribed tamoxifen, since aromatase inhibitors are more expensive. Cost-effectiveness studies should be done in order to support the implementation of CYP2D6 genotyping in clinical practice.

In conclusion, our population-based study does confirm that tamoxifen users with decreased CYP2D6 activity have an increased risk of breast cancer mortality. Other drugs may have to be considered in these patients.

References

1. Louwman WJ, Voogd AC, van Dijck JA et al. On the rising trends of incidence and prognosis for breast cancer patients diagnosed 1975-2004: a long-term population-based study in southeastern Netherlands. *Cancer Causes Control* 2008;19:97-106.
2. Osborne CK. Tamoxifen in the treatment of breast cancer. *N Engl J Med* 1998;339:1609-1618.
3. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;365:1687-1717.
4. Jordan VC, Collins MM, Rowsby L, Prestwich G. A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J Endocrinol* 1977;75(2):305-316.
5. Stearns V, Johnson MD, Rae JM et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst* 2003;95:1758-1764.
6. Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther* 2004;310:1062-1075.
7. 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(3):496-526.
8. 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* 60(2):284-295
9. Bradford LD (2002) CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 1997;3:229-243.
10. Gaedigk A, Gotschall RR, Forbes NS, Simon SD, Kearns GL, Leeder JS. Optimization of cytochrome P4502D6 (CYP2D6) phenotype assignment using a genotyping algorithm based on allele frequency data. *Pharmacogenetics* 1999;9:669-682.
11. Jin Y, Desta Z, Stearns V et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J Natl Cancer Inst* 2005;97(1):30-39.
12. Borges S, Desta Z, Li L 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.
13. Goetz MP, Rae JM, Suman VJ et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol* 2005;23:9312-9318.
14. Goetz MP, Knox SK, Suman VJ et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res Treat* 2007;101:113-121.
15. Schroth W, Antoniadou L, Fritz P et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol* 2007;25:5187-5193.
16. Nowell SA, Ahn J, Rae JM et al. Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. *Breast Cancer Res Treat* 2005;91:249-258.
17. Wegman P, Vainikka L, Stal O et al. Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. *Breast Cancer Res* 2005;7:R284-290.
18. Wegman P, Elingarami S, Carstensen J, Stal O, Nordenskjold B, Wingren S. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. *Breast Cancer Res* 2007;9:R7.
19. Lash TL, Ahern TP, Cronin-Fenton D et al. Modification of tamoxifen response: what have we learned? *J Clin Oncol* 2008;26:1764-1765.
20. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7:403-422.

21. Hofman A, Breteler MM, van Duijn CM et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22:819-829.
22. Bijl MJ, Visser LE, Hofman A et al. Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *Br J Clin Pharmacol* 2008;65:558-564.
23. Bonneterre J, Buzdar A, Nabholz JM et al. Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma. *Cancer* 2001;92:2247-2258.
24. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol* 2008;9:45-53.
25. Punglia RS, Burstein HJ, Winer EP, Weeks JC. Pharmacogenomic variation of CYP2D6 and the choice of optimal adjuvant endocrine therapy for postmenopausal breast cancer: a modeling analysis. *J Natl Cancer Inst* 2008;100:642-648.

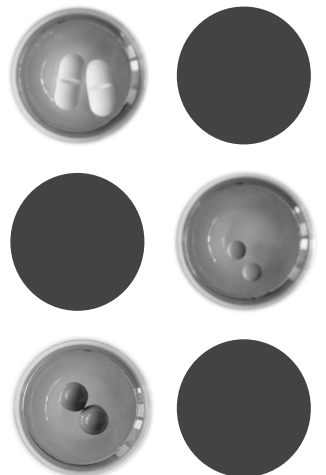


*CYP2D6**4 genotype and susceptibility to basal cell carcinoma

CHAPTER 5.2

Monique J. Bijl, Tamar Nijsten, Ville Kiiski,
Loes E. Visser, Ron H.N. van Schaik, Albert
Hofman, Arnold G. Vulto, Teun van Gelder,
Bruno H.Ch. Stricker.

Submitted



Abstract

Background: Genetic factors may contribute to the susceptibility of basal cell carcinoma (BCC) or modify the course and characteristics. Several studies described an association between *CYP2D6* genotype, encoding an enzyme involved in drug metabolism, and multiple BCC. However, the role of *CYP2D6* in the onset and number of basal cell carcinomas is still unclear and was investigated in only one study population.

Aim: To study the association between the *CYP2D6**4 polymorphism and incident and multiple BCC.

Methods: This study was embedded in the Rotterdam Study, a population-based cohort study among people aged 55 years and older. BCC cases were extracted from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) gathered from general practitioners and hospitals. The association between the *CYP2D6**4 polymorphism and first BCC was evaluated using a Cox's proportional hazards model. The association between multiple BCC was analyzed with an Andersen-Gill's model.

Results: In total, 369 (5.8%) of 6,382 patients developed a BCC and 128 patients multiple BCCs. In *CYP2D6* *1/*1 (wild type), the risk of first BCC was comparable to *CYP2D6**4 carriers (HR=1.01; 95% CI 0.82-1.25; p=0.91). In a nested cohort of BCC patients, the adjusted HR of multiple BCC was 0.78 (CI 95% 0.58-1.05; p=0.10) in *CYP2D6* wild type patients compared to patients with the *1/*4 or *4/*4 genotype. In men, the risk of multiple BCC was lower in persons with *CYP2D6* *1/*1 than in those with one or two variant alleles (HR= 0.60; 95% CI 0.41-0.90; p=0.013). This difference was not demonstrated in women.

Conclusion: In general, this study demonstrated that *CYP2D6* genotype is not associated with first occurrence of BCC. Possibly, males with the wild type genotype have a reduced risk of developing multiple BCC.

Introduction

Basal cell carcinoma (BCC) is the most common cancer in Caucasians and its incidence is increasing [1]. In the Netherlands, approximately 30,000 people develop an incident BCC annually and the incidence rate of BCC is around 500/100,000 person-years [2]. Multiple risk factors are associated and are likely to interact with BCC development including demographic characteristics such as age and gender, phenotypic factors (e.g. hair and eye colour and susceptibility to sun burn) and environmental factors including UV exposure. Differences in known risk factor profiles do not fully explain interindividual susceptibility and variability in tumor numbers, site and accrual of tumors [3,4]. Genetic factors may contribute to the susceptibility of BCC or may be risk factors for specific tumor characteristics (i.e., number, site, accrual). Several genodermatoses such as naevoid basal cell carcinoma syndrome and xeroderma pigmentosum are associated with an increased risk of developing (multiple) skin cancers including BCC [5]. In addition to genetic disorders, single nucleotide polymorphisms in genes concerning melanocortin, immune response (eg, TNF and IL-10), DNA repair, folate and iron metabolism, glutathione S-transferase family and vitamin D receptor may be related to BCC development [6].

The cytochrome P450 enzymes are a family of drug-metabolizing enzymes that catalyze phase 1 drug metabolism (i.e. oxidation, reduction and hydrolysis). Cytochrome P450 2D6 (CYP2D6) is an important member of this family, responsible for the metabolism of approximately 25% of all drugs metabolized by CYPs [7]. CYP2D6 is mainly expressed in the liver, but can also be found in small amounts in brain, intestine and skin [8-10]. CYP2D6 may cause formation of toxic metabolites or reactive oxygen species, which may damage cellular function and may, therefore, be carcinogenic [11]. The *CYP2D6* gene is highly polymorphic with more than 70 variant alleles [12]. Several of these variants encode an inactive protein or lead to the absence of an enzyme product (e.g. *CYP2D6**3, *4, *5, *6). Individuals carrying two of these non-functional alleles lack CYP2D6 enzyme activity and are classified as 'poor metabolizer' (PM). This PM phenotype occurs in 5-10% of the Caucasian population [13]. Carriers of two functional alleles (wild type) are classified as 'extensive metabolizer' (EM) and exhibit 'normal' CYP2D6 enzyme activity. Heterozygous carriers can be considered as 'intermediate metabolizer' (IM).

Lear et al. studied the influence of glutathione S-transferase and cytochrome P450 polymorphisms on tumor numbers and accrual, and found that CYP2D6 extensive metabolizers (EM) had significantly increased numbers of BCC and a faster accrual [14]. In the same UK study population, an association between CYP2D6 EM genotype and development of multiple BCC (MPP or multiple presentation phenotype) has been found [15,16]. The risk of multi cluster BCC was significantly increased in individuals carrying the wild type genotype (OR=15.5; CI 95% 1.34-178.5), but the number of patients in subgroup was small (n=32) and the subgroups were not well defined and not clinically relevant [16]. Another study did not confirm the association between CYP2D6 polymorphisms and number of BCCs in families with naevoid basal cell carcinoma syndrome [17]. Although several papers describe a positive association between

the *CYP2D6* wildtype genotype and number of BCCs, they are all based on the same study population of BCC patients recruited from dermatology outpatient clinics in England [3, 14-16].

The objective of this study was to investigate the association between the *CYP2D6**4 polymorphism and BCC susceptibility and multiplicity in a Dutch population-based cohort study.

Methods

Setting

This study was embedded in the Rotterdam Study, a prospective population-based cohort study among inhabitants of Ommoord, a suburb of Rotterdam [18,19]. In 1990, all inhabitants aged 55 years or older were invited to participate. Of the 10,275 eligible subjects, 7,983 took part in the baseline examination, consisting of an extensive home interview followed by two visits to the research center. The Medical Ethics Committee of the Erasmus Medical Center approved the study, and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993–1996, 1997–1999, and 2002–2004. In addition, the total cohort is continuously monitored for major morbidity and mortality (including cancers) through linkage with the records of each patient's general practitioner, discharge letters from medical specialists or linkage with regional pathology databases.

Study population and case identification

The study population consisted of all subjects in the Rotterdam Study for whom a blood sample was available and the *CYP2D6* genotype could be determined. Patients with a BCC prior to their study entry (between 1982 and 1990) were excluded from the analyses. Subjects were followed from study entry until diagnosis of basal cell carcinoma, death or the end of the study period whichever came first. BCC cases were gathered via general practitioners, hospital data, and through linkage with the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). The first index date was defined as the earliest date found in the pathology data during the study period between baseline and December 2007.

Reports of BCC within the same location with pathology sample dates or excision dates < 6 months apart were considered as being the same BCC. Subsequent lesions at the same location with the terms 'recurrence' or 're-excision' mentioned in the pathology report were considered as recurrent tumors and not subsequent tumors.

Genotyping

At baseline examination of the Rotterdam Study, blood was taken, from which DNA was isolated. Genotyping for the *CYP2D6**4 polymorphism (1846G>A) was performed using Taqman

allelic discrimination assays on the ABI Prism 9700 HT Sequence detection system (Applied Biosystems, Foster City, USA), as described earlier [20]. Briefly, 1 ng of genomic DNA was amplified in 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute. Individuals were classified as poor metabolizer (PM) if they were homozygous for the *4 allele. Heterozygous individuals were classified as intermediate metabolizer (IM). All individuals without a *4 allele were considered to have the wild-type allele (*1).

Statistical analysis

Genotype frequency was tested for deviations from Hardy–Weinberg equilibrium by using a χ^2 -test. The association between *CYP2D6* genotype and first BCC was examined using a Cox proportional hazard model that calculated hazard ratios (HR) and 95% confidence intervals (CI). In a subcohort of BCC patients where follow-up started at the first diagnosis of BCC, an Andersen–Gill model was used to analyze the association between the *CYP2D6**4 polymorphism and multiple BCC. The Andersen–Gill approach models the repeated tumor episodes for each person as separate observations in contrast to the standard Cox model, where cases are censored at the moment of the study outcome, discarding any information past that point [21,22].

The following covariates were tested as potential confounders or effect modifiers: age, gender, skin type, sun exposure, eye colour (brown, intermediate, blue), hair colour (blond, brown, red, black), smoking (never, current, past) and alcohol use (gram/day). Skin type was determined on the basis of tendency towards sunburn. Sun exposure was based on outdoor work or years living in a sunny country. Confounders were defined as covariates associated with the outcome at a *p*-value of 0.1 in the univariate analysis and if they changed the point estimate by 10% or more in the multivariate model in addition to age and gender. In order to study effect modification, an interaction term was included in the analyses and patients were stratified according to the effect modifier if the *p*-value for the term was significant. The analyses presented here were performed with SPSS for Windows, version 15.0 and SAS, version 9.13, using the phreg procedure.

Results

Of the 7,983 participants in the Rotterdam Study, no DNA sample was available for genotyping in 1,414 patients, and in 125 patients suboptimal blood samples had led to difficulties with *CYP2D6**4 genotyping. Furthermore, 62 patients with a BCC before their study entry were excluded. In total, 369 of the remaining 6,382 (incidence 519/100,000 person years) patients developed one or more BCC during the study period. Several demographic, phenotypic and life-style characteristics of the BCC cases and controls are given in table 1. There were 4,096 individuals (64.2%) with the *CYP2D6* *1/*1 genotype, 1913 individuals (30.0%) were heterozygous, and 373 individuals (5.4%) were homozygous for the *4 allele. The allele frequency

of the *CYP2D6**4 allele was 20.8%. Genotype frequencies significantly deviated from Hardy-Weinberg Equilibrium ($\chi^2 = 53.1$; $p < 0.001$).

Table 1. Description of study population

Characteristics	Entire study population (n=6382)	BCC cases (n=369)
Gender		
male	2578 (40.4%)	165 (44.7%)
female	3804 (59.6%)	204 (55.3%)
Age (SD)*	69.5 (9.1)	69.0 (7.8)
CYP2D6 genotype#		
*1/*1 (EM)	4096 (64.2%)	238 (64.5)
*1/*4 (IM)	1913 (30.0%)	109 (29.5%)
*4/*4 (PM)	373 (5.8%)	22 (6.0%)
Tumor count (total)	-	
1		241 (65.3%)
2		71 (19.2%)
3		21 (5.7%)
4		14 (3.8%)
≥ 5		22 (6.0%)
Tendency for sunburns*		
low	3986 (62.5%)	221 (59.9%)
high	1994 (31.2%)	137 (37.1%)
missing	402 (6.3%)	11 (3.0%)
Hair colour*		
brown/black	4517 (70.8%)	245 (66.4%)
blond	1360 (21.3%)	93 (25.2%)
red	188 (2.9%)	22 (6.0%)
missing	317 (5.0%)	9 (2.4%)
Eye colour*		
brown	1364 (21.4%)	71 (19.2%)
intermediate	588 (9.2%)	27 (7.3%)
blue	4055 (63.5%)	256 (69.4%)
missing	375 (5.9%)	15 (4.1%)
Smoking*		
never	2208 (34.6%)	121 (32.8%)
past	2581 (40.4%)	175 (47.4%)
current	1414 (22.2%)	68 (18.4%)
missing	179 (2.8%)	5 (1.4%)
Alcohol intake*		
no	1004 (15.7%)	56 (15.2%)
0.01-9.99 g/day	2200 (34.5%)	156 (42.3%)
10.0- 19.99 g/day	751 (11.8%)	48 (13.0%)
≥ 20 g/day	957 (15.0%)	65 (17.6%)
missing	1470 (23.0%)	44 (11.9%)

* These covariates were assessed at baseline.

Hardy Weinberg equilibrium; $\chi^2 = 53.1$ ($p < 0.001$)

Table 2. Association between CYP2D6*4 genotype or other risk factors and first BCC.

	BCC cases	HR* (95% CI)	p-value
Overall			
CYP2D6 genotype			
*1/*1 (EM)	238	1.01 (0.82-1.25)	0.905
*1/*4 + *4/*4 (IM + PM)	131	1.00 (ref)	-
Age < 65			
CYP2D6 genotype			
*1/*1 (EM)	69	0.68 (0.48-0.97)	0.04
*1/*4 + *4/*4 (IM + PM)	55	1.00 (ref)	-
Age 65-75			
CYP2D6 genotype			
*1/*1 (EM)	111	1.19 (0.85-1.65)	0.31
*1/*4 + *4/*4 (IM + PM)	53	1.00 (ref)	-
Age > 75			
CYP2D6 genotype			
*1/*1 (EM)	58	1.42 (0.88-2.31)	0.15
*1/*4 + *4/*4 (IM + PM)	23	1.00 (ref)	-
Tendency for sunburns			
low	221	1.00 (ref)	-
high	137	1.29 (1.04-1.60)	0.02
Hair colour			
blond	93	1.00 (ref)	-
red	22	1.80 (1.13-2.86)	0.01
brown	222	0.81 (0.64-1.03)	0.09
black	23	0.55 (0.35-0.86)	0.01
Eye colour			
blue	256	1.00 (ref)	-
intermediate	27	0.76 (0.51-1.14)	0.18
brown	71	0.84 (0.65-1.10)	0.20
Smoking			
never	121	1.00 (ref)	-
past	175	1.17 (0.89-1.52)	0.26
current	68	0.96 (0.69-1.32)	0.79
Alcohol intake			
no	56	1.00 (ref)	-
0.01-9.99 g/day	155	1.30 (0.95-1.77)	0.10
10.0- 19.99 g/day	48	1.19 (0.80-1.77)	0.39
≥ 20 g/day	65	1.27 (0.87-1.86)	0.21

* Hazard ratios (HR) were calculated using cox-proportional hazard models adjusted for age and gender.

BCC susceptibility

The association between CYP2D6 genotype and first BCC is shown in table 2. In CYP2D6 *1/*1 the risk of first BCC was not statistically different from individuals carrying the CYP2D6*4 and *4/*4 genotype together (HR=1.01; 95% CI 0.82-1.25; p=0.91). The risk of first BCC in individuals with CYP2D6 *4/*4 was 1.03 (95% 0.66-1.59; p=0.91) compared to those with the

wild type genotype. In individuals heterozygous for the *CYP2D6**4 allele, there was also no difference in risk of BCC in comparison with the wild type (HR=0.98; 95% CI 0.78-1.23; p=0.86).

Skin type, sun exposure, eye colour, hair colour, smoking and alcohol use did not influence the association between *CYP2D6* genotype and BCC susceptibility. A high tendency for sunburns, blond/red hair colour and male sex were significantly associated with an increased risk of first BCC (table 2).

The interaction term between *CYP2D6* genotype and age was statistically significant (p=0.019). Stratified analyses according to different age groups resulted in a higher hazard ratio of the *CYP2D6* *1/*1 genotype on first BCC occurrence in the highest age group and a lower risk in the youngest age group (table 2).

Multiple BCC

In the nested cohort of 369 BCC patients, time from first BCC till subsequent BCCs was analyzed. The influence of genetic variation in *CYP2D6* gene and risk of multiple BCC is given in table 3. With an Andersen-Gill model, the adjusted hazard ratio of multiple BCC was 0.78 in *CYP2D6* wild type patients compared *CYP2D6**4 carriers (CI 95% 0.58-1.05; p=0.10). The interaction term between *CYP2D6* genotype and sex was borderline significant (p=0.061). Stratified analyses according to sex resulted in a decreased hazard ratio for the *CYP2D6* wild type genotype on multiple BCC in men (HR= 0.60; 95% CI 0.41-0.90; p=0.013) but not in women (HR=1.06; 95% CI 0.68-1.65; p=0.79).

Table 3. Association between *CYP2D6**4 genotype and multiple BCC.

	BCC cases	HR* (95% CI)	p-value
<i>CYP2D6</i> genotype			
*1/*1 (EM)	74	0.78 (0.58-1.05)	0.099
*1/*4 + *4/*4 (IM + PM)	54	1.00 (ref)	-

* Hazard ratios (HR) were calculated using an Andersen-Gill model adjusted for age and gender. The reference group were IMs and PMs together.

Discussion

Overall, this study demonstrated that individuals with the *CYP2D6* *1/*1 genotype (wild type) are not at an increased risk for one or multiple BCC. However, in the youngest age group (<65 years), the risk of first BCC was significantly lower in *1/*1 patients than in patients carrying a *CYP2D6**4 allele. No difference in frequencies of *CYP2D6* genotypes between BCC cases and controls was found in another study [14], but the results were not stratified by age. A possible explanation for a lower risk at younger age could be that genes explain a higher proportion of the variability in BCC susceptibility at younger age than other risk factors such as skin type and UV exposure. In contrast to genetic predisposition that is constant in time, the degree of

exposure to exogenous factors cumulates with age and may overrule the genetic predisposition at a certain point in time.

In BCC patients with the wild type genotype, the risk of subsequent tumors (multiple BCC) is not significantly different from patients carrying one or more *CYP2D6**3 and *4 alleles. These findings are consistent with Yang et al, who did not find an association between the *CYP2D6**4 genotype and number of BCCs in patients with naevoid basal cell carcinoma [23]. Several other studies using the same study population showed that the *CYP2D6* wild type genotype is associated with BCC tumor numbers and development of multiple tumors. The risk of increased tumor numbers in *CYP2D6* *1/*1 genotype was relatively small (RR=1.27 CI 95% 1.13-1.43) [14]. Ramachadran et al. did find a higher relative risk of the multiple cluster multiple presentation phenotype (MPP), but the number of patients was small [16]. We were unable to analyze multiple cluster MPP, since patients in our study were not classified into clusters of multiple presentation phenotype at examination. Interestingly, in another study, there was an increased frequency of *CYP2D6**3 and *4 variant alleles in patients with malignant melanoma and red/blond hair [24], suggesting a protective effect of the *CYP2D6* wild type genotype in these patients instead of an increased risk. However, in men we found a lower risk of multiple BCC in *CYP2D6* *1/*1 than in *1/*4 and *4/*4, which is consistent with Ramachandran et al, who found that males carrying a *CYP2D6**4 allele with skin type 1 developed more BCC per year than *CYP2D6* wild type [15]. The explanation for this gender difference may be due to true pathophysiological differences of *CYP2D6* in men and women and/or different gene-environmental interactions. For example, men in our study were significantly more extensively exposed to sunlight, smoking and alcohol than women and the effect of these exposures may interact with *CYP2D6* status, although interaction terms were not significant in our analyses.

The role of *CYP2D6* in basal cell carcinomas remains unclear. *CYP2D6* is mainly expressed in the liver, but can also be found in small amounts in brain, intestine and skin [8-10]. *CYP2D6* may be involved in the formation of dopamine from tyramine [25]. Earlier, it was suggested that *CYP2D6* enzyme activity can influence melanin synthesis, since melanin is a derivate of tyrosine, which level can be affected by *CYP2D6*. *CYP2D6* seems to be involved in the formation of epinephrine and norepinephrine from octapamine and synephrine, which might influence immune function [16,25]. Besides, *CYP2D6* is a xenobiotic-metabolizing enzyme and may cause formation of toxic metabolites or reactive oxygen species, which may damage cellular function [11]. Therefore, it might have a role in detoxification of environmental exposure to harmful substances and it seems possible that extensive metabolizers would form more of such intermediates in the skin than poor metabolizers. This phenomenon would explain the difference in the influence of *CYP2D6* on multiple BCC between women and men in our study, since men were more extensively exposed to UV radiation. We admit, however, that such an explanation is speculative and requires confirmation.

The strengths of this cohort study is the large study population with its long follow-up of more than 15 years. The Rotterdam Study contains detailed information on patient character-

istics including hair and eye colour, sun exposure and genetics, and clinical and pathological details. Nevertheless, some potential limitations of our cohort study should be considered. Only pathology confirmed cases were included in this study. Lesions not biopsied or removal without pathological samples were missed in this study, but occurred sporadically, since patients frequently underwent surgery at the department of dermatology of a community hospital, which usually required pathological confirmation. Nevertheless, the number of BCC tumors is likely to be an underestimation. Because there is no reason to assume that this false-negative misclassification of BCC would differ between genotypes, this random misclassification would tend to underestimate the true hazard ratio rather than produce spurious associations. Second, in this study we only determined *CYP2D6*4*, because this polymorphism is the most common variant allele in Caucasians. Determination of *CYP2D6*4* in our population should predict > 75% of PMs [13]. The allele frequency of the *CYP2D6*4* allele was in concordance with the literature, but significantly deviated from Hardy–Weinberg equilibrium (HWE). The genotyping assay was validated by DNA sequencing, but the assay identified individuals heterozygous for the gene deletion *CYP2D6*5* as homozygous **4*: **4/*5* individuals will be identified as **4/*4* and **1/*5* individuals as **1/*1*, leading to an overestimation of the number of **4* homozygous individuals in HWE. Information bias is not likely, since data on genotype and BCC cases were collected prospectively without prior knowledge of the study hypothesis. We assessed potential confounding factors such as age, gender, skin type, sun exposure, eye colour, hair colour, smoking and alcohol use in the analyses, but no association was found between *CYP2D6* and these covariates.

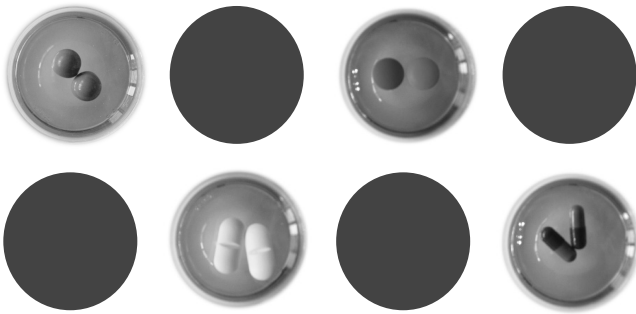
The Andersen-Gill analysis models the repeated tumor episodes as separate observations, but makes a strong assumption of independence among multiple observations per person over time. However, the Andersen-Gill approach has been shown to be a valid method to analyze multiple basal cell carcinoma [22].

In conclusion, our study showed that individuals with the *CYP2D6 *1/*1* genotype (wild type) did not have an overall increased risk of BCC. However, in those below 65 years of age, the risk of first BCC was lower in **1/*1* patients than in patients carrying a *CYP2D6*4* allele. In BCC patients having the wild type genotype, the risk of subsequent tumors (multiple BCC) was not significantly different from patients carrying the *CYP2D6*4* allele. In men, we found a lower risk of multiple BCC in *CYP2D6* wild type. This suggests that the *CYP2D6* genotype may interact with certain environmental factors such as UV exposure or cigarette smoking, leading to an altered risk of multiple BCC.

References

1. Rubin AI, Chen EH, Ratner D. Basal-cell carcinoma. *N Engl J Med* 2005;353:2262-9.
2. de Vries E, van de Poll-Franse LV, Louwman WJ et al. Predictions of skin cancer incidence in the Netherlands up to 2015. *Br J Dermatol* 2005;152:481-8.
3. Ramachandran S, Lear JT, Ramsay H et al. Presentation with multiple cutaneous basal cell carcinomas: association of glutathione S-transferase and cytochrome P450 genotypes with clinical phenotype. *Cancer Epidemiol Biomarkers Prev* 1999;8:61-7.
4. Wong CS, Strange RC, Lear JT. Basal cell carcinoma. *Br Med J* 2003;327:794-8.
5. Tilli CM, Van Steensel MA, Krekels GA et al. Molecular aetiology and pathogenesis of basal cell carcinoma. *Br J Dermatol* 2005;152:1108-24.
6. Festa F, Kumar R, Sanyal S et al. Basal cell carcinoma and variants in genes coding for immune response, DNA repair, folate and iron metabolism. *Mutat Res* 2005;574:105-11.
7. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005; 5: 6-13.
8. Paine MF, Hart HL, Ludington SS et al. The human intestinal cytochrome P450 "pie". *Drug Metab Dispos* 2006;34:880-6.
9. Siegle I, Fritz P, Eckhardt K et al. Cellular localization and regional distribution of CYP2D6 mRNA and protein expression in human brain. *Pharmacogenetics* 2001;11:237-45.
10. Yengi LG, Xiang Q, Pan J et al. Quantitation of cytochrome P450 mRNA levels in human skin. *Anal Biochem* 2003;316:103-10.
11. Jiang Y, He X, Ortiz de Montellano PR. Radical intermediates in the catalytic oxidation of hydrocarbons by bacterial and human cytochrome P450 enzymes. *Biochemistry* 2006;45:533-42.
12. Human Cytochrome P450 (CYP) Allele Nomenclature Committee <http://www.cypalleles.ki.se/>, 2009.
13. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3:229-43.
14. Lear JT, Heagerty AH, Smith A et al. Multiple cutaneous basal cell carcinomas: glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. *Carcinogenesis* 1996;17:1891-6.
15. Ramachandran S, Fryer AA, Lovatt TJ et al. Combined effects of gender, skin type and polymorphic genes on clinical phenotype: use of rate of increase in numbers of basal cell carcinomas as a model system. *Cancer Lett* 2003;189:175-81.
16. Ramachandran S, Fryer AA, Smith AG et al. Basal cell carcinomas: association of allelic variants with a high-risk subgroup of patients with the multiple presentation phenotype. *Pharmacogenetics* 2001;11:247-54.
17. X RY, Pfeiffer RM, Goldstein AM. Influence of glutathione-S-transferase (GSTM1, GSTP1, GSTT1) and cytochrome p450 (CYP1A1, CYP2D6) polymorphisms on numbers of basal cell carcinomas (BCCs) in families with the naevoid basal cell carcinoma syndrome. *J Med Genet* 2006;43:e16.
18. Hofman A, Breteler MM, van Duijn CM et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22:819-29.
19. Hofman A, Grobbee DE, de Jong PT et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;7:403-22.
20. Bijl MJ, Visser LE, Hofman A et al. Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *Br J Clin Pharmacol* 2008;65:558-64.
21. Kelly PJ, Lim LL. Survival analysis for recurrent event data: an application to childhood infectious diseases. *Stat Med* 2000;19:13-33.
22. Pandeya N, Purdie DM, Green A et al. Repeated occurrence of basal cell carcinoma of the skin and multifaailure survival analysis: follow-up data from the Nambour Skin Cancer Prevention Trial. *Am J Epidemiol* 2005;161:748-54.

23. Yang X, Pfeiffer RM, Goldstein AM. Influence of glutathione-S-transferase (GSTM1, GSTP1, GSTT1) and cytochrome p450 (CYP1A1, CYP2D6) polymorphisms on numbers of basal cell carcinomas (BCCs) in families with the naevoid basal cell carcinoma syndrome. *J Med Genet* 2006;43:e16.
24. Strange RC, Ellison T, Ichii-Jones F et al. Cytochrome P450 CYP2D6 genotypes: association with hair colour, Breslow thickness and melanocyte stimulating hormone receptor alleles in patients with malignant melanoma. *Pharmacogenetics* 1999;9:269-76.
25. Hiroi T, Imaoka S, Funae Y. Dopamine formation from tyramine by CYP2D6. *Biochem Biophys Res Commun* 1998;249:838-43.



Discussion

CHAPTER 6

General Discussion

Patients differ in their response to drugs. Not all patients will benefit from a particular drug. Some patients will experience adverse drug reactions, while others will not. Although variability in drug response can be explained by age, gender, renal and liver function, underlying diseases or drug interactions, genetic factors also contribute to differences in drug response [1]. Influence on interindividual variability in drug response is partly exerted by differences in drug metabolism caused by genetic polymorphisms or by inhibition or induction of drug metabolism [2]. Several polymorphisms were investigated in genes coding for cytochrome P450, a family of drug-metabolizing enzymes that catalyze phase 1 drug metabolism, among which CYP2D6 is the most extensively studied representative. CYP2D6 is one of the most important enzymes in the metabolism of therapeutic drugs, because it converts 25% of all drugs metabolized by CYPs including antidepressants, β -blockers, antiarrhythmics and antipsychotics. Genetic polymorphisms in the *CYP2D6* gene have a relatively high impact on the metabolism of drugs compared to polymorphisms in other CYP450 enzymes, since many dysfunctional and non-functional variant alleles are known and the amount of CYP2D6 enzyme in the liver is relatively small (about 2%) compared to the expression of other CYPs. Despite the fact that the influence of CYP2D6 polymorphisms on plasma concentration of drugs metabolized by CYP2D6 is quite clear, the relevance of these polymorphisms on drug efficacy and toxicity in daily practice is still limitedly described.

This thesis contains several studies examining the influence of genetic variation in *CYP2D6* on drug response and disease susceptibility in a daily practice setting. In this section, the main findings will be discussed and placed in broader perspective. Furthermore, clinical implementation of pharmacogenetic tests for CYP2D6 enzyme activity will be discussed.

Main findings

Antipsychotics and antidepressants (tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs)) are widely used in psychiatry. However, pharmacotherapy in depression is rather inefficient, as approximately 50% of the patients will not respond sufficiently to first treatment [3]. This large interindividual variability in antidepressant response can be partly explained by pharmacogenetics.

In chapter 2.1 we studied the effect of the *CYP2D6**4 polymorphism on dose, switching and discontinuation of antidepressants. In users of TCAs, the risk of switching to any other antidepressant within 45 days was significantly higher in CYP2D6 PMs (*4/*4) than in EMs and this possibly reflects a higher incidence of adverse events in PMs. These findings are in accordance with Mulder et al, who also found an increased risk of switching to another drug in the same therapeutic class in PMs versus EMs [4]. The increased risk of switching to another antidepressant was not seen in SSRI users. The maintenance doses of TCAs as well as SSRIs

were significantly lower in PMs, showing that PMs require lower doses of antidepressants to achieve the same effectiveness with minimum adverse drug reactions as seen in EMs.

In chapter 2.2 the association between *CYP2D6* genotype and serum sodium concentration in antidepressant users was studied. Hyponatremia can be provoked by antidepressant use and is thought to occur due to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) [5]. The serum sodium concentration in PMs was lower in users of an antidepressant, especially in TCA users and *CYP2D6* PMs might be at increased risk of developing symptoms of hyponatremia.

In contrast to TCAs for which a narrow therapeutic range exists, no clear relationship between clinical effect and plasma concentration was found for SSRIs. ADRs seemed not to be associated with high plasma concentrations of SSRIs [6]. Furthermore, our results indicate that the contribution of *CYP2D6* to the metabolism of TCAs is greater than to the metabolism of SSRIs. Most tricyclic antidepressants are (partly) metabolized by *CYP2D6*, whereas some SSRIs are not metabolized by *CYP2D6* (e.g. citalopram, escitalopram and sertraline). Kirchheiner et al. suggested dose recommendations for antidepressants based on literature. For TCAs dose reductions of 50% were recommended for PMs of *CYP2D6* and *CYP2C19*, whereas reductions were smaller for the SSRIs [7].

The critical question is whether knowledge of an individual's *CYP2D6* genotype could contribute to the optimization of antidepressant therapy. Our data in chapter 2.1 show that starting doses of antidepressants prescribed to the elderly general population are carefully low and are titrated to the optimum dose. Moreover, antidepressants are not only metabolized by *CYP2D6*, other enzymes are also involved. *CYP2C19* plays a role in the demethylation of amitriptyline, imipramine, clomipramine, sertraline and citalopram, *CYP1A2* and *CYP3A4* contribute to a lesser extent [7, 8]. However, *CYP2D6* exerts a stronger influence on antidepressants metabolism than *CYP2C19* [9].

Although our studies demonstrated that the *CYP2D6*4* polymorphism is likely associated with increased antidepressant toxicity, the question remains whether genotyping prior to the start of antidepressant therapy will contribute substantially to the optimization of pharmacotherapy. Further prospective studies, in which standard pharmacotherapy is compared with genotype-based adjusted pharmacotherapy, are required to answer this question.

Several studies showed an association between the *CYP2D6* PM phenotype and an increased metoprolol plasma concentration and more intense and sustained receptor blockade, but the number of subjects in these studies were small and some results were controversial [10-16]. Therefore, we studied in chapter 3 the influence of the *CYP2D6*4* polymorphism on heart rate and blood pressure in patients treated with β -blockers in a population-based cohort. We found that β -blocker users homozygous for the *CYP2D6*4* allele had a significantly lower heart rate and diastolic blood pressure than users with the wild-type genotype. The adjusted heart rate in users of metoprolol, the β -blocker most extensively metabolized by *CYP2D6* (70-80%), was 8.5 beats/min lower in PMs than in EMs, leading to an increased risk of bradycardia in PMs.

In clinical practice, β -blocker dose is usually titrated on the basis of heart rate and blood pressure. Indeed, in our study we saw that PMs required lower β -blocker doses. We observed that titrating was not properly performed in our study, because PMs had a lower heart rate and an increased risk of bradycardia after adjusting for β -blocker dose, suggesting that genotyping prior to the start of pharmacotherapy might have an additive value above dosing on clinical effect. In our study, the risk of bradycardia in metoprolol users was almost four times higher in PMs than in EMs. Bradycardia is a known ADR of β -blockers and is dose dependent. Bradycardia can be life threatening and can be avoided in PMs by reducing the dose.

Unfortunately, we were unable to show a difference between CYP2D6 PMs and EMs in cardiovascular morbidity (e.g. myocardial infarction, stroke, angina pectoris, heart failure) or mortality, but numbers were small.

Apart from an effect of the CYP2D6 genotype on drug toxicity due to increased plasma concentrations, drug efficacy and effectiveness can be influenced by the CYP2D6*4 polymorphism in case the therapeutic agent is a pro-drug. In chapter 4 we studied variation in the CYP2D6, CYP3A4/3A5 and UGT2B7 gene in relation to co-prescription of other analgesics and switching to opioids in codeine users. The analgesic effect of codeine is mostly dependent on its metabolism to morphine by CYP2D6. Interestingly, a decreased effectiveness of codeine was not observed in patients with the CYP2D6*4/*4 genotype, while morphine concentrations are expected to be low in these subjects. In patients carrying a CYP3A4*1B allele the risk of switching to an opioid was increased and CYP3A4*1B carriers required more co-prescription of other analgesics after start of codeine use. The finding that codeine is less active in patients with enhanced CYP3A4 activity is supported by other studies [17,18]. The effect of the CYP3A4*1B polymorphism on CYP3A activity is controversial and CYP3A enzyme activity can be influenced by a large number of inducing and inhibiting factors beside genetic variation. In our study, we did not find an association between rs4274916 as a proxy for UGT2B7*2 and codeine effectiveness. We were not surprised by this finding, since UGT2B7*2 does not seem to influence enzyme activity in vitro, and moreover the clinical relevance is still unclear [19]. Unfortunately, we did not have information on plasma concentrations or data from pain models or pain scores as outcome. We used switching and co-prescription as a proxy for codeine effectiveness. This study did not clarify the impact of CYP2D6 polymorphisms on codeine effectiveness. Further studies are needed to elucidate the influence of impaired CYP2D6 metabolism and should take possible influence of CYP3A4 polymorphisms into account.

At present, decreased efficacy of tamoxifen therapy in breast cancer patients with reduced cytochrome P450 2D6 (CYP2D6) activity is a hot topic. CYP2D6 plays an important role in the formation of endoxifen, the active metabolite of tamoxifen. Differences exist in today's literature on the effect of the CYP2D6*4 polymorphism on breast cancer survival, with contradictory results. Our population-based study, as presented in chapter 5, showed that tamoxifen users with a decreased CYP2D6 enzyme activity have an increased breast cancer mortality

despite the small group of PMs ($n=4$). Intermediate metabolizers tended to have an increased risk of breast cancer mortality. These results were consistent with another study in which IMs did not have a shorter time to breast cancer recurrence, but tended to have a reduced relapse-free survival ($p=0.07$) [20]. Improved efficacy of the aromatase inhibitor anastrozole compared to tamoxifen in metastatic breast cancer and as adjuvant treatment for breast cancer was reported in other studies [21,22]. The difference could be explained by a worse outcome in CYP2D6 PMs using tamoxifen. Modelling suggested that breast cancer survival outcomes in tamoxifen EMs are indeed similar or even superior to those in users of aromatase inhibitors [23]. Genotyping of the *CYP2D6* gene before start of endocrine treatment in breast cancer could identify PMs who may better respond to aromatase inhibitors than to tamoxifen therapy. CYP2D6 EMs could be prescribed tamoxifen, since aromatase inhibitors are more expensive. Prospective studies are now ongoing, including one at the Leiden University Medical Center, the Netherlands (LUMC), in order to evaluate the benefit of prospective CYP2D6 genotyping in the treatment of breast cancer [24].

From genotype to phenotype

The *CYP2D6* gene is highly polymorphic with more than 70 variant alleles [25], leading to a wide range of enzymatic activities, from absent to normal or even increased activity. In contrast to some other drug metabolizing enzymes (e.g., CYP3A4), the total expression of CYP2D6 is low and not inducible, and therefore genetic variation contributes substantially to the interindividual variability in enzyme activity [26]. However, not all interindividual variability in drug response is explained by genes. Other factors such as age, gender, environmental factors, poor compliance, and drug-drug interactions may affect drug response. CYP2D6 enzyme activity can be decreased due to co-administration of inhibitors (such as fluoxetine, paroxetine, bupropion, quinidine, sertraline, duloxetine, cimetidine, terbinafine, amiodaron). Neither age, gender, menstrual cycle phase, nor smoking appear to affect CYP2D6 activity [27,28]. And besides genetic variation in drug metabolizing enzymes, there is also genetic variation in drug transporters, receptors and other drug targets that can influence drug disposition and efficacy.

The phenotype of a person is a combination of his or her genotype and the influence of environmental factors. An individual's CYP2D6 enzyme activity can also be determined by phenotyping after administering a single dose of a probe drug such as debrisoquine, sparteine, dextromethorphan, tramadol, metoprolol – which are metabolized by CYP2D6 to a high extent. The metabolic ratio of the parent compound over its CYP2D6-mediated metabolite can be calculated when concentrations of both substances in body fluids are known [28, 29]. Based on the metabolic ratio, four groups of metabolizers, displaying gradually decreasing CYP2D6 activity, can be identified in the population: ultra-rapid (UMs), extensive (EMs), intermediate (IMs) and poor metabolizers (PMs). Phenotyping determines an individual's actual enzymatic activity taking renal and liver function, drug-drug interactions (CYP2D6 inhibitors) and environmental factors into account. However, phenotyping requires exposure to a probe

drug, multiple urine or plasma samples, is time consuming and is not stable throughout a person's life. In addition, in patients lacking the CYP2D6 enzyme (PM), the influence of hepatic impairment or co-administration of CYP2D6 inhibitors is limited.

Genotyping *CYP2D6**3, *4, *5, and *6 would predict the PM phenotype by about 98% [30], but predictions of IMs and UMs are less sensitive. Discussions are ongoing whether classification into PMs, IMs, EMs and UMs is the most accurate approach. Gaedigk et al. and Steimer et al. proposed another classification system on the presumed activity of individual CYP2D6 alleles [31,32]. In this CYP2D6 activity score system, also known as the semiquantitative gene dose classification system, non-functional alleles are assigned an activity score of 0, functional alleles are assigned 1, and decreased activity alleles (e.g. *10, *41) are assigned a score between 0 and 1. This system was shown to be more accurate in prediction of the in vivo activity than the traditional phenotype system. However, further research in large groups of patients using CYP2D6 probe drugs is needed to validate these scoring systems.

Therapeutic Drug Monitoring

Why determine a patient's *CYP2D6* genotype with expensive techniques if it is possible to measure the concentration of a drug in plasma, bridging the genotype-phenotype gap? In clinical practice, therapeutic drug monitoring (TDM) is recommended for drugs with a narrow therapeutic range, including antiepileptics, tricyclic antidepressants (TCAs), lithium, antipsychotics, digoxin, antiretroviral drugs and aminoglycosides. TDM is a tool to optimize pharmacotherapy by minimizing the risk of adverse events and enhancing therapeutic response. An advantage of TDM is that it takes compliance into account in addition to genetic variation, renal or hepatic insufficiency, drug-drug interactions and environmental factors. However, also TDM has its limitations. First, TDM can only be performed if the patient is exposed to the drug. This may be undesirable when the patient is susceptible to toxicity. By genotyping a patient prior to exposure to a drug the starting dose can be individualized, potentially avoiding extensive over- or underexposure in the initial treatment phase. Measurement of the plasma concentration of a drug is a snapshot in time, while a person's genotype is highly stable throughout a person's life. Furthermore, assessing a person's genotype when this person is taking multiple drugs is less cumbersome than TDM of each of the drugs. It should be mentioned that therapeutic drug monitoring is no routine care for the gross of drugs, including drugs studied in this thesis: SSRIs, β -blockers, codeine and tamoxifen. Genotyping may provide information in case of non-response or toxicity at normal dose level.

As for CYP2D6, several studies showed good agreement between CYP2D6 metabolizer status, plasma concentrations of antidepressants and response [9,33,34]. In another study, 13% of antidepressant serum levels outside the therapeutic range were concordant with aberrant CYP2D6 or CYP2C19 genotypes, but these results were not adjusted for dose [35].

Methodological considerations

Design

Pharmacoepidemiology may be defined as the study of drug use as determinant of the frequency of disease in large numbers of people. In the past, the association between SNPs in drug metabolizing enzymes and drug response was mainly described in small patient populations. The prevalence of genetic variation is usually low and can have a small effect size. Large sample sizes are required to support previous findings or generate new hypotheses. All studies in this thesis were embedded in the Rotterdam Study, a population-based cohort study among 7983 inhabitants of Ommoord, a district in Rotterdam, aged 55 years or over.

In general, two study types of pharmacogenetic epidemiology can be distinguished. First, candidate gene approach studies, in which the effect of a single gene is studied in relation to drug response. Second, genome-wide analysis (GWA) studies are increasingly performed to generate new hypotheses and unravel complex diseases. In this thesis, a candidate gene approach was followed with the *CYP2D6* gene as important determinant of drug response and adverse events.

In a cohort study, preferably 'time till event' or 'survival' analyses are performed in which cases are censored at the occurrence of the study outcome. The Cox proportional hazard model calculates an accurate estimate of the relative risk with adjustment for relevant confounding factors. In chapter 5, we used this model to calculate the breast cancer mortality risk between EMs and PMs in tamoxifen users. In the same chapter, we used an extension of the Cox proportional hazard model to analyze repeated tumor episodes as separate observations in the same subject (Andersen-Gill analysis) [36]. Cross-sectional analyses were performed when repeated measurements were not available (e.g. chapter 2.2 serum sodium concentration), precise event dates were lacking (e.g. chapter 2.1 discontinuation date of antidepressants, 2.3 diagnosis of depression). Cross-sectional studies lack any information on timing of relationships between exposure and outcome. Since genotypes do not change over time and are therefore present at birth before first drug use, a cross-sectional design may be used to examine the association between *CYP2D6* genotype and drug response and disease susceptibility.

Misclassification

A pharmacogenetic test should not only be able to detect a variation in DNA sequence, it should also provide a valid predictable result and be reproducible. False positive tests result in identification of responders instead of non-responders or those at high risk of developing adverse drug reactions instead of low-risk. False negatives are the opposite; responders identified as non-responders or patients at risk identified as low-risk [37]. The ideal pharmacogenetic test has a high sensitivity (the proportion of true positives which are correctly identified as such),

high specificity (the proportion of true negatives which are correctly identified), adds new information (post-test probability > pre-test probability) and is non-invasive and inexpensive.

In December 2004, the FDA approved the AmpliChip™ CYP450 Test as the first pharmacogenetic test using microarray technology [38]. The genotype results of the AmpliChip™ CYP450 Test for the CYP2D6 non-functional alleles (*3, *4, *5, *6) and gene duplication (*xN) were verified with existing methods (real time PCR) [39]. In addition, the predicted patient's phenotype was compared with the classical dextromethorphan phenotyping method, a specific CYP2D6 probe drug. The AmpliChip Test genotyping accuracy was 100% compared to routine methods for the five variant alleles analyzed. There was a perfect correlation observed between genotype results, phenotype prediction and measured phenotype for CYP2D6 PMs. Among 114 measured EMs, 108 were correctly predicted (95% sensitivity). The sensitivity of the AmpliChip Test was 42% in predicting IMs and only 6% in predicting UMs. The AmpliChip™ CYP450 Test appears to be a useful tool to discriminate PMs from EMs and may help clinicians to optimize pharmacotherapy by improving drug efficacy and reducing adverse drug reactions. Disadvantage of the AmpliChip Test are the high costs (ca. € 615; proposed CTG rate 2009) per patient. Standard pharmacogenetic testing using real time PCR for *CYP2D6**3, *4, *5, *6 and gene duplication cost ca. € 192 (proposed CTG rate 2009) per patient in our laboratory, but additional rarer variant alleles are missed in this analysis.

In our studies, we only determined *CYP2D6**4, because this polymorphism should predict >75% of PMs in a Caucasian population [30] and is the most cost-effective in this large number of subjects. However, other less frequent variant alleles (*3, *5, *6, *7, *8, *10, *41), were not assessed, which led to some misclassification in our reference (EM) group.

Clinical implementation

In the last few years, it became more and more obvious that pharmacogenetic testing can improve the clinical outcome of pharmacotherapy. The FDA even recently revised the drug labelling of mercaptopurine, azathioprine, irinotecan, warfarin and cetuximab to include pharmacogenetic recommendations. Why is genotyping not yet regularly performed in daily practice? Several aspects influence the clinical implementation of pharmacogenetic testing. These aspects are summarized in figure 1.

First of all, the concept that genetic variation leads to an altered drug plasma concentration should be clear, as is the case for *CYP2D6**4, when the metabolism of the drug is mainly via the CYP2D6 pathway. However, for genetic variation in other CYP450 enzymes (e.g., CYP3A4) or UGT2B7 this relationship is less clear. The correlation between genotype and phenotype should be high. Genotyping *CYP2D6**3, *4, *5, and *6 would predict the PM phenotype in Caucasians by about 98% [30]. The impact of a genetic polymorphism on drug response should have clinical consequences. If genetic variation predicts survival (e.g., cetuximab, tamoxifen) to a large extent or prevents serious adverse drug reactions (e.g., TPMT and myelosuppression),

acceptance of pharmacogenetic testing is more likely. At present, several barriers to the clinical implementation of pharmacogenetics exist. Some of these barriers were already discussed above, others will be reviewed below.

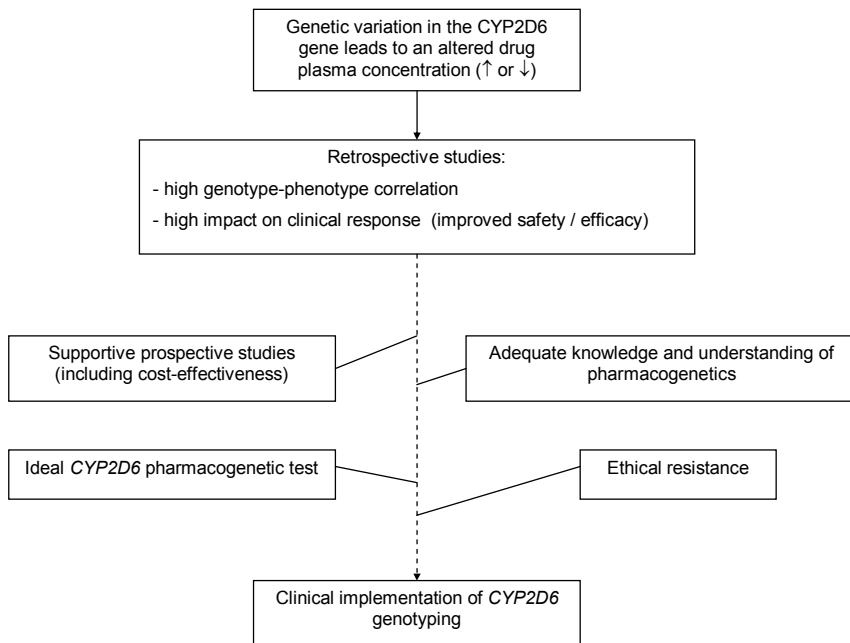


Figure 1. Flow chart of steps which should be undertaken to implement CYP2D6 testing in clinical practice [40-42]

Supportive prospective studies

In order to implement pharmacogenetic testing supportive prospective studies should be performed. In prospective studies, the added value of pharmacogenetic compared with conventional pharmacotherapy could be determined. For example, in one arm, genotyping is executed before the start of pharmacotherapy and doses of drugs metabolized by CYP2D6 are adjusted according to genotype. In the other arm the 'one dose fits all' approach is followed (standard pharmacotherapy). Drug efficacy and adverse drug reactions are monitored during the duration of the trial. Both arms should preferably be blinded to treatment as well as the treating physician.

However, large double-blind clinical trials are expensive and mainly carried out by the pharmaceutical industry. The pharmaceutical industry is usually not very interested to carry out these studies for drugs that already have marketing approval, although there are some exceptions e.g., cetuximab and pharmacogenetic testing for *KRAS* genotype.

Costs

Some experts believe that good evidence of comparative effectiveness is lacking for more than half of all medical care that is delivered in the United States [43]. For example, physicians' treatment choice often depends on own experience or judgement instead of evidence. Expensive imaging techniques are often requested, even if their benefits do not compensate additional costs. Although the FDA approved the AmpliChip™ CYP450 Test for pharmacogenetic testing of the most important CYP450 enzymes, marketplace adoption is not ensured. Since health care costs are rapidly increasing, the relative value of a new health-care technology should be determined in relation to additional costs [41]. Cost-effectiveness analysis (CEA) is a form of economic analysis that compares a new strategy with current practice. The result is expressed as an incremental cost-effectiveness ratio (ICER), e.g., the incremental cost to avoid one serious adverse drug reaction, or to achieve one additional therapeutic response for a genetic test [44]. If a pharmacogenetic test results in lower costs and greater health benefits compared with the standard strategy, it is considered 'cost-effective'.

There have been only a few pharmacoeconomic analyses for genetic variations performed so far, regarding polymorphisms in several cytochrome P450 (CYP) enzyme genes, thiopurine S-methyltransferase (TPMT) and angiotensin-converting enzyme (ACE) insertion deletion (ACE I/D). Most economic analyses reported that genetic testing was cost effective and often even clearly dominated standard strategies [45]. An important limitation of several of these studies was that a sufficient evidence-based rationale for an association between genotype and phenotype was lacking. The uncertainty of these pharmacoeconomic studies leads to postponement of implementation of pharmacogenetic testing in clinical practice.

Insurance companies will only pay for additional costs of pharmacogenetic testing if there is a proven benefit over standard therapies. In the Netherlands, the Dutch Health Insurance Board (CVZ) requests a cost-effectiveness analysis from the pharmaceutical industry for inclusion of new medicines in the reimbursement system to estimate costs and budget impact. Nowadays, pharmacogenetic testing is mainly integrated in the field of oncology, where targeted therapy is desirable and expensive. Insurance companies only reimburse the costs of trastuzumab (Herceptin®) in breast cancer patients expressing HER2 (human epidermal growth factor receptor 2), a member of the epidermal growth factor receptor (EGFR) family of tyrosine kinases and is involved in regulation of cell proliferation. Another example is cetuximab (Erbix®) or panitumumab (Vectibix®), monoclonal antibodies targeting the Epidermal Growth Factor Receptor (EGFR) in colorectal cancer. Recent data demonstrate that patients with *KRAS* wild-type metastatic colorectal cancer have a significantly higher chance to benefit from treatment with cetuximab or panitumumab. The costs are only reimbursed if a patient has the wild-type *KRAS* genotype determined by genetic testing.

Education

Understanding the mechanisms of genetic variation and its impact on drug efficacy and toxicity is the key to implement pharmacogenetic testing. Since pharmacogenetics constitutes a rapidly evolving research field, problems arise with knowledge. Both pharmacists as well as physicians require education at both undergraduate and postgraduate level in order to translate this kind of sophisticated information into practical prescribing information [40]. The Erasmus Medical University incorporated pharmacogenetic education in the curriculum of medical students. Pharmacy students are also trained in this field during their curriculum.

The G-standard of the Royal Dutch Association for the advancement of Pharmacy (KNMP) is the Dutch national drug database and contains drug (safety) information for all drugs registered in the Netherlands. Recently, pharmacogenetic information was added to the G-standard and gives recommendations for the extremes of metabolism (PM, UM). All computerized physician order entry (CPOE) systems are coupled to the G-standard and have integrated computerized clinical decision support. In this way pharmacists and physicians are informed about the influence of metabolizer status on drug response. However, genotyping is not yet regularly performed and education is definitely necessary.

Ethics

Is it ethical to continuously expose individuals to drugs with adverse drug reactions that can be potentially avoided, or expose them to drugs that are most likely inefficacious in some, while we have the knowledge and diagnostic opportunities to improve pharmacotherapy?

For example, in chapter 5 we found that breast cancer mortality is increased in tamoxifen users carrying the *CYP2D6**4 variant allele. Should we still prescribe tamoxifen to these patients? Contradictory results exist in the literature and further prospective studies should be performed in order to answer this question properly.

One of the ethical drawbacks for the introduction of a pharmacogenetic test might occur if the test may also predict which diseases we may get. Since cytochrome P450 enzymes metabolize not only drugs but also endogenous substances such as hormones, environmental chemicals and toxins, one might expect that variability in enzyme activity could result in an altered susceptibility to certain diseases. CYP metabolism may cause formation of toxic metabolites or reactive oxygen species, which may damage cellular function [46,47].

Therefore, we examined the influence of the *CYP2D6* genotype on the development of (multiple) basal cell carcinoma (chapter 5.2) and susceptibility to depression or anxiety (chapter 2.3), because these associations were suggested in medical literature. In none of them, we found an association between *CYP2D6* and disease susceptibility. At this moment, there is no drawback on the basis of ethical consideration to implement *CYP2D6* genotyping in daily practice, since no relevant association between *CYP2D6* genotype and disease has been found.

Personalized medicine in the future

Many drugs are still prescribed according to the model that 'one dose fits all', although we know that some patients may not benefit from the drug or experience adverse drug reactions. A better understanding of why people do not respond to drug therapy or experience adverse drug reactions could improve the safety and efficacy of pharmacotherapy. In personalized medicine, medical care is tailored to an individual's needs on the basis of patient's specific characteristics, such as age, gender, renal and liver function, underlying diseases or drug interactions and genetic information. However, not only patient's characteristics play a role in optimizing pharmacotherapy, also the precision of a disease diagnosis is important [48]. When the diagnosis of an underlying disease is not properly determined, response to treatment is often uncertain. For example, the etiology of depression is complex and unclear, while antidepressant pharmacotherapy is rather inefficacious: 50% of the patients will not respond adequately to first treatment [3]. On the other hand, some diseases, like HER2 positive breast cancer, can be precisely diagnosed, which can be targeted with trastuzumab (Herceptin®), a monoclonal antibody that binds selectively to the HER2 protein. Diseases in between are defined as empirical medicine, in which patterns of symptoms are recognized and the result of therapy can be predicted (e.g. myocardial infarction, stroke). In the ideal situation of 'personalized medicine' the disease can be diagnosed precisely and drug response can be predicted accurately, leading to improved health care [48].

Conclusion

Although pharmacogenetic testing can improve the clinical outcomes of pharmacotherapy, it is only performed for a very limited number of drugs in clinical practice. In order to implement CYP2D6 genotyping in clinical practice some barriers have to be overcome. Further supportive prospective studies should be done, in which standard pharmacotherapy is compared with genotype-based adjusted pharmacotherapy. In these studies, cost-effectiveness of pharmacogenetic testing should be investigated as well. If genetic variation in drug-metabolizing enzymes would accurately predict clinical response at low costs, implementation is more likely. Both pharmacists and physicians require education at both undergraduate and postgraduate level in order to translate genetic information into practical prescribing information.

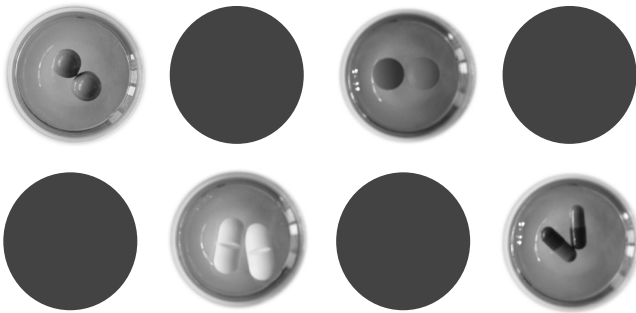
This thesis provides more evidence that pharmacogenetic testing could contribute to the quality and safety of pharmacotherapy, but we should keep in mind that not all variation in drug response is explained by genes.

References

1. Evans WE, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348(6):538-49.
2. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5(1):6-13.
3. Roose SP, Schatzberg AF. The efficacy of antidepressants in the treatment of late-life depression. *J Clin Psychopharmacol* 2005;25(4 Suppl 1):S1-7.
4. Mulder H, Wilmink FW, Belitser SV, Egberts AC. The association between cytochrome P450-2D6 genotype and prescription of antiparkinsonian drugs in hospitalized psychiatric patients using antipsychotics: a retrospective follow-up study. *J Clin Psychopharmacol* 2006;26(2):212-5.
5. Adroque HJ, Madias NE. Hyponatremia. *N Engl J Med* 2000;342(21):1581-9.
6. Rasmussen BB, Brosen K. Is therapeutic drug monitoring a case for optimizing clinical outcome and avoiding interactions of the selective serotonin reuptake inhibitors? *Ther Drug Monit* 2000;22(2):143-54.
7. Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, et al. CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. *Acta Psychiatr Scand* 2001;104(3):173-92.
8. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev* 2006;58(3):521-90.
9. Grasmader K, Verwohlt PL, Rietschel M, Dragicevic A, Muller M, Hiemke C, et al. Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. *Eur J Clin Pharmacol* 2004;60(5):329-36.
10. Fux R, Morike K, Prohmer AM, Delabar U, Schwab M, Schaeffeler E, et al. Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. *Clin Pharmacol Ther* 2005;78(4):378-87.
11. Kirchheiner J, Heesch C, Bauer S, Meisel C, Seringer A, Goldammer M, et al. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2004;76(4):302-12.
12. Lennard MS, Silas JH, Freestone S, Ramsay LE, Tucker GT, Woods HF. Oxidation phenotype--a major determinant of metoprolol metabolism and response. *N Engl J Med* 1982;307(25):1558-60.
13. Nozawa T, Taguchi M, Tahara K, Hashimoto Y, Igarashi N, Nonomura M, et al. Influence of CYP2D6 genotype on metoprolol plasma concentration and beta-adrenergic inhibition during long-term treatment: a comparison with bisoprolol. *J Cardiovasc Pharmacol* 2005;46(5):713-20.
14. Rau T, Heide R, Bergmann K, Wuttke H, Werner U, Feifel N, et al. Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. *Pharmacogenetics* 2002;12(6):465-72.
15. Wuttke H, Rau T, Heide R, Bergmann K, Bohm M, Weil J, et al. Increased frequency of cytochrome P450 2D6 poor metabolizers among patients with metoprolol-associated adverse effects. *Clin Pharmacol Ther* 2002;72(4):429-37.
16. Zineh I, Beitelshes AL, Gaedigk A, Walker JR, Pauly DF, Eberst K, et al. Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. *Clin Pharmacol Ther* 2004;76(6):536-44.
17. Caraco Y, Sheller J, Wood AJ. Pharmacogenetic determinants of codeine induction by rifampin: the impact on codeine's respiratory, psychomotor and miotic effects. *J Pharmacol Exp Ther* 1997;281(1):330-6.
18. Fromm MF, Eckhardt K, Li S, Schanzle G, Hofmann U, Mikus G, et al. Loss of analgesic effect of morphine due to coadministration of rifampin. *Pain* 1997;72(1-2):261-7.
19. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 2003;3(3):136-58.

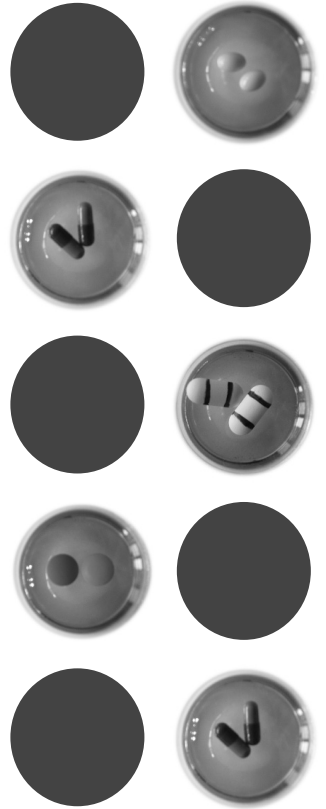
20. Goetz MP, Knox SK, Suman VJ, Rae JM, Safgren SL, Ames MM, et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res Treat* 2007;101(1):113-21.
21. Bonnetterre J, Buzdar A, Nabholz JM, Robertson JF, Thurlimann B, von Euler M, et al. Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma. *Cancer* 2001;92(9):2247-58.
22. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol* 2008;9(1):45-53.
23. Punglia RS, Burstein HJ, Winer EP, Weeks JC. Pharmacogenomic variation of CYP2D6 and the choice of optimal adjuvant endocrine therapy for postmenopausal breast cancer: a modeling analysis. *J Natl Cancer Inst* 2008;100(9):642-8.
24. American Society of Clinical Oncology (ASCO). In: May 29-June 2; 2009; Orlando, Florida; 2009.
25. Human Cytochrome P450 (CYP) Allele Nomenclature Committee <http://www.cypalleles.ki.se/> 2009.
26. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoeconomic and clinical aspects. *Pharmacol Ther* 2007;116(3):496-526.
27. Bebia Z, Buch SC, Wilson JW, Frye RF, Romkes M, Cecchetti A, et al. Bioequivalence revisited: influence of age and sex on CYP enzymes. *Clin Pharmacol Ther* 2004;76(6):618-27.
28. Frank D, Jaehde U, Fuhr U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur J Clin Pharmacol* 2007;63(4):321-33.
29. Streetman DS, Bertino JS, Jr., Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics* 2000;10(3):187-216.
30. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002;3(2):229-43.
31. 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(2):234-42.
32. 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(9):1623-33.
33. Mulder H, Herder A, Wilimink FW, Tamminga WJ, Belitser SV, Egberts AC. The impact of cytochrome P450-2D6 genotype on the use and interpretation of therapeutic drug monitoring in long-stay patients treated with antidepressant and antipsychotic drugs in daily psychiatric practice. *Pharmacoeconomic Drug Saf* 2006;15(2):107-14.
34. Sjoqvist F, Eliasson E. The convergence of conventional therapeutic drug monitoring and pharmacogenetic testing in personalized medicine: focus on antidepressants. *Clin Pharmacol Ther* 2007;81(6):899-902.
35. Kootstra-Ros JE, Van Weelden MJ, Hinrichs JW, De Smet PA, van der Weide J. Therapeutic drug monitoring of antidepressants and cytochrome p450 genotyping in general practice. *J Clin Pharmacol* 2006;46(11):1320-7.
36. Kelly PJ, Lim LL. Survival analysis for recurrent event data: an application to childhood infectious diseases. *Stat Med* 2000;19(1):13-33.
37. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends Mol Med* 2001;7(5):201-4.
38. Roche Diagnostics. AmpliChip CYP450 for in vitro diagnostic use. <http://www.amplichip.us/>.

39. Rebsamen MC, Desmeules J, Daali Y, Chiappe A, Diemand A, Rey C, et al. The AmpliChip CYP450 test: cytochrome P450 2D6 genotype assessment and phenotype prediction. *Pharmacogenomics J* 2009;9(1):34-41.
40. Clemerson JP, Payne K, Bissell P, Anderson C. Pharmacogenetics, the next challenge for pharmacy? *Pharm World Sci* 2006;28(3):126-30.
41. Deverka PA, McLeod HL. Harnessing economic drivers for successful clinical implementation of pharmacogenetic testing. *Clin Pharmacol Ther* 2008;84(2):191-3.
42. McKinnon RA, Ward MB, Sorich MJ. A critical analysis of barriers to the clinical implementation of pharmacogenomics. *Ther Clin Risk Manag* 2007;3(5):751-9.
43. Orszag PR, Ellis P. Addressing rising health care costs--a view from the Congressional Budget Office. *N Engl J Med* 2007;357(19):1885-7.
44. Dervieux T, Bala MV. Overview of the pharmacoeconomics of pharmacogenetics. *Pharmacogenomics* 2006;7(8):1175-84.
45. Vegter S, Boersma C, Rozenbaum M, Wilffert B, Navis G, Postma MJ. Pharmacoeconomic evaluations of pharmacogenetic and genomic screening programmes: a systematic review on content and adherence to guidelines. *Pharmacoeconomics* 2008;26(7):569-87.
46. Bast A, Haenen GR, Doelman CJ. Oxidants and antioxidants: state of the art. *Am J Med* 1991;91(3C):2S-13S.
47. Villeneuve JP, Pichette V. Cytochrome P450 and liver diseases. *Curr Drug Metab* 2004;5(3):273-82.
48. Christensen CM, Grossman H, Hwang J. *The Innovator's prescription*. New York: McGraw-Hill Companies; 2009.



Summary

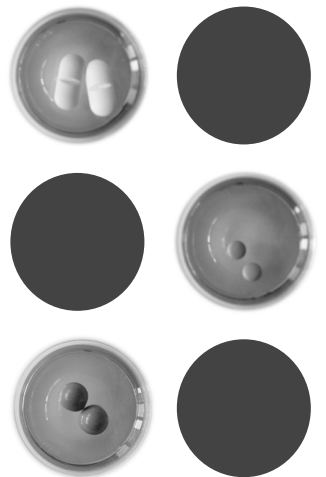
CHAPTER 7



Summary



CHAPTER 7



Summary

Patients differ in their response to drugs. On average only 40% of all patients will benefit from a particular drug. Some patients will experience adverse drug reactions, while others will not. Although variability in drug response can be explained by age, gender, renal and liver function, underlying disease or drug interactions, genetic factors also contribute to differences in drug response. CYP2D6 is responsible for the metabolism of approximately 25% of all drugs metabolized by the cytochrome P450 system. Approximately 5-10% of the Caucasian population completely lacks CYP2D6 enzyme activity, and is therefore at increased risk of suffering from adverse drug reactions or ineffectiveness.

Since cytochrome P450 enzymes metabolize not only drugs but also endogenous substances such as hormones, environmental chemicals and toxins, one might expect that variability in enzyme activity could result in an altered susceptibility to certain diseases.

Therefore, the aim of this thesis was to study the influence of genetic variation in the *CYP2D6* gene on drug response and disease susceptibility from an epidemiological perspective. The studies described in this thesis are embedded in the Rotterdam Study, a population-based cohort study among 7983 inhabitants of Ommoord, a district in Rotterdam, aged 55 years or over.

Chapter 1 provides a general introduction to this thesis. In **chapter 2** the influence of CYP2D6 variant alleles on the response to antidepressants is described as well as the association between *CYP2D6* genotype and risk of depression. In patients with decreased CYP2D6 enzyme activity, plasma concentrations of some antidepressants are higher, which could lead to adverse drug reactions requiring dose reduction or discontinuation of the drug. In **chapter 2.1** the influence of the *CYP2D6**4 polymorphism on dose, switching and discontinuation of antidepressants is studied in a cohort of 1198 incident antidepressant users from the Rotterdam Study. In users of tricyclic antidepressants (TCAs) the risk of switching to another antidepressant within 45 days was significantly higher in poor metabolizers (*4/*4) than in extensive metabolizers (*1/*1). A switch within 45 days is assumed to occur due to intolerance of the drug, since the efficacy of an antidepressant can only be assessed after at least 6 weeks of therapy. The increased risk of switching to another antidepressant was not seen in SSRI users. After titrating the dose of an antidepressant to an optimal level of effectiveness with minimal ADRs, the mean antidepressant dose in both TCA and SSRI users was significantly lower in PMs than in EMs. No association was found between *CYP2D6* genotype and discontinuation of antidepressants.

Chapter 2.2 focuses on the association between *CYP2D6* genotype and serum sodium concentration in antidepressant users. Hyponatremia can be caused by antidepressant use and is thought to occur due to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). The serum sodium concentration in PMs was significantly lower in users of an antidepressant in comparison to CYP2D6 EMs, especially in TCA users. CYP2D6 PMs might be at increased risk of developing symptoms of hyponatremia.

In **chapter 2.3**, we studied the association between *CYP2D6* genotype and risk of depression and anxiety, since it was hypothesized by others that *CYP2D6* PMs have a lower baseline serotonin concentration in various brain regions, due to impaired metabolism of 5-methoxytryptamine to serotonin, and are therefore more prone to depression or anxiety. The risk of major depression in *CYP2D6* *4/*4 was not significantly different from extensive metabolizers (OR=0.85; 95%CI 0.36-2.00). Neither did we find an association between *CYP2D6* genotype and minor depression (OR=1.56; 95%CI 0.69-3.52) or anxiety disorders (OR=1.19; 95% CI 0.68-2.09).

The association between the *CYP2D6**4 polymorphism and heart rate and blood pressure in β -blocker users is described in **chapter 3**. *CYP2D6**4 genotype in combination with information on heart rate and blood pressure was available in 1430 and 1533 subjects on β -blockers respectively. The most frequently used β -blockers were atenolol, metoprolol and bisoprolol during the total study period.

In *CYP2D6* *4/*4 PMs the adjusted heart rate in users of metoprolol, the β -blocker most extensively metabolized by *CYP2D6* (70-80%), was 8.5 beats/min lower than in *1/*1 extensive metabolizers (EMs) ($p < 0.001$), leading to an increased risk of bradycardia in PMs. These effects were also observed for β -blockers selectively metabolized by *CYP2D6* (metoprolol, carvedilol, nebivolol, propranolol and alprenolol). The diastolic blood pressure in PMs was significantly lower in users of β -blockers metabolized by *CYP2D6*. *CYP2D6* PMs should be carefully monitored in clinical practice, since they have an increased risk of bradycardia.

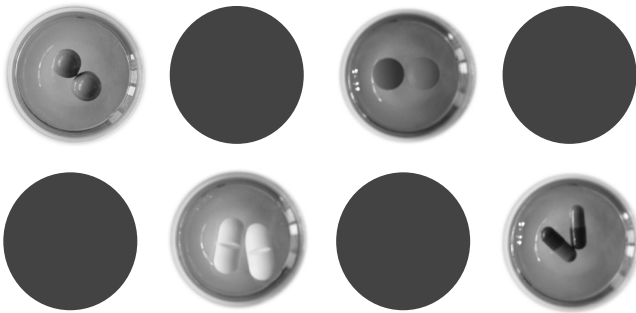
In **chapter 4** variation in the *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* gene is studied in relation to co-prescription of other analgesics and switching to opioids in codeine users. The analgesic effect of codeine is mostly dependent on its metabolism to morphine by *CYP2D6*. Apart from *CYP2D6*, codeine is metabolized by *CYP3A4* and *UGT2B7*. Interestingly, a decreased effectiveness of codeine was not observed in patients with the *CYP2D6**4/*4 genotype, while morphine concentrations are expected to be low in these subjects. Patients carrying a *CYP3A4**1B allele more often switch to an opioid, and have more co-prescription of other analgesics than non-carriers. In this study, we did not find an association between rs4274916 as a proxy for *UGT2B7**2 and codeine effectiveness.

In **chapter 5** we investigated *CYP2D6* genotype in relation to cancer. **Chapter 5.1** describes the impact of impaired *CYP2D6* metabolism on breast cancer survival in tamoxifen users. Tamoxifen users with absent *CYP2D6* enzyme activity are at increased risk of breast cancer mortality (HR=4.1, CI 95% 1.1-15.9, $p=0.041$) compared to extensive metabolizers. Intermediate metabolizers tended to have an increased risk of breast cancer mortality. These results strengthen the hypothesis that endoxifen formation is strongly dependent on *CYP2D6*. In **chapter 5.2** the influence of *CYP2D6* genotype on basal cell carcinoma (BCC) susceptibility and subsequent BCCs was investigated. BCC cases were extracted from the nationwide

network and registry of histo- and cytopathology in the Netherlands (PALGA). In total, 369 (5.8%) out of 6,382 patients developed a BCC and 128 patients multiple BCCs. This study demonstrated that *CYP2D6* genotype is not associated with first occurrence of BCC (HR=1.01; 95% CI 0.82-1.25; p=0.91). Possibly, males with the wild type genotype have a reduced risk of developing multiple BCC.

Finally, in **chapter 6** we discuss the main findings of this thesis and speculate on the implementation of pharmacogenetic testing in clinical practice.

Overall, in this thesis we have provided more evidence that pharmacogenetics could contribute to the quality and safety of pharmacotherapy. Pharmacogenetic testing should be part of overall quality improvement in healthcare for a number of drugs (e.g. tamoxifen). However, we should keep in mind that not all variation in drug response is explained by pharmacogenetics. Accurate interpretation of data is necessary and pharmacists could play a role in this.



Appendices

CHAPTER 8

Samenvatting

Patiënten reageren verschillend op geneesmiddelen. Gemiddeld genomen zal 40% van alle patiënten een goede respons op geneesmiddelen hebben. Echter, sommige patiënten zullen bijwerkingen ondervinden, terwijl anderen hier geen last van hebben. Variabiliteit in geneesmiddelrespons kan onder andere verklaard worden door leeftijd, geslacht, nier- en leverfunctie, onderliggende aandoening(en) en geneesmiddel interacties. Daarnaast kunnen genetische factoren ook bijdragen aan de verschillen in geneesmiddel respons. CYP2D6 is verantwoordelijk voor het metabolisme van circa 25% van alle geneesmiddelen, die door het CYP450 systeem gemetaboliseerd worden. Ongeveer 5-10% van de Kaukasische bevolking brengt geen CYP2D6 enzym activiteit tot expressie, zogenaamde poor metabolizers (PMs). Deze verminderde enzymactiviteit kan verklaard worden door genetische variatie in CYP2D6. Door verandering van 1 basepaar in het DNA coderend voor het CYP2D6 enzym (een zogenaamd polymorfisme), wordt geen of minder eiwit geproduceerd. Het belangrijkste polymorfisme dat het poor metabolizer fenotype veroorzaakt is een mutatie G > A in het *CYP2D6* gen, ook wel *4 genoemd.

Door de afwezigheid van het CYP2D6 enzym zijn plasmaconcentratie en totale blootstelling van geneesmiddelen, die gemetaboliseerd worden door CYP2D6, hoger dan in extensive metabolizers, waardoor PMs een verhoogde kans op bijwerkingen hebben. In geval van prodrugs waarbij CYP2D6 betrokken is bij de activatie van het geneesmiddel, hebben CYP2D6 PMs juist een lagere concentratie van de actieve metaboliet, wat kan leiden tot verminderde effectiviteit.

Aangezien cytochroom P450 enzymen niet alleen geneesmiddelen metaboliseren, maar ook endogene stoffen (hormonen), chemicaliën en toxinen in het milieu omzetten, zou variabiliteit in enzymactiviteit ook kunnen leiden tot een veranderde kans op bepaalde ziekten.

Het doel van dit proefschrift was het bestuderen van de invloed van genetische variatie in het *CYP2D6* gen op geneesmiddel respons en het ontstaan van ziekten. Alle studies die hier worden beschreven zijn uitgevoerd binnen het Rotterdamse ERGO-onderzoek (Erasmus Rotterdam Gezondheid en Ouderen), internationaal bekend als 'the Rotterdam Study'. Dit is een prospectief bevolkingsonderzoek naar de frequentie en oorzaken van chronische ziekten bij ouderen.

Hoofdstuk 1 geeft een algemene inleiding op dit proefschrift. In **hoofdstuk 2** wordt de invloed van het *CYP2D6* variant genotype op antidepressiva respons beschreven evenals de associatie tussen het *CYP2D6* genotype en risico op depressie. Plasma concentraties van een aantal antidepressiva zijn hoger in patiënten met verminderde CYP2D6 enzym activiteit, wat bijwerkingen zou kunnen geven. Dit zou kunnen leiden tot dosisverlaging of staken van het antidepressivum. In **hoofdstuk 2.1** wordt de invloed van het *CYP2D6**4 genotype op dosering, switchen en staken van antidepressiva bestudeerd in een cohort van 1198 incidente antidepressiva gebruikers in ERGO. In gebruikers van tricyclische antidepressiva (TCA's) was het risico op switchen naar een ander antidepressivum binnen 45 dagen in CYP2D6 'poor meta-

bolizers' significant verhoogd ten opzichte van patiënten met een normale enzymactiviteit. Er wordt verwacht dat een switch binnen 45 dagen optreedt als gevolg van geneesmiddelintolerantie, aangezien de werkzaamheid van een antidepressivum pas na 6 weken kan worden beoordeeld. Een verhoogd risico op switchen werd niet gezien voor SSRI gebruikers.

Nadat de antidepressiva dosering was getitreerd tot een optimale effectiviteit met minimale bijwerkingen, was de dosering significant lager in PMs dan in EMs in zowel TCA als SSRI gebruikers. Er werd geen associatie gevonden tussen *CYP2D6* genotype en het staken van de therapie met antidepressiva.

Hoofdstuk 2.2 focust op de associatie tussen *CYP2D6* genotype en plasma natriumspiegel in antidepressiva gebruikers. Antidepressiva kunnen hyponatriemie veroorzaken door het optreden van het syndroom van inadequate secretie van antidiuretisch hormoon (SIADH). In deze studie vonden wij dat de natrium concentratie in PMs significant lager was in vergelijking met *CYP2D6* EMs in antidepressiva gebruikers, met name in gebruikers van TCA's. Hierdoor zouden *CYP2D6* PMs een verhoogd risico op het ontwikkelen van symptomen van hyponatriemie kunnen hebben.

In **hoofdstuk 2.3** wordt de associatie tussen het *CYP2D6* genotype en het risico op depressie en angst bestudeerd. In de literatuur werd gesuggereerd dat *CYP2D6* PMs een lagere concentratie serotonine hebben in diverse gebieden in de hersenen door verminderd metabolisme van 5-methoxytryptamine in serotonine, waardoor zij vatbaarder voor depressie of angst kunnen zijn. Het risico op major depressie in *CYP2D6* *4/*4 was niet verschillend van extensive metabolizers (OR=0.85; 95%CI 0.36-2.00). We vonden ook geen associatie tussen *CYP2D6* genotype en minor depressie (OR=1.56; 95%CI 0.69-3.52) of angststoornissen (OR=1.19; 95% CI 0.68-2.09).

De associatie tussen het *CYP2D6**4 polymorfisme en hartslag en bloeddruk in β -blokker gebruikers wordt beschreven in **hoofdstuk 3**. In totaal hadden wij gegevens over het *CYP2D6**4 genotype in combinatie met informatie over hartslag en bloeddruk in respectievelijk 1430 en 1533 β -blokker gebruikers. De meest gebruikte β -blokkers in deze studie waren atenolol, metoprolol en bisoprolol.

In *CYP2D6* *4/*4 PMs was de gecorrigeerde hartslag in gebruikers van metoprolol, de β -blokker die in hoge mate door *CYP2D6* wordt gemetaboliseerd (70-80%), 8.5 slag/minuut lager dan in *1/*1 extensive metabolizers (EMs) ($p < 0.001$). Hierdoor hebben deze PMs een verhoogd risico op bradycardie. Deze effecten werden ook geobserveerd voor β -blokkers, die selectief door *CYP2D6* werden gemetaboliseerd (metoprolol, carvedilol, nebivolol, propranolol and alprenolol). De diastolische bloeddruk was significant lager in *CYP2D6* PMs in gebruikers van β -blokkers gemetaboliseerd door *CYP2D6*. *CYP2D6* PMs zouden in de dagelijkse praktijk intensiever moeten worden gevolgd, aangezien zij een verhoogd risico op bradycardie hebben.

In **hoofdstuk 4** wordt de genetische variatie in het *CYP2D6*, *CYP3A4/3A5* en *UGT2B7* gen bestudeerd in relatie tot co-prescriptie van andere analgetica en switchen naar opioïden in

codeïne gebruikers. Het analgetisch effect van codeïne is voornamelijk afhankelijk van de omzetting van codeïne in het actieve morfine door CYP2D6. Naast CYP2D6 wordt codeïne gemetaboliseerd door CYP3A4 en UGT2B7. Opmerkelijk was dat een verlaagde effectiviteit van codeïne niet werd gevonden in patiënten met het *CYP2D6**4/*4 genotype, terwijl verwacht wordt dat morfine concentraties in deze patiënten laag zijn. Draggers van het *CYP3A4**1B allel switchen vaker naar een ander opioïd en hadden meer co-prescriptie van andere analgetica dan niet-dragers. In deze studie werd geen relatie gevonden tussen rs4274916 als een proxy voor UGT2B7*2 en codeïne effectiviteit.

In **hoofdstuk 5** werd het *CYP2D6* genotype onderzocht in relatie tot kanker. **Hoofdstuk 5.1** beschrijft de impact van verstoorde *CYP2D6* metabolisme op borstkanker overleving in tamoxifen gebruikers. Tamoxifen gebruikers met een verminderde *CYP2D6* enzymactiviteit hebben een verhoogd risico op borstkanker mortaliteit (HR=4.1, CI 95% 1.1-15.9, p=0.041) vergeleken met extensive metabolizers. Intermediate metabolizers lijken een verhoogd risico te hebben op borstkanker mortaliteit. Genotypen voor aanvang van endocriene therapie bij borstkanker patiënten zou *CYP2D6* PMs kunnen identificeren, die waarschijnlijk beter reageren op aromataseremmers dan op therapie met tamoxifen. Prospectieve studies, waaronder studies naar de kosteneffectiviteit, zouden moeten worden uitgevoerd om de implementatie van *CYP2D6* genotypen in de kliniek te ondersteunen.

In **hoofdstuk 5.2** wordt de invloed van het *CYP2D6* genotype op het ontstaan van basaalcelcarcinomen (BCC) bestudeerd. BCC gevallen werden geëxtraheerd uit het Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA). In totaal ontwikkelden 369 van de 6382 patiënten (5.8%) een basaalcelcarcinoom, waarvan 128 patiënten meer dan eenmaal. Onze studie laat zien dat het *CYP2D6* genotype niet geassocieerd is met het ontstaan van een BCC (HR=1.01; 95% CI 0.82-1.25; p=0.91). Mannen met het wildtype genotype hebben mogelijk een verminderd risico op het ontwikkelen van meerdere basaalcelcarcinomen.

In **hoofdstuk 6** worden de belangrijkste onderzoeksresultaten bediscussieerd en wordt er gespeculeerd over de implementatie van genotypen in de kliniek.

Concluderend geeft dit proefschrift meer bewijs dat *CYP2D6* genotyping bijdraagt aan de kwaliteit en veiligheid van de farmacotherapie. Farmacogenetische testen zouden voor sommige geneesmiddelen (bv. tamoxifen) onderdeel moeten uitmaken van de dagelijkse praktijk om de gezondheidszorg te verbeteren. Echter, men moet rekening houden met het feit dat niet alle variatie in geneesmiddelrespons wordt verklaard door de farmacogenetica. Interpretatie van de verkregen data is noodzakelijk, waarbij apothekers een belangrijke rol kunnen spelen.

Dankwoord

Promoveren is naast het behalen van de academische graad van doctor ook een reis door de wonderlijke wereld van wetenschap. Ik ben iedereen die mij heeft geholpen bij het tot stand komen van dit proefschrift bijzonder dankbaar.

Allereerst wil ik mijn beide promotoren, Prof. dr. Bruno Stricker, hoogleraar farmaco-epidemiologie en Prof. dr. Arnold Vulto, hoogleraar Ziekenhuisfarmacie en Praktische Farmacotherapie, bedanken voor het mogelijk maken van deze reis. Bruno, jouw enorme kennis, levenservaring, betrokkenheid bij mijn onderzoek en precisie heb ik zeer gewaardeerd. Jij zorgde er altijd voor dat ik zeker wist dat alles tot in detail was uitgezocht. Arnold, meer vanaf de zijlijn gaf jij richting aan mijn proefschrift. Jouw enthousiasme voor de ziekenhuisfarmacie, wetenschap en Washington is zeer aanstekelijk.

Mijn copromotoren, dr. L.E. Visser, beste Loes, de snelheid waarmee je mijn manuscripten las en mijn e-mail beantwoordde, ondanks je volle agenda, was uniek en stelde ik zeer op prijs. Ik kon altijd bij jou terecht voor een gezellig praatje, promotiezaken en advies. Dr. T. (Teun) van Gelder, als internist-nefroloog gaf jij meer klinische inbreng aan mijn onderzoek. Naast onze gezamenlijke liefde voor Heavenly Hazels (DE koffie) en swirl-ijsjes, blijken we nog veel meer gemeen te hebben.

De begeleiding vanuit meerdere invalshoeken (farmaco-epidemiologie, klinische farmacie, interne geneeskunde en klinische chemie) heb ik als zeer waardevol en vruchtbaar ervaren.

Prof. dr. J. Lindemans, prof. dr. A.G. Uitterlinden en prof. dr. A.C.G. Egberts, dank ik voor hun bereidheid om zitting te nemen in de kleine commissie en voor de inhoudelijke beoordeling van dit proefschrift.

Sehr geehrte prof. dr. Julia Kirchheiner, es ist uns eine Ehre eine Expertin auf dem Gebiet der Pharmakogenetik am Tisch begrüßen zu dürfen. Ihre Arbeit war immer beispielhaft!

Ook dank aan prof. dr. W.M.A. Verhoeven voor zijn bereidheid om zitting te nemen in de grote commissie om de psychiatrische visie te vertegenwoordigen.

Ron van Schaik en Marianne van Fessem ben ik zeer erkentelijk voor het bepalen van het CYP2D6*4 polymorfisme. Ron, jij kwam altijd even buurten of ik nog iets spannends had gevonden. Bedankt voor je interesse in mijn onderzoek en het indienen van mijn abstract bij ASCPT. Zonder jou had ik dit mooie congres waarschijnlijk moeten missen. Ik hoop op een vruchtbare samenwerking in de toekomst.

Alle co-auteurs ben ik dankbaar voor hun bijdrage aan de diverse papers.

Alle collega's van de farmaco-epidemiologie Albert-Jan, Ana, Ann, Anne, Bert, Carmen, Caroline, Charlotte, Claire, Daan, Dika, Eline, Emine, Eva, Fatma, Gianluca, Jeanne, Katia, Laura, Marissa, Mark, Marlies, Martina, Matthijs, Mendel, Miriam, Rikje, Roelof, Sabine, Sandra, Seppe, Toke, Vera en Yannick. Wil ik bedanken voor de gezellige tijd en vele discussies tijdens de staf. En uiteraard hebben we leuke tijden beleefd tijdens de ICPE!

Charlotte, samen de NIHES cursussen doorlopen, koffie gedronken in de DE-koffiebar, vele statistiek problemen opgelost, farmacologische mechanismen ontrafeld, etc. Ik kon bij jou altijd binnenlopen voor advies en gezelligheid. Bedankt dat je mij terzijde wilt staan bij de verdediging.

Mijn vele kamergenoten, die ik door de jaren heen heb gehad. Allereerst Matthijs, in het begin een stille jongen, die ik bijna niet van zijn werk wilde afhaken, maar waar ik altijd met mijn vragen en SPSS problemen terecht kon. Bedankt voor alle steun de afgelopen jaren! Leuk dat we nu met z'n drieën bijna tegelijkertijd promoveren.....

Mark, jij zorgde altijd voor de nodige afleiding op het werk en wist altijd het laatste nieuws. Als mijn computer vast liep, draaide je je gelijk om en keek over mijn schouder mee. Jammer, dat je er vandaag niet bij kunt zijn vanwege je onderzoek in Boston. Laura, jij bent het levende bewijs dat onderzoek erg afhangt van de kwaliteit van de data. Succes met het afronden van jouw promotie-onderzoek! Toke, als ijverige, ietwat onzekere student kwam je bij ons binnen op de epidemiologie. Ik denk dat jij je het afgelopen jaar enorm ontwikkeld hebt. Rikje, leuk dat jij met mijn data gelijk aan de slag kon. Onze samenwerking heeft tot een mooie paper geleid. En ook jij hebt een passie voor verre reizen (hoeveel procent van de Wereld hebben we nu gezien?).

Alle andere collega-onderzoekers van de afdeling Epidemiologie Abbas, Arfan, Ben, Elisabeth, Frank-Jan, Germaine, Isabella, Janine, Jory, Julia, Lintje, Marielle, Meike, Monika, wil ik bedanken voor de gezellige lunches en borrels.

Mijn (oud-)collega's in de apotheek Andras, Anouk, Asmar, Bart, Bregje, Els, Carolien, Claartje, Delia, Frederike, Heleen, Jan-Dietert, Laureen, Lidwien, Lyonne, Maarten, Maren, Margreet, Mila, Paul, Patricia, Peter, Pieter, Reinier, Ryan, Ron, Satu, Sonja, Valentina en Yves. Bedankt voor jullie interesse in mijn onderzoek en begrip. Sonja, dank voor de succesvolle samenwerking en betrokkenheid bij mijn onderzoek. Bedankt nog voor alle tips bij de voorbereiding van ons huwelijk....

Collega onderzoekers Anna, Brenda, Ferdi, Maurice en sinds kort Rachida. Veel succes met het afronden van jullie promotie-onderzoek. Liselotte, als ik het even niet zag zitten, kon ik altijd mijn hart bij jou storten (en andersom). Bedankt voor alle gezellige lunches bij WP!

Caroline, Else en Susan, bedankt voor de gezellige meiden-weekendjes, etentjes, discussies en perspectief van mede-apothekers. Else, succes met het afronden van jouw promotieonderzoek in Zweden. Caroline (hoogzwanger), ik hoop dat je er vandaag bij kunt zijn! Inge en Esther, mijn sportmaatjes, voor het stimuleren om toch te gaan sporten; ook al had ik mijn eten 5 minuten daarvoor naar binnen gewerkt. Froukje en Brechtje, vriendinnen sinds de lagere school, ik heb nu meer tijd om af te spreken! Alle andere vrienden en vriendinnen wil ik bedanken voor alle steun en gezelligheid.

Mijn ouders wil ik bedanken voor hun interesse in mijn onderzoek. Trots op hun dochter, maar ook bezorgd om de lange werkdagen. Mam, uiteindelijk ben ik toch nog 'doctor' geworden! (al is dat nog geen dokter...). Ook mijn schoonouders ben ik dankbaar voor alle steun en gezelligheid. Mijn broer en zus, die altijd zorgen voor de nodige afleiding en ontspanning (wanneer gaan we weer wii-en?). Lisette, bedankt dat je mij bij wilt staan bij de verdediging van dit proefschrift (ik zadel je wel op met een hoop taken: eerste getuige, nu weer paranimf...).

Lieve Dave, na bijna 11 jaar samen ken je inmiddels vele medische en farmaceutische termen, maar ZIP2D6 blijft lastig.... Jij hielp mij altijd de dingen te relativieren, als het onderzoek even niet mee zat. Ondanks mijn promotie/opleiding tot ziekenhuisapotheker en jouw master aan de VU in de avonduren vonden we toch altijd tijd voor elkaar. You make my life more colourful!

Monique



List of Publications

Bijl MJ, Visser LE, Hofman A, Vulto AG, van Gelder T, Stricker BH, van Schaik RH. Influence of the CYP2D6*4 polymorphism on dose, switching and discontinuation of antidepressants. *British Journal of Clinical Pharmacology* 2008; 65: 558-64.

Bijl MJ, Visser LE, van Schaik RH, Kors JA, Witteman JCM, Hofman A, Vulto AG, van Gelder T, Stricker BH. Genetic variation in the CYP2D6 gene is associated with a lower heart rate and blood pressure in β -blocker users. *Clinical Pharmacology and Therapeutics* 2009; 85: 45-50.

Bijl MJ, van Schaik RH, Lammers LA, Hofman A, Vulto AG, van Gelder T, Stricker BH, Visser LE. The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users. *Breast Cancer Research and Treatment*; in press.

Bijl MJ, Luijendijk HJ, van den Berg JF, Visser LE, van Schaik RH, Hofman A, Vulto AG, van Gelder T, Tiemeier H, Stricker BH. Association between the CYP2D6*4 polymorphism and depression or anxiety in the elderly. *Pharmacogenomics* 2009; 10(4): 541-7.

Kwadijk-de Gijssel S, **Bijl MJ**, Visser LE, van Schaik RHN, Hofman A, Vulto AG, van Gelder T, Stricker BH. Variation in the CYP2D6 gene is associated with a lower serum sodium concentration in patients on antidepressants. *British Journal of Clinical Pharmacology* 2009; 68: 221-5.

Bijl MJ, Visser LE, van Schaik RHN, Eijgelsheim M, Hofman A, Uitterlinden AG, Vulto AG, van Gelder T, Stricker BH. The influence of CYP2D6, CYP3A4/3A5 and UGT2B7 genetic polymorphisms on prescription of other analgesics in codeine users. Submitted.

Bijl MJ, Nijsten T, Kiiski V, Visser LE, van Schaik RHN, Hofman A, Vulto AG, van Gelder T, Stricker BH. CYP2D6*4 genotype and susceptibility to basal cell carcinoma. Submitted.

Ruiter R, **Bijl MJ**, van Schaik RHN, Berns EMJJ, Hofman A, Coebergh JW, van Noord C, Visser LE, Stricker BH. CYP2C19*2 polymorphism is associated with increased survival in breast cancer patients using tamoxifen. Submitted.

Bijl MJ, van Schaik RHN. Research Highlights. Evaluation of the AmpliChip™ CYP450 Test. *Personalized Medicine* 2009; 6(3): 247-249.

Lammers LA, Mathijssen RHJ, van Gelder T, **Bijl MJ**, Seynaeve C, van Fessem MAC, Berns EMJJ, Vulto AG, van Schaik RHN. Effects of CYP2D6 phenotype on tamoxifen-treatment outcome in patients with metastatic breast cancer. Submitted.

PhD Portfolio

Name	Monique Johanna Bijl
Erasmus MC department	Hospital Pharmacy, Epidemiology
Research school	Netherlands Institute for Health Sciences
PhD period	2006-2009
Promotores	Prof. dr. B.H.Ch. Stricker and Prof. dr. A.G. Vulto

Research skills

Statistics and methodology

- 2006-2008 Master of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences (NIHES), Rotterdam, the Netherlands.
- 2008 Biomedical English Writing and Communication.

Presentations

Oral presentations

- 2009 The influence of *CYP2D6*, *CYP3A4/3A5* and *UGT2B7* genetic polymorphisms on prescription of other analgesics in codeine users. 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, USA.
- 2008 The *CYP2D6*4* polymorphism affects breast cancer survival in tamoxifen users. 3rd Dutch Hospital Pharmacy Day, Rotterdam, The Netherlands.
- 2008 Genetic variation in the *CYP2D6* gene is associated with a lower heart rate and blood pressure in β -blocker users. FIGON Dutch Medicine Days, Lunteren, The Netherlands.
- 2007 The influence of the *CYP2D6*4* polymorphism on dose, switching and discontinuation of antidepressants. 2nd Dutch Hospital Pharmacy Day, Leiden, The Netherlands.
- 2007 Influence of the *CYP2D6*4* polymorphism on dose and switching of antidepressants. 23rd International Conference on Pharmacoepidemiology and Therapeutic Risk in Quebec, Canada.

Poster presentations

- 2009 The *CYP2D6*4* polymorphism affects breast cancer survival in tamoxifen users. American Society for Clinical Pharmacology and Therapeutics, Washington DC, USA.
- 2007 Influence of the *CYP2D6*4* polymorphism on dose and switching of antidepressants. 8th Congress of the European Association for Clinical Pharmacology and Therapeutics, Amsterdam, The Netherlands.
- 2007 The influence of the *CYP2D6*4* polymorphism on dose, switching and discontinuation of antidepressants. Dutch Society of Clinical Pharmacology and Biopharmacy, Utrecht, The Netherlands.

International conferences

- 2009 25th International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, USA.
- 2009 110th annual meeting of the American Society for Clinical Pharmacology and Therapeutics, Washington DC, USA.
- 2007 23rd International Conference on Pharmacoepidemiology and Therapeutic Risk, Quebec, Canada.
- 2007 12th Congress of the European Association of Hospital Pharmacists, Bordeaux, France.

Seminars and workshops

- 2009 Introduction to Pharmacogenetics. International Conference on Pharmacoepidemiology and Therapeutic Risk in Providence, USA.
- 2009 Clinical Pharmacology Curriculum Review Course. American Society for Clinical Pharmacology and Therapeutics, Washington DC, USA.
- 2006-2009 Research seminars, department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands
- 2007 Advanced topics in Pharmacoepidemiological methods, special skill workshop. International Conference of Pharmacoepidemiology and Therapeutic Risk, Quebec, Canada.

Others

- 2006-2008 Organisation and programme coordinator of the research seminars, department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

Teaching

Lecturing

- 2009-current Education in hospital pharmacy, pharmacists assistants, Zadkine/Alberda, Rotterdam, The Netherlands.
- 2005-current Education in pharmacy, 5th/6th year medical students. Erasmus MC, Rotterdam, The Netherlands.
- 2007-2009 Clinical epidemiology, 4th year medical students. Erasmus MC, Rotterdam, The Netherlands.
- 2007-2009 Data-analysis in pharmacoepidemiology practicals, 4th year medical students. Erasmus MC, Rotterdam, The Netherlands.
- 2007-2008 Statistics practicals, 4th year medical students. Erasmus MC, Rotterdam, The Netherlands.