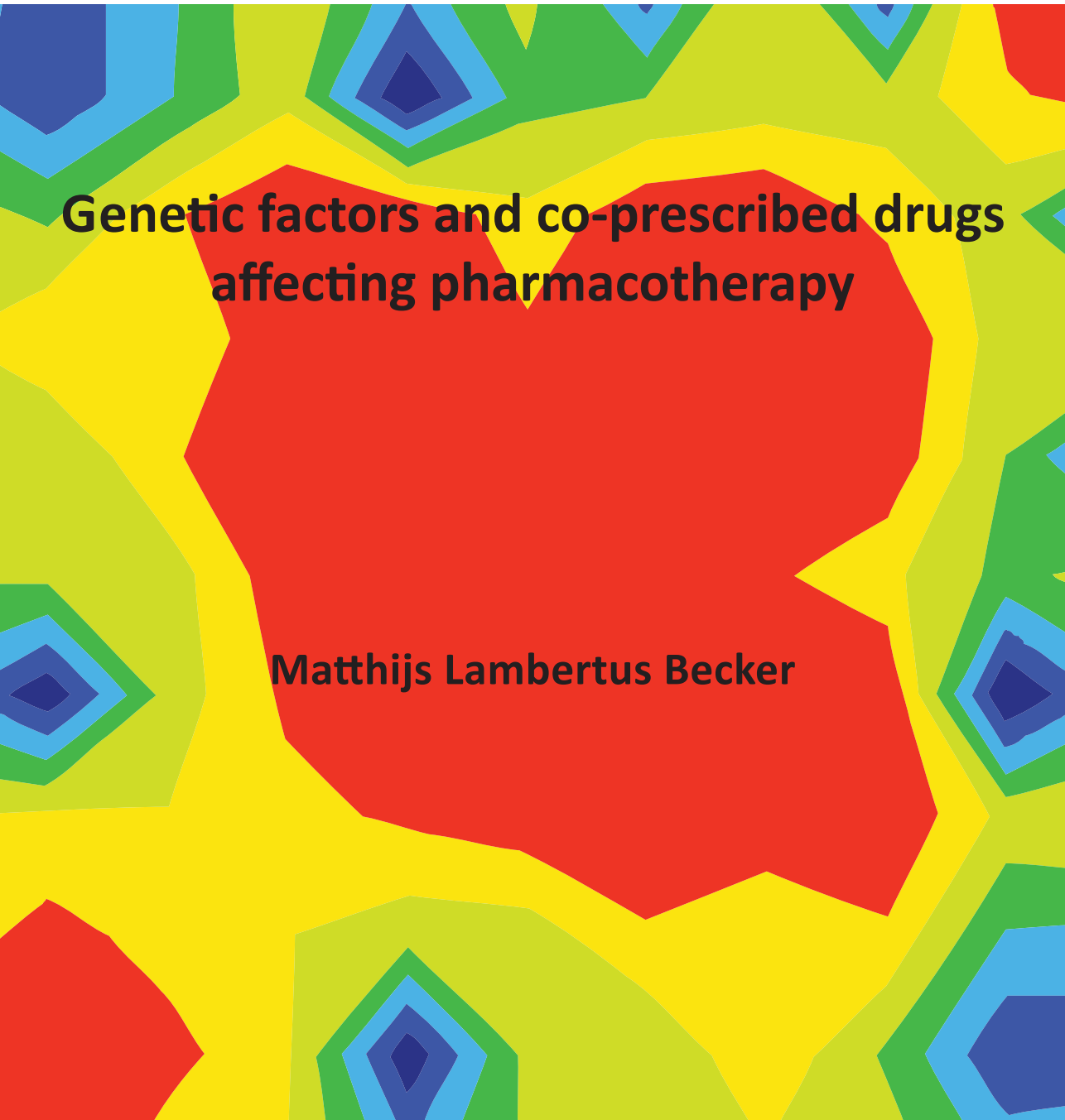
INDIVIDUALIZING PHARMACOTHERAPY



Genetic factors and co-prescribed drugs affecting pharmacotherapy

Matthijs Lambertus Becker

Individualizing Pharmacotherapy

Genetic factors and co-prescribed drugs affecting pharmacotherapy

Matthijs Lambertus Becker

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 Sport, the European Commission (DG XII), and the Municipality of Rotterdam.

The contributions of the study participants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged.

The work in this thesis was financially supported by the Inspectorate for Health Care.

Cover: Linkage disequilibrium between single nucleotide polymorphisms in the *SLC22A1* gene, visually presented by the software package Graphical Overview of Linkage Disequilibrium (GOLD), Center for Statistical Genetics, University of Michigan.

Printed by Optima Grafische Communicatie, Rotterdam
ISBN: 978-90-8559-574-8

INDIVIDUALIZING PHARMACOTHERAPY

GENETIC FACTORS AND CO-PRESCRIBED DRUGS AFFECTING PHARMACOTHERAPY

Individualiseren van farmacotherapie

De invloed van genetische factoren en co-medicatie op farmacotherapie

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
woensdag 4 november 2009 om 15.30 uur

door

Matthijs Lambertus Becker

geboren te Nijmegen

ERASMUS UNIVERSITEIT ROTTERDAM

PROMOTIECOMMISSIE

Promotoren:	Prof. dr. B.H.Ch. Stricker
	Prof. dr. A.G. Vulto
Overige leden:	Prof. dr. A.G. Uitterlinden
	Prof. dr. A. de Boer
	Prof. dr. P.A.B.M. Smits
Copromotor:	Dr. L.E. Visser

Contents

Chapter 1.	**General introduction**	**7**
Chapter 2.	**Co-prescribed drugs affecting pharmacotherapy**	**21**
Chapter 2.1.	Increasing exposure to drug-drug interactions between 1992 and 2005 in people aged ≥55 years	23
Chapter 2.2.	Potential determinants of drug-drug interaction associated dispensing in community pharmacies: a literature review	37
Chapter 2.3.	Determinants of potential drug-drug interaction associated dispensing in community pharmacies in the Netherlands	49
Chapter 2.4.	Hospitalizations and emergency department visits due to drug-drug interactions: a literature review	61
Chapter 3.	**Genetic factors affecting pharmacotherapy for type 2 diabetes mellitus**	**75**
Chapter 3.1.	Cytochrome P450 2C9 *2 and *3 polymorphisms and the dose and effect of sulfonylurea in type 2 diabetes mellitus	77
Chapter 3.2.	Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with type 2 diabetes mellitus	91
Chapter 3.3.	Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose lowering effect of metformin in patients with type 2 diabetes mellitus	103
Chapter 3.4.	Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response	115
Chapter 3.5.	Common variation in the *NOS1AP* gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea	127
Chapter 4.	**Genetic factors affecting cardiovascular pharmacotherapy**	**141**
Chapter 4.1.	Common genetic variation in the *ABCB1* gene is associated with the cholesterol lowering effect of simvastatin in males	143
Chapter 4.2.	Influence of genetic variation in *CYP3A4* and *ABCB1* on dose decrease or switching during simvastatin and atorvastatin therapy	155
Chapter 4.3.	Genetic variation in the *NOS1AP* gene is associated with the incidence of diabetes mellitus in users of calcium channel blockers	169
Chapter 4.4.	A common *NOS1AP* genetic polymorphism is associated with increased cardiovascular mortality in users of dihydropyridine calcium channel blockers	175

Chapter 5.	**Genetic factors affecting pharmacotherapy for Parkinson's disease**	**189**
Chapter 5.1.	The OCT1 polymorphism rs622342 A>C is associated with decreased drug response and shorter survival time in Parkinson's disease	191
Chapter 6.	**General discussion**	**203**
Chapter 7.	**Summary**	**225**
Chapter 7.1.	Summary	227
Chapter 7.2.	Samenvatting voor niet ingewijden	233
	Abbreviations	241
	Dankwoord	243
	Bibliography	247
	PhD portfolio	251
	About the author	253

Chapter 1.

General introduction

INTRODUCTION

The goal of pharmacotherapy is, in general, to cure a disease or to eliminate or reduce symptoms. In daily practice, predefined goals of pharmacotherapy are often not met for various reasons, such as ineffectiveness of the drug or adverse drug reactions. Estimations of the proportion of patients without clinically significant efficacy to important classes of therapeutic drugs range from 30 to 60 percent.[1] Conversely, around two to four percent of all hospital admissions result from adverse drug reactions with a quarter to half of these admissions being preventable.[2-4] In the United States, adverse drug reactions are the fourth leading cause of hospitalization and result in roughly 100.000 deaths annually.[5,6]

Many factors are involved in the variation in drug response and a better understanding of these factors can improve the effectiveness of pharmacotherapy and reduce the incidence of adverse drug reactions. Healthcare relies, more often than is desirable, on the 'one-dose-fits-all' approach. The initial starting dose is the same for all patients, irrespective of the patient's individual characteristics. Personalized medicine, or tailoring drug therapy to the characteristics of the individual patient, is a useful tool in reducing the number of ineffective therapies and adverse drug reactions.[5]

PHARMACOKINETICS AND PHARMACODYNAMICS

The process from drug intake to drug response is complicated, and many factors are involved. A distinction can be made between pharmacokinetic and pharmacodynamic factors. Pharmacokinetics concerns the fate of a drug when it is administered to the body. The first part of the pharmacokinetic process consists of the absorption of the drug into the body, distribution throughout the body tissues and fluids and then subsequent elimination. During these stages, some drugs can diffuse through membranes passively, without the help of energy consuming enzymes. These drugs are in most cases uncharged, lipophilic and unbound. Other drugs cannot cross membranes passively and rely on active carriage by transporter proteins. A large number of different transporter proteins are present throughout the body and regulate the plasma levels of substances in tissues and fluids.[7-9] Two important transporter families are the ATP Binding Cassette (ABC) family and the solute carrier (SLC) family. These two families have important roles in the pharmacokinetics of both drugs and endogenous compounds.[10-12]

The second part of the pharmacokinetic process is the irreversible transformation of drugs into metabolites. Metabolism is divided into two phases. In phase I, drugs are metabolized into more water-soluble substances through oxidation and reduction. The main phase I metabolizing enzymes are the cytochrome P450 (CYP) enzymes, although other enzymes such as xanthine oxidase (metabolizing 6-mercaptopurine) and alcohol dehydrogenase

(metabolizing ethanol) are also involved.[13-15] The CYP enzymes are responsible for around 75 percent of total drug metabolism.[13]

The phase II metabolizing enzymes conjugate polar groups, such as glucuronyl (UDP glucoronosyltransferases, UGT) and acetyl (acetyl-CoA) to apolar substances.[15,16] These reactions result in a further increase in hydrophilicity and a more efficient excretion by the kidney or on some occasions via bile secretion. Most drugs are inactivated by phase I and phase II reactions, although some drugs, such as codeine and tamoxifen, are administered as the inactive pro-drug and metabolized to the active compound.[17,18]

Besides pharmacokinetics, pharmacodynamics plays a major role in drug response. Pharmacodynamics relates to the biochemical or physiological effects of a drug on the body. One of the mechanisms of pharmacodynamics is the binding of a drug to a receptor.[15,19] Ligand binding may result in activation of the receptor (agonism) or in blocking the effect of an agonist (antagonism). This leads to a change in the intracellular transduction pathways, which can trigger events such as the release of substances stored in vesicles, a change in the gene transcription rate or activation of intracellular messengers. These processes may result in the intended effects of drug therapy, although they may also produce adverse drug reactions. A drug can exert its effects in a large number of other ways. For example, a drug may bind to an ion channel, changing the ion current through the channel, a drug may bind to an enzyme protein, altering the functioning of this enzyme or may directly react with a substance in the body.

FACTORS INVOLVED IN DRUG RESPONSE

In all the pharmacokinetic and pharmacodynamic processes described previously, variations in drug response do occur. A possible response to a drug may be an adverse drug reaction. Adverse drug reactions can be divided into two groups. Type A adverse drug reactions are related to the drug, resulting from an unexpectedly strong or unintended pharmacological effect. These reactions are dose dependent, as their incidence and severity increases with increasing dose. Type B adverse drug reactions are unrelated to the drug's pharmacological effect and include hypersensitivity reactions. Apart from the intended pharmacological activity, pharmacokinetics and pharmacodynamics are involved in both non-response to drugs and type A adverse drug reactions. However, they do not play a major role in type B adverse drug reactions.

Factors, which are involved in the variation in drug response, are age, gender, co-morbidity (e.g. renal or liver dysfunction), environmental factors, body weight, co-prescribed drugs and genetic factors. In table 1, an overview is given as to how these factors affect drug response. As mentioned before, many factors are involved in many different ways, making it impossible to give a complete overview of the relations applicable to all drugs.

Table 1 Factors involved in variation of drug response

	Processes	Examples of proteins	Examples of factors involved in variation in drug response
Pharmacokinetics			
Transportation	Absorption	SLC, ABC	Drugs, genetics
	Distribution	SLC, ABC	Age, body weight, drugs, genetics
	Excretion	SLC, ABC	Age, co-morbidity, drugs, genetics
Metabolism	Oxidation (Phase I)	Cytochrome P450	Age, co-morbidity, drugs, genetics
	Conjugation (Phase II)	UGT, acetyl-CoA, SAM	Genetics
Pharmacodynamics			
	Binding to receptors	β-receptor	Age, drugs, genetics
	Interaction with ion channels	Calcium channel	Drugs, genetics
	Interaction with enzyme proteins	HMG-CoA reductase	Genetics
	Chemical reaction	- [a]	-
Type B adverse drug reactions			
	Hypersensitivity	HLA	Genetics

[a] No proteins are involved in these chemical reactions. SLC: solute carrier; ABC: ATB binding cassette; UGT: UDP glucoronosyltransferases; SAM: S-adenosyl methionine; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-CoA; HLA: human leukocyte antigen.

The impact of age

In the elderly, lowering prescribed doses of drugs with a narrow therapeutic window is often indicated. Age is associated with changes in body composition, such as a relative increase in body fat, a decrease in drug clearance, combined with a higher sensitivity to pharmacodynamic processes.[20] Renal clearance is decreased due to a reduction in renal functioning. The functioning of CYP enzymes tends to be lower with increasing age, although results from studies are conflicting.[20-22] However, enzymatic clearance by phase II pathways is not affected by age.[20]

The impact of gender

Gender affects drug response in two ways. First of all, differences exist in pharmacokinetic properties between men and women. For example, the clearance of drugs metabolized by CYP3A4 is higher in women than in men.[21] It has been suggested that this is caused by lower P-gp efflux transporter activity in women. P-gp is co-expressed in hepatic cells and in the cells in the intestinal wall and a reduction in efflux results in more substrate becoming available for CYP3A4 and thus higher CYP3A4 clearance.[23,24] Secondly, there is a difference in pharmacodynamic actions of a drug between genders. For example, aspirin has a major role in the prevention of myocardial infarction in men, in contrast many women do not respond to aspirin therapy and several studies have failed to show a protective effect.[25]

The impact of co-morbidity

Many people suffer, apart from the disease they are treated for, from co-morbidities and these can also affect drug response. The kidney and the liver are the major organs involved in drug metabolism and excretion, and therefore co-morbidities in these organs may influence drug response. For example, the risk of adverse drug reactions is increased in patients with reduced kidney function who use drugs with a narrow therapeutic window and which are excreted unchanged by the kidney.[26] Much less is known about the effect of liver impairment on the metabolism of drugs undergoing hepatic metabolism.[27,28]

The impact of environmental factors

Countless environmental factors, such as smoking, hygiene, stress and exercise, contribute to the variation in drug response. For instance diet can have an important effect on drug response. Many patients with Parkinson's disease are treated with oral levodopa therapy to suppress symptoms. Since the amino acids phenylalanine, leucine and isoleucine competitively inhibit the absorption of levodopa into the brain, high-protein meals reduce the inhibitory effect of levodopa on symptoms of the disease.[29] Another such example is that of grapefruit juice, which contains ingredients that inhibit CYP3A4 enzymes, ATP-binding cassette B1 (ABCB1) transporters and transporters in the solute carrier organic anion (SLCO) transporter family.[24,30] Therefore, combining grapefruit juice with drugs that are metabolized by the CYP3A4 enzyme will result in higher plasma levels and possibly adverse drug reactions.

The impact of body weight

In obese people, the distribution of drugs throughout body tissues differs from lean people. This especially applies to drugs that have a high fat-solubility and those which are dosed per kilogram body weight.[31,32] Prescribing doses irrespective of body weight may in obese people lead to both too low doses, if the same dose is used in lean and obese people, and too high doses if drugs are dosed per kilogram body weight. An example of this can be found in the predominance of neuropsychiatric adverse reactions to mefloquine, which is used the for prevention of malaria, in women with a low body mass index.[33] Racial differences are obviously also linked to body weight due to differences in body stature.

The impact of co-prescribed drugs

Polypharmacy, the use of multiple drugs by one patient, is common. These drugs may influence each other resulting in drug-drug interactions (DDIs). Apart from the intended effects, DDIs may also lead to reduced effectiveness or increased toxicity.[34] Many drugs either induce or inhibit CYP enzymes and combining these drugs with ones which are metabolized by that CYP enzyme may result in ineffective or toxic plasma levels.[35,36] Whether these effects really do occur depends on the degree of CYP induction or inhibition, the therapeutic window of

the drug and the availability of alternative metabolizing enzymes. Drugs may also induce or inhibit transporters, resulting in DDIs with substrates for these transporters.[37]

Two drugs may also exert their effects via the same pathway, resulting in a pharmacodynamic DDI. Drugs may either be both agonists, resulting in additional effects or if the total effect is larger than the two separate effects in synergism, or an agonist and an antagonist, resulting in a reduction of drug response. One such DDI is the combination of drugs which are agonists for the human ether-a-go-go-related gene (hERG) ion channel. Inhibition of the hERG ion channel lengthens the QTc-interval duration on the ECG and increases the risk of ventricular arrhythmias. A single drug may lead to a minor, but not clinically relevant increase in QTc interval, while a combination of these drugs may result in a synergistic effect and a much larger life-threatening QTc prolongation than the sum of the separate QTc prolongations.[38]

The impact of genetic factors

Genetic variation in the DNA encoding proteins can result in a change in amino acid sequence in the protein or differences in transcription rates. These deviations may result in the increased or reduced effectiveness of drugs. The estimations of variation in drug response that can be explained by genetics varies from 12 to 98 percent.[39-42] Genetic variation in both CYP enzymes and transporters has been extensively described.[43,44] A single nucleotide polymorphism (SNP) is a variation in nucleotide sequence within the DNA. SNPs in coding regions of the DNA (exons) may result in an alternative amino acid incorporated in the protein. These amino acid changes may result in decreased or increased activity of the protein. SNPs in the non-coding regions (introns) may result in changes in transcription rates and gene expression, resulting in higher or lower enzyme concentrations. SNPs affect the activity of CYP enzymes, transporters and receptors and explain part of the variation in drug response.[45] Genetic variation also applies to duplications (copy number variations or multiplicons) or deletions of DNA fragments.[46] Duplications result in more genes being expressed and higher enzyme expressions, while deletions result in the absence of the enzyme.

The impact of other factors

Innumerable other factors are involved. Biological variation throughout the day is involved in many physiological processes and modifies drug response, for example the variation in hormone and glucose levels.[47-49] Comparing two glucose levels, measured at different times during the day, will result in a variation, which is not attributable to the effect of a drug.

Although obvious, non-adherence is a major contributor to the non-response of a drug and may result in hospital admissions and deaths.[50-51] In clinical trials, the adherence is relatively high when compared to daily practice due to the attention study patients receive. Yet even in clinical trials, the reported average adherence rates vary from 43 to 78 percent in patients

receiving chronic medication.[50] In daily practice, there is an inverse relationship between the prescribed number of doses per day and compliance rates.[52]

At last, variation in drug response may also result from methodological issues. For example, measurement errors do contribute to the variation. Part of the change between two time points will be due to measurement errors and are erroneously attributed to drug response.

AIM AND OUTLINE OF THIS THESIS

The variation in drug reactions, including adverse drug reactions, response and non-response, is mostly unpredictable before the start of therapy. Pharmacotherapy would be much more efficient and safer if a better prediction of drug response was possible. With this objective in mind, the subject of this thesis is the impact of co-prescribed drugs and genetic variation on drug response.

In a review, published in 1993, it was estimated that up to three percent of all hospital admissions were due to DDIs.[53] It can therefore be assumed, that co-prescribed drugs have a substantial impact on the occurrence of adverse drug reactions and possibly on drug response in general. Although drug use and most likely the prevalence of DDIs have increased in the meantime, no reviews have been published after 1993 on the proportion of hospital admissions due to DDIs.

In this thesis, we also studied the effects of genetic variation, although the effects of co-prescribed drugs and genetic variation seem unrelated. However, both co-prescribed drugs and genetic variation can either induce or inhibit metabolizing enzymes and transporters. It would be expected that the clinical effects of induction or inhibition either by co-prescribed drugs or genetic variation will be similar. Studying the effect of genetic variation on drug response has the advantage over the effect of co-prescribed drugs that genetic variation is stable over time, while co-prescribed drugs do vary over time. A better knowledge on the effect of genetic variation may also be beneficial in predicting the effect of co-prescribed drugs.

Studies of twins and comparison of inter- and intra-individual variation have given us some insight into the degree to which genetic variation contributes to variation in drug response.[40-42] In these studies, the contribution of genetic variation varies from 12 to 98 percent, although it is questionable whether these studies could really distinguish between genetic and other factors and these percentages may be an overestimation.

Many SNPs have been identified that are associated with variation in plasma level or drug response. However, the amount of variation explained by these SNPs is much less than the contribution of all genetic factors. This discrepancy suggests that a large number of as yet unidentified SNPs and other genetic variations do contribute. Further research in this area has the potential to improve the prediction in drug response.

The following examples illustrate this point. It is estimated that 95 percent of the variation in renal clearance of metformin is due to genetic variation. A limited number of SNPs in the gene coding for the organic cation transporter 2 (OCT2) have been associated with renal clearance of metformin, but these associations were too weak to explain the majority of variation in renal clearance.[54-57] This suggests that many more SNPs and other genetic determinants of variation, still unknown, do contribute.

The same applies for the glucuronidation of oxazepam. Around 98 percent of glucuronidation is under genetic control [40] and SNPs in the gene coding for the UDP-glucuronosyltransferase enzyme, conjugating oxazepam, have been identified.[58] However, the variation explained by these SNPs is far less than 98 percent.

In figure 1, the major compartments in the human body, involved in variation in drug response, are presented, as well as the enzymes that were studied in the thesis. The liver and intestinal wall have a major impact on pharmacokinetic processes, due to their metabolism of a large number of drugs. From the circulation, drugs are often distributed to organs other than the target organ. In these organs, the drug may trigger receptors other than those

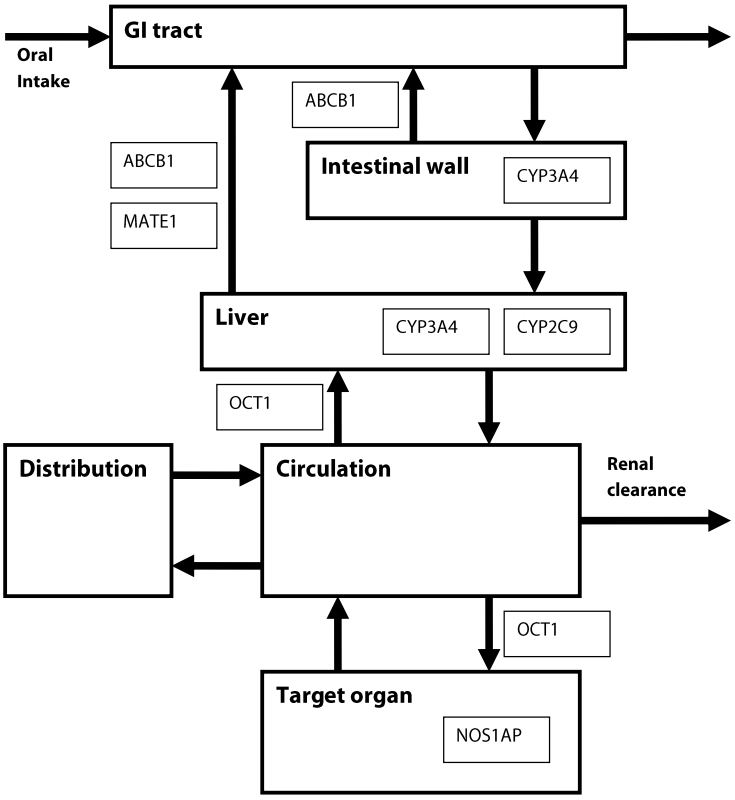


Figure 1 Diagram representing the distribution and elimination of drugs in the human body and the role of transporters and enzymes, studied in this thesis

intended, resulting in adverse drug reactions. The pharmacodynamic processes in the target organ are too diverse and complicated to represent in a model. Moreover, the target organ could be one of the other organs in the model, such the liver, intestinal wall or the circulation itself.

The goal of this thesis is to improve the prediction in drug response due to both genetic variation and co-prescribed drugs. In chapter two, exposure to and clinical consequences of DDIs were assessed. In this chapter, we also studied determinants that are associated with high risk DDI dispensings by community pharmacies. In chapter three, the effect of genetic variation on the response to antidiabetic drugs was studied. Both the effect genetic variation in *CYP2C9* and *nitric oxide synthase 1 adaptor protein* (*NOS1AP)* has on sulfonylurea response was studied, as well as the effect of genetic variation in the genes coding for the OCT1 and MATE1 transporter on metformin response.

The studies in chapter four assessed the effect of genetic variation in the *ABCB1* and *CYP3A4* gene on adverse drug reactions and the cholesterol lowering effect of statins, as well as the effect of genetic variation in the *NOS1AP* gene on calcium channel blocker response. In chapter five, the effect of genetic variation in the gene encoding OCT1 on anti-Parkinson drug response was studied.

REFERENCES

1. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends Mol Med* 2001;7(5):201-4.
2. Leendertse AJ, Egberts AC, Stoker LJ, van den Bemt PM. Frequency of and risk factors for preventable medication-related hospital admissions in the Netherlands. *Arch Intern Med* 2008;168(17):1890-6.
3. Beijer HJ, de Blaey CJ. Hospitalisations caused by adverse drug reactions (ADR): a meta-analysis of observational studies. *Pharm World Sci* 2002;24(2):46-54.
4. van der Hooft CS, Sturkenboom MC, van Grootheest K, Kingma HJ, Stricker BH. Adverse drug reaction-related hospitalisations: a nationwide study in The Netherlands. *Drug Saf* 2006;29(2):161-8.
5. Piquette-Miller M, Grant DM. The art and science of personalized medicine. *Clin Pharmacol Ther* 2007;81(3):311-5.
6. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 1998;279(15):1200-5.
7. Shitara Y, Horie T, Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* 2006;27(5):425-46.
8. Yin OQ, Tomlinson B, Chow MS. Variability in renal clearance of substrates for renal transporters in chinese subjects. *J Clin Pharmacol* 2006;46(2):157-63.
9. Tsuji A. Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems. *NeuroRx* 2005;2(1):54-62.
10. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 2002;71:537-92.
11. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteinsIntroduction. *Pflugers Arch* 2004;447(5):465-8.
12. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 2007;24(7):1227-51.
13. Guengerich FP. Cytochrome P450 and chemical toxicology. *Chem Res Toxicol* 2008;21(1):70-83.
14. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005;352(21):2211-21.
15. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. London: Elsevier Limited; 2003.
16. Finel M, Kurkela M. The UDP-glucuronosyltransferases as oligomeric enzymes. *Curr Drug Metab* 2008;9(1):70-6.
17. Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. *Lancet* 2006;368(9536):704.
18. Goetz MP, Kamal A, Ames MM. Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. *Clin Pharmacol Ther* 2008;83(1):160-6.
19. Lees P, Cunningham FM, Elliott J. Principles of pharmacodynamics and their applications in veterinary pharmacology. *J Vet Pharmacol Ther* 2004;27(6):397-414.
20. Schwartz JB. The current state of knowledge on age, sex, and their interactions on clinical pharmacology. *Clin Pharmacol Ther* 2007;82(1):87-96.
21. Cotreau MM, von Moltke LL, Greenblatt DJ. The influence of age and sex on the clearance of cytochrome P450 3A substrates. *Clin Pharmacokinet* 2005;44(1):33-60.

22. Parkinson A, Mudra DR, Johnson C, Dwyer A, Carroll KM. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* 2004;199(3):193-209.
23. Cummins CL, Wu CY, Benet LZ. Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein. *Clin Pharmacol Ther* 2002;72(5):474-89.
24. Dresser GK, Bailey DG. The effects of fruit juices on drug disposition: a new model for drug interactions. *Eur J Clin Invest* 2003;33 Suppl 2:10-6.
25. Yerman T, Gan WQ, Sin DD. The influence of gender on the effects of aspirin in preventing myocardial infarction. *BMC Med* 2007;5:29.
26. Matzke GR, Frye RF. Drug administration in patients with renal insufficiency. Minimising renal and extrarenal toxicity. *Drug Saf* 1997;16(3):205-31.
27. Verbeeck RK, Horsmans Y. Effect of hepatic insufficiency on pharmacokinetics and drug dosing. *Pharm World Sci* 1998;20(5):183-92.
28. Klotz U. Antiarrhythmics: elimination and dosage considerations in hepatic impairment. *Clin Pharmacokinet* 2007;46(12):985-96.
29. Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The "on-off" phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. *N Engl J Med* 1984;310(8):483-8.
30. Kiani J, Imam SZ. Medicinal importance of grapefruit juice and its interaction with various drugs. *Nutr J* 2007;6:33.
31. Green B, Duffull SB. What is the best size descriptor to use for pharmacokinetic studies in the obese? *Br J Clin Pharmacol* 2004;58(2):119-33.
32. Han PY, Duffull SB, Kirkpatrick CM, Green B. Dosing in obesity: a simple solution to a big problem. *Clin Pharmacol Ther* 2007;82(5):505-8.
33. van Riemsdijk MM, Sturkenboom MC, Ditters JM, et al. Low body mass index is associated with an increased risk of neuropsychiatric adverse events and concentration impairment in women on mefloquine. *Br J Clin Pharmacol* 2004;57(4):506-12.
34. Becker ML, Kallewaard M, Caspers PW, Schalekamp T, Stricker BH. Potential determinants of drug-drug interaction associated dispensing in community pharmacies. *Drug Saf* 2005;28(5):371-8.
35. Cytochrome P450 drug-interaction table. Indiana University School of Medicine. Available from http://www.medicine.iupui.edu/flockhart/. Accessed 26 October 2008.
36. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev* 2002;34(1-2):83-448.
37. Drug evelopment and drug interactions: table of substrates, inhibitors and inducers. U.S. Food and Drug Administration. Available from http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm. Accessed 26 October 2008.
38. De Ponti F, Poluzzi E, Cavalli A, Recanatini M, Montanaro N. Safety of non-antiarrhythmic drugs that prolong the QT interval or induce torsade de pointes: an overview. *Drug Saf* 2002;25(4):263-86.
39. Weinshilboum R. Inheritance and drug response. *N Engl J Med* 2003;348(6):529-37.
40. Kalow W, Tang BK, Endrenyi L. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics* 1998;8(4):283-9.
41. Leabman MK, Giacomini KM. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* 2003;13(9):581-4.
42. Kalow W, Endrenyi L, Tang B. Repeat administration of drugs as a means to assess the genetic component in pharmacological variability. *Pharmacology* 1999;58(6):281-4.

43. Zair ZM, Eloranta JJ, Stieger B, Kullak-Ublick GA. Pharmacogenetics of OATP (SLC21/SLCO), OAT and OCT (SLC22) and PEPT (SLC15) transporters in the intestine, liver and kidney. *Pharmacogenomics* 2008;9(5):597-624.
44. Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee. Human Cytochrome P450 (CYP) Allele Nomenclature Committee. Available from http://www.cypalleles.ki.se/. Accessed 26 October 2008.
45. Evans WE, McLeod HL. Pharmacogenomics-drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348(6):538-49.
46. Freeman JL, Perry GH, Feuk L, et al. Copy number variation: new insights in genome diversity. *Genome Res* 2006;16(8):949-61.
47. Lemmer B. The clinical relevance of chronopharmacology in therapeutics. *Pharmacol Res* 1996;33(2):107-15.
48. Bruguerolle B. Clinical chronopharmacology in the elderly. *Chronobiol Int* 2008;25(1):1-15.
49. Bruguerolle B, Boulamery A, Simon N. Biological rhythms: a neglected factor of variability in pharmacokinetic studies. *J Pharm Sci* 2008;97(3):1099-108.
50. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med* 2005;353(5):487-97.
51. Simpson SH, Eurich DT, Majumdar SR, et al. A meta-analysis of the association between adherence to drug therapy and mortality. *BMJ* 2006;333(7557):15-18.
52. Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. *Clin Ther* 2001;23(8):1296-310.
53. Jankel CA, Fitterman LK. Epidemiology of drug-drug interactions as a cause of hospital admissions. *Drug Saf* 1993;9(1):51-9.
54. Song I, Shin H, Shim E, et al. Genetic Variants of the Organic Cation Transporter 2 Influence the Disposition of Metformin. *Clin Pharmacol Ther* 2008;84(5):559-62.
55. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet Genomics* 2008;18(7):637-45.
56. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;117(5):1422-31.
57. Shu Y, Brown C, Castro RA, et al. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
58. Court MH, Hao Q, Krishnaswamy S, et al. UDP-glucuronosyltransferase (UGT) 2B15 pharmacogenetics: UGT2B15 D85Y genotype and gender are major determinants of oxazepam glucuronidation by human liver. *J Pharmacol Exp Ther* 2004;310(2):656-65.

Chapter 2.

Co-prescribed drugs affecting pharmacotherapy

Chapter 2.1.

Increasing exposure to drug-drug interactions between 1992 and 2005 in people aged ≥55 years

ABSTRACT

Background: Drug-drug interactions (DDIs) are responsible for a variety of adverse reactions, particularly in an elderly population. The objective of our study was to identify the frequency and potential clinical relevance of DDIs in a population aged 55 years and over.

Methods: Exposure to DDIs was assessed in 7,842 people, participating in the Rotterdam Study, a population-based cohort study. These people were followed between January 1st 1992 and July 1st 2005. The DDI list of the Royal Dutch Association for the Advancement of Pharmacy, in which DDIs were categorized by potential clinical relevance and quality of evidence, was used. Simultaneous use of interacting drug combinations was calculated on the basis of drug dispensing data from community pharmacies.

Results: The incidence of a first dispensing of DDIs in the study period was 10.5 per 100 person-years and 2.7 per 100 person-years for potentially life-threatening DDIs. The prevalence of DDIs in people aged 70 years and older increased from 10.5% in 1992 to 19.2% in 2005. Ten DDIs comprised two-third of the total exposure time to DDIs. The prevalence of potential life-threatening DDIs in people aged 70 years and older increased from 1.5% to 2.9%. This increase was most likely caused by an increase in use of spironolactone combined with renin-angiotensin-aldosteron system inhibitors.

Conclusion: A large number of people in the Netherlands aged 55 years and older are exposed to DDIs and this number has increased sharply between 1992 and 2005. Healthcare professionals should pay special attention to the potential risks of DDIs in these people, particularly if spironolactone is involved.

INTRODUCTION

Drug-drug interactions (DDIs) play an important role in current healthcare and are a potential cause of adverse reactions. The estimates of hospital admissions caused by DDIs vary from 0.1 to 2.6%.[1-3] For several DDIs, such as those arising from the combination of erythromycin with strong cytochrome P450 3A inhibitors and glibenclamide with co-trimoxazole, higher incidences of death or hospital admissions have been reported.[4, 5] In the elderly population the risk of adverse reactions caused by DDIs is higher due to polypharmacy and changes in pharmacokinetics and pharmacodynamics.[6] The number of hospital admissions in the elderly population as a consequence of DDIs is higher than in the general population. The estimates vary between 2.9 and 6.2%.[7, 8] Whether DDIs are a potential threat to public health depends both on the frequency and the potential clinical relevance of the DDIs.

In the Netherlands, one of the tasks of the pharmacist is to intervene when combinations of prescriptions induce a high risk of patient harm, in order to prevent adverse reactions caused by DDIs. Each prescription dispensed by a pharmacy is recorded in the medication history. Most patients receive their drugs from the same pharmacy, and consequently medication histories are usually complete. With each new prescription for the same patient the medication history is screened for other drugs used at that moment, and checked as to whether potential DDIs might occur. The pharmacist evaluates the seriousness of the DDI on the basis of the patient's characteristics and the medication history. Just as the clinical consequences of DDIs vary greatly, so too the management of DDIs differs. Where the benefits of both drug therapies outweigh the risks of the DDIs, the drug can be dispensed unless there are safer alternatives. If not, an intervention is required and appropriate measures, such as changing doses or monitoring blood levels, should be taken.

Several studies have been conducted to estimate the incidence and prevalence of DDIs.[8-14] Most studies have focused on populations at risk for DDIs and adverse reactions, such as in hospital settings. As a result of the large differences in study design, exposure rates to DDIs range from 2 to 60%. The aim of this study was to analyze the frequency and potential clinical relevance of exposure to DDIs and the change in frequency over time in a population aged 55 years and older in the Netherlands.

METHODS

Setting

Data were obtained from the Rotterdam Study, a prospective population-based closed cohort study in Ommoord, a suburb of Rotterdam, the Netherlands. Between 1990 and 1993, all inhabitants aged 55 years and older who had lived in the district for more than one year were invited to participate in the study. Of the 10,275 eligible persons, 7,983 (78%) participated.

All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC.

The rationale and design of the study have been described before.[15] In short, the aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases. At baseline, trained interviewers administered a questionnaire during a home visit. The seven pharmacies in this suburb dispense the prescriptions of more then 99% of the participants. The pharmacy dispensing records from January 1st 1991 until July 1st 2005 were available and included the product name of the drug, the anatomical therapeutical chemical code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.

Cohort definition

The study cohort consisted of all subjects in the Rotterdam Study. The dispensing data between January 1st 1992 and July 1st 2005 were analyzed to obtain complete medication histories of at least 1 year. As 141 participants in the cohort died during 1991, the medication histories of 7,842 people were analyzed. The cohort was followed until death, removal or the end of the study period.

Procedure

The Royal Dutch Association for the Advancement of Pharmacy publishes a list of DDIs that require a potential intervention by healthcare providers to prevent adverse reactions as a result of exposure to a combination of drugs.[16] This list is used for computerized drug interaction surveillance systems used in community and hospital pharmacies and updates are sent out monthly. For this study, we used this list as updated to March 2006.[17] The Royal Dutch Association for the Advancement of Pharmacy categorizes both the quality of evidence and the potential clinical relevance of the DDIs. The quality of evidence for the DDI is categorized from 0 to 4, with 4 being the highest quality of evidence, and from A to F, reflecting the increasing potential clinical relevance of the DDI (table 1).[16] The DDI list included 451 DDIs. Of these, 101 DDIs were not subdivided into the categories 0A-4F, since formal classification of these interactions was still in progress. This occurred, for example, in the case of DDIs with newly marketed drugs such as antiviral drugs, or because of new understandings of DDIs. These DDIs occurred infrequently and therefore had little influence on the results.

For all dispensed prescriptions in the database, the duration of use was calculated by dividing the number of dispensed drug units by the number of units used per day. When the regimen was unknown or no duration could be calculated, the duration was replaced by the average valid durations of all dispensing of that drug in the study population. This was done, for example, in the use of 'as needed' drugs, or if the total prescription length was for more then 168 days. This period was chosen because the dispensing of six cycles of 28 days for oral contraceptive drugs is the longest period for which drugs are regularly dispensed. For all

Table 1 Categories for the quality of evidence and potential clinical relevance of drug-drug interactions published by the Royal Dutch Association for the Advancement of Pharmacy[16]

Quality of evidence	
0	Pharmacodynamic animal studies, in vitro studies
1	Incomplete published case reports
2	Well documented published case reports
3	Controlled published interaction studies with surrogate endpoints
4	Controlled published interaction studies with clinical relevant endpoints
Potential clinical relevance of adverse reactions	
A	Clinically irrelevant effect
B	Short acting adverse reactions (<24-48 hours) without sequel
C	Long lasting adverse reactions (48-168 hours) without sequel
D	Very long lasting adverse reactions (>168 hours) or adverse reactions with sequel
E	Increased risk of failure of life-saving therapy
F	Death

patients, we recorded the period that they were exposed to simultaneous drug use that was listed as causing a DDI. The DDIs were listed by group, such as β-adrenoreceptor antagonists or insulin. Therefore, switching from one β-adrenoreceptor antagonists to another while using drugs that interact with β-adrenoreceptor antagonists was recorded as the same DDI, and using two different types of insulin interacting with another drug was counted as one DDI.

Analysis

We used three types of outcome in our study, the incidence rate, the point prevalence and the exposure time to the DDIs. For the incidence rate the first dispensing of a DDI or category of DDIs after the start of the study period was considered an event. Endpoints were an event, death or end of the study period. For people exposed to DDIs on January 1st 1992, no first dispensing date could be calculated and therefore these people were not taken into account for the calculation of the incidence rate. We also calculated the incidence rate for the first dispensing of both drugs on the same day because an overlap of usage periods does not guarantee simultaneous use. Prevalences were calculated on January 1st of every year and presented per age stratum. As people aged 55 years and older were included in the closed cohort, after ten years of follow up the cohort consisted only of people aged 65 and older. Therefore trends from 1992 to 2005 could be analyzed only in the population aged 70 years and older. Trends were standardized to the composition of the population at January 1st 2005. We also analyzed differences in prevalence between socio-economic status groups at January 1st 2005. We divided the population into low-, middle- and high-income groups, which were equal in size, based on reported income at the baseline interview. A chi-squared (χ^2) test was used to test for differences in prevalence between income groups. For each DDI the exposure time and the number of exposed people were calculated. Linear regression was used to test whether changes over time were significant. These analyses were performed with SPSS software (version 11.0.1; SPSS, Chicago, IL).

RESULTS

The average age in the study population on January 1st 1992 was 70.3 years (standard deviation (SD) 9.8 years) and 39% were men. 3,728 people (48%) were over 70 years of age. The average follow-up time was 10.4 years (SD 4.2 years) and the total follow-up time of the study cohort was 81,310 person-years. In the study cohort 3,732 of the 7,842 people (48%) died during follow-up and loss to follow up was minimal. 280 of the 451 listed DDIs were not dispensed at all.

The incidence rate of a first dispensing of an interacting drug combination was 10.5 per 100 person-years and 6.7 per 100 person-years for a first dispensing of both drugs on the same day. The prevalence of any DDI at January 1st 2005 was 18.4% (table 2) in a population with an average age of 78.2 years. The prevalence in low-, middle- and high-income groups were 19.6, 16.9 and 19.0%, respectively. These differences were not statistically significant (χ^2=2.46, p=0.29). The incidence of a first potentially life-threatening (failure of therapy) DDI (category E or F) was 2.7 per 100 person-years and the prevalence at January 1st 2005 was 2.8%.

Table 2 Incidence, prevalence and exposure to drug-drug interactions

Level of potential clinical relevance	Incidence rate (95% CI) [a]		Incidence rate, dispensed on the same day (95% CI) [a]		Prevalence (%) (95% CI) [b]		Exposure time (% of total exposure time)
A	2.22	(2.12, 2.33)	1.51	(1.43, 1.60)	4.61	(3.99, 5.24)	14.26
B	1.64	(1.55, 1.73)	1.16	(1.08, 1.23)	5.70	(5.01, 6.39)	16.52
C	5.38	(5.20, 5.57)	2.59	(2.47, 2.71)	3.11	(2.59, 3.62)	15.49
D	5.49	(5.31, 5.68)	3.48	(3.34, 3.62)	7.39	(6.61, 8.18)	39.84
E	1.77	(1.67, 1.86)	0.73	(0.67, 0.79)	1.51	(1.14, 1.87)	8.83
F	1.16	(1.09, 1.24)	0.48	(0.43, 0.52)	1.32	(0.98, 1.66)	3.45
Any [c]	10.52	(10.22, 10.82)	6.67	(6.45, 6.88)	18.36	(17.20, 19.51)	100.00

[a] Per 100 person-years. [b] At January 1st 2005. [c] The number of the categories do not add up to the number in the any category because people could receive multiple interacting drug combinations and uncategorized DDIs are not represented separately.

The cumulative exposure time to DDIs was 14,823 person-years or 18.2% of total follow-up. The twenty DDIs with the longest duration in the study period are given in table 3. The first ten of these DDIs were responsible for 67.0% of the total exposure time to DDIs. Combinations of cardiovascular drugs causing hypotension, NSAIDs counteracting the blood pressure lowering effects of antihypertensives and combinations of drugs influencing potassium levels were most often involved. Table 4 lists the potentially life-threatening DDIs (category F) and DDIs with a potential risk of failure of life-saving therapy (category E). The exposure to potentially life-threatening DDIs (category F) was 3.5% and exposure to DDIs with a potential risk of failure of life-saving therapy (category E) was 8.8% of the total exposure time to DDIs.

Table 3 Twenty drug-drug interactions with the largest exposure time

	Drug-drug interaction	Category	Users	Duration as % of exposure time
1.	ACE-inhibitors + diuretics	3D	1,587	19.91
2.	Digoxin + potassium losing diuretics	3A	785	9.66
3.	Diuretics + NSAIDs	3D	2,061	7.44
4.	β-adrenoreceptor antagonists + oral blood glucose lowering drugs	3B	354	6.66
5.	β-adrenoreceptor antagonists + NSAIDs	3C	1,679	5.24
6.	RAAS inhibitors + NSAIDs	3D	1,271	4.28
7.	NSAIDs (not COXIBs) + corticosteroids	3C	1,394	3.65
8.	β-adrenoreceptor antagonists + verapamil/diltiazem	3E	437	3.59
9.	α-adrenoreceptor antagonists + β-adrenoreceptor antagonists or calcium channel blockers	3B	307	3.45
10.	Angiotensin II antagonists + diuretics	3B	387	3.15
11.	Bisphosphonates + antacids/iron/calcium	0A	451	3.01
12.	RAAS inhibitors + potassium or potassium sparing drugs	2F	422	3.01
13.	Simvastatin/atorvastatin + verapamil/diltiazem	3E	161	2.64
14.	Digoxin + verapamil/diltiazem	3D	203	2.09
15.	NSAIDs (not COXIBs) + SSRIs/trazodone	4C	403	2.05
16.	Thyroid preparations + antacids/calcium	3C	121	1.91
17.	Vitamin K antagonists + amiodarone/propafenone	3D	211	1.73
18.	QT prolongating drugs + QT prolongating drugs (not erythromycin, clarithromycin, voriconazole)	1E	614	1.47
19.	Vitamin K antagonists + thyroid preparations	1B	100	1.33
20.	Digoxin + amiodarone	3D	144	1.32

COX-2: cyclo-oxygenase-2; ACE: angiotensin converting enzyme; NSAIDs: non-steroid anti-inflammatory drugs; RAAS: renin-angiotensin-aldosterone system; SSRIs: selective serotonin reuptake inhibitors.

The prevalence of DDIs increases with age (figure 1). In people aged 70 years and older the prevalence rose between 1992 and 2005 from 10.5% to 19.2% ($p<0.001$, figure 2). There was no increase of prevalence in people younger then 70 years of age between 1992 and 2002 ($p=0.29$). In 2005 the risk of exposure to DDIs was lower in the age category of 84 years and older compared with the age categories 70-74 years and 75-79 years. The prevalence of potentially life-threatening (failure of therapy) DDIs (category E and F) also increased during the study period (figure 3). This increase was present in all age categories, although there was a small decrease from 2004 to 2005. The prevalence of these DDIs in people aged 70 years and older increased from 1.5% in 1992 to 2.9% in 2005 ($p<0.001$, figure 4). This increase was caused by an increase in potentially life-threatening DDIs between spironolactone and renin-angiotensin-aldosteron system (RAAS) inhibitors and between spironolactone and potassium. The prevalence of these DDIs was below 0.3% between 1992 and 1999 in people aged 70 years and older but increased between 1999 and 2004 to 1.2% ($p=0.004$). A small decrease to 1.1% was seen in 2005. The overall prevalence of DDIs with a potential risk of failure of life-saving therapy and the other potentially life-threatening DDIs increased between 1992 and 2005 from 1.5% to 1.9% ($p=0.001$).

Table 4 Exposure to potentially life-threatening (failure of therapy) drug-drug interactions

Drug-drug interaction		Category	Users	Duration as % of exposure time	Duration as % of E or F
Potentially life-threatening DDIs (category F)					
1.	RAAS Inhibitors + potassium or potassium sparing drugs	2F	422	3.01	87.34
2.	Potassium + potassium sparing diuretics	3F	41	0.11	3.11
3.	Coumarin + tamoxifen	1F	20	0.10	2.81
4.	Ibopamine + amiodarone	3F	13	0.08	2.28
5.	SSRIs + tramadol	1F	46	0.05	1.41
	Other		492	0.10	3.04
	Total		911 [a]	3.45	100.00
Potential risk of failure of life-saving therapy (category E)					
1.	Beta-blockers + verapamil/diltiazem	3E	437	3.59	40.68
2.	Simva/atorvastatin + verapamil/diltiazem	3E	161	2.64	29.88
3.	QT-prolongators + QT-prolongators (excl. ery / clarithromycin/voriconazole)	1E	614	1.47	16.64
4.	Methotrexate + NSAIDs	3E	38	0.52	5.93
5.	Statins + gemfibrozil	3E	44	0.28	3.16
6.	Ketanserin + potassium losing diuretics	3E	24	0.19	2.19
	Other		495	0.14	1.53
	Total		1,359 [a]	8.83	100.00

[a] As one person can be exposed to more than one DDI, the total is not the sum of the separate DDIs. NSAIDs: non-steroid anti-inflammatory drugs; RAAS: renin-angiotensin-aldosterone system; SSRIs: selective serotonin reuptake inhibitors.

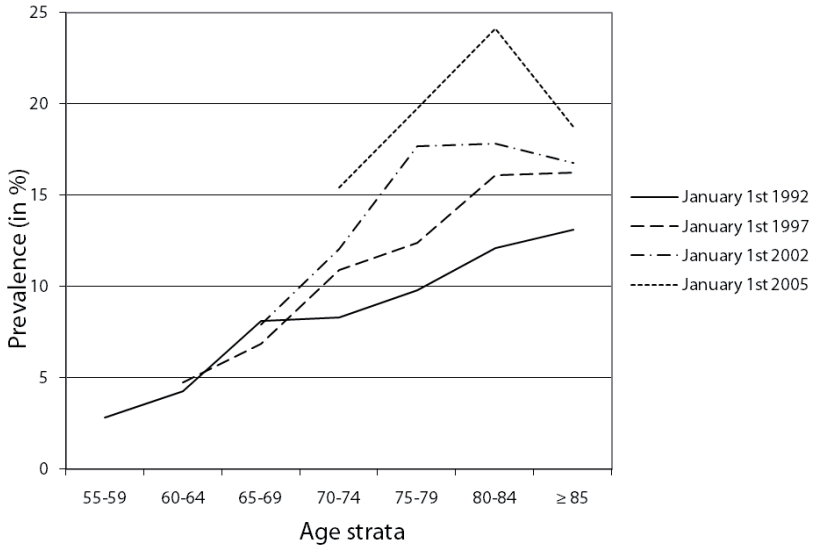


Figure 1 Prevalence of drug-drug interactions over time per age stratum

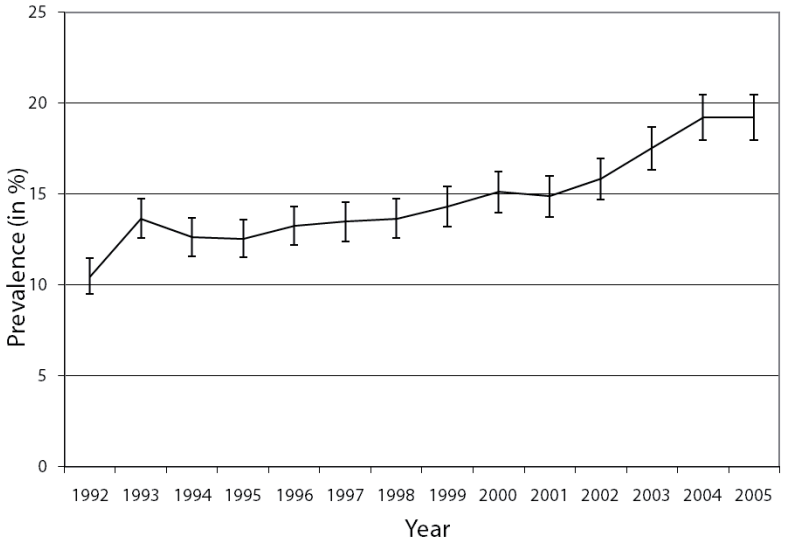


Figure 2 Prevalence (95% CI) of drug-drug interactions over time in people aged ≥70 years

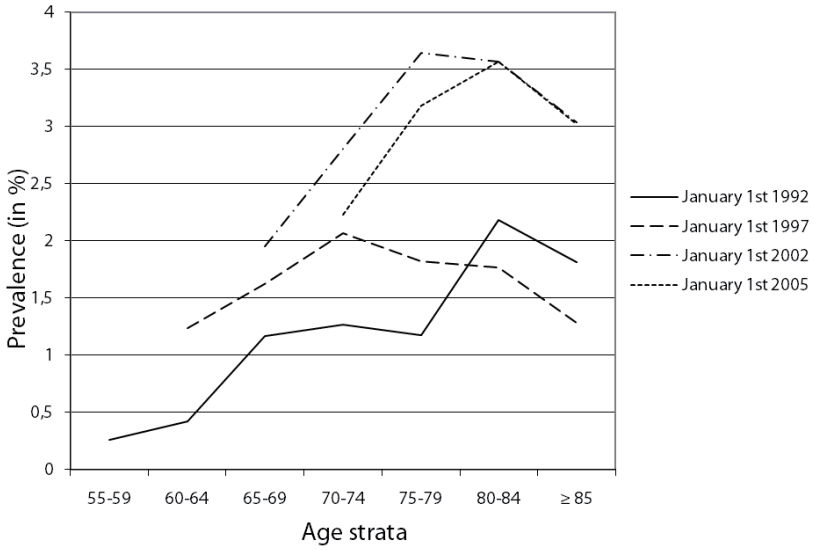


Figure 3 Prevalence of potentially life-threatening (failure of therapy) drug-drug interactions (category E and F) over time per age stratum

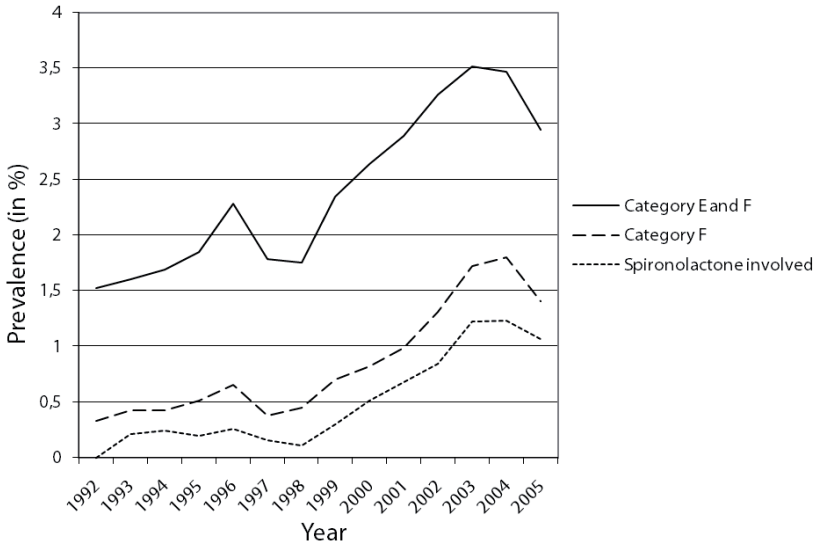


Figure 4 Prevalence over time of potentially life-threatening drug-drug interactions (category F), drug-drug interactions with a potential risk of failure of life-saving therapy (category E), and of potentially life-threatening drug-drug interactions (category F) in which spironolactone was involved, in people aged 70 years and older.

DISCUSSION

In our study, many people were exposed to DDIs during the study period. Furthermore, in the study period, the prevalence of DDIs in people aged 70 years and older doubled. In 1992, one in ten people was exposed to DDIs compared with one in five in 2005. The prevalence of potentially life-threatening DDIs in people aged 70 years and older also doubled from 1.5% in 1992 to 2.9% in 2005. The main cause was an increase in the prevalence of DDIs between spironolactone and RAAS inhibition therapy. This increased use of spironolactone followed the publication of RALES (Randomized Aldactone Evaluation Study), in which the beneficial effects of spironolactone in the treatment of heart failure were shown.[18] In 2004, an increase in hospitalizations due to hyperkalaemia was associated with more frequent use of spironolactone after publication of RALES and this may have caused the observed decrease in 2005.[19]

Although the results of this study might suggest that a large part of the population aged 55 years and older is exposed to a potential threat, dispensing of drugs that result in DDIs is often inevitable.[20] Avoiding the drugs causing the DDI is often not possible because alternative drugs that do not interact are not available and the drugs are clinically necessary. The majority of exposure to DDIs was caused by DDIs that were clinically relevant, although not life-threatening. With these DDIs serious adverse events may happen, although the risks are

often acceptable. A small number of well known DDIs were responsible for the majority of exposure time, such as DDIs between ACE inhibitors and diuretics and between NSAIDs and β-adrenoreceptor antagonists. Guidelines to reduce the risks of these inevitable DDIs exist and, if followed appropriately, the risk of adverse reactions is acceptable. An example is the DDI between NSAIDs and β-adrenoreceptor antagonists. This DDI can be managed by checking blood pressure regularly at start of therapy and adepting therapy if necessary.[21] The DDI between RAAS inhibitors and potassium or potassium-sparing drugs is responsible for most of the exposure time to life-threatening DDIs. These drug combinations are indicated if potassium levels are low, as may happen when patients are concomitantly treated with loop diuretics.[22] Again, the risks of adverse events are acceptable, when potassium levels are monitored regularly. However, while guidelines exist to reduce the risks associated with the use of interacting drug combinations, the absolute risk of adverse reactions caused by DDIs remains considerable when the prevalence of DDIs is high.

Medication surveillance systems alert for all DDIs that do occur, resulting in a high number of signals, of which the majority is clinically irrelevant. A large portion of the irrelevant signals can be suppressed, if clinical rules for these DDIs are implemented in the system. For example, the risk of a sudden strong reduction in blood pressure caused by the frequently used combination of ACE-inhibitors and diuretics is high if therapy with ACE-inhibitors is started during diuretic therapy.[23] Thus, many signals can be avoided if the system generates an alert only when an ACE inhibitor is added to diuretic therapy. The same applies to the DDI between RAAS inhibitors or diuretics and NSAIDs. These DDIs are mostly clinically irrelevant in the case of normal renal functioning and in the absence of heart failure. However, while a medication surveillance system can identify some irrelevant signals, the judgment as to whether a DDI can be used safely must be tailored to every individual case. The risk of adverse reactions is dependent on many patient characteristics, such as age, co-morbidity and renal function, and precise rules for deciding which DDIs can be dispensed safely or should be avoided in all cases cannot be given.

This study has some potential limitations. First, we had information only on dispensed prescriptions. As we do not have other information, for example, on the counseling of the prescriber or patient by the pharmacist, we did not know whether guidelines were followed to reduce the risk of adverse reactions. Precautionary measures can be taken, for example by measuring potassium levels, stopping one of the drugs and adjusting the dose regimen. As it is likely that dispensing two drugs on the same day meant that these drugs are used concomitantly, we therefore also determined the incidence rates of simultaneous dispensing. We also did not know how often a DDI was cancelled following contact between the pharmacist and the prescribing physician. Second, in this study we use the DDI list from 2006. As the dispensings were between 1992 and 2005, it is possible that a combination of prescribed drugs was not recognized or categorized as a DDI at that time. However, such misclassification was probably modest. Third, in this study we included only prescribed drugs. Some drugs with

potential DDIs, such as NSAIDs and hypericum (St. John's Wort), could be obtained without prescriptions. DDIs involving these drugs were not included in the analysis.

To conclude, a large number of people aged 55 years and older in the Netherlands are exposed to DDIs and this number has increased sharply between 1992 and 2005. A limited number of DDIs are responsible for the majority of the exposure time, and most of these drugs are part of normal pharmacotherapy. Because of their high prevalence and the sharp increase in this prevalence over the last decade, healthcare professionals should pay special attention to the potential risks of DDIs in people aged 70 years and older. This is particularly the case for DDIs involving spironolactone, which may cause potentially life-threatening elevated potassium levels.

REFERENCES

1. McDonnell PJ, Jacobs MR. Hospital admissions resulting from preventable adverse drug reactions. *Ann Pharmacother* 2002;36(9):1331-6.
2. Peyriere H, Cassan S, Floutard E, et al. Adverse drug events associated with hospital admission. *Ann Pharmacother* 2003;37(1):5-11.
3. Pirmohamed M, James S, Meakin S, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ* 2004;329(7456):15-9.
4. Juurlink DN, Mamdani M, Kopp A, Laupacis A, Redelmeier DA. Drug-drug interactions among elderly patients hospitalized for drug toxicity. *JAMA* 2003;289(13):1652-8.
5. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. *N Engl J Med* 2004;351(11):1089-96.
6. Routledge PA, O'Mahony MS, Woodhouse KW. Adverse drug reactions in elderly patients. *Br J Clin Pharmacol* 2004;57(2):121-6.
7. Lindley CM, Tully MP, Paramsothy V, Tallis RC. Inappropriate medication is a major cause of adverse drug reactions in elderly patients. *Age Ageing* 1992;21(4):294-300.
8. Doucet J, Chassagne P, Trivalle C, et al. Drug-drug interactions related to hospital admissions in older adults: a prospective study of 1000 patients. *J Am Geriatr Soc* 1996;44(8):944-8.
9. Merlo J, Liedholm H, Lindblad U, et al. Prescriptions with potential drug interactions dispensed at Swedish pharmacies in January 1999: cross sectional study. *BMJ* 2001;323(7310):427-8.
10. Bergendal L, Friberg A, Schaffrath A. Potential drug-drug interactions in 5,125 mostly elderly outpatients in Gothenburg, Sweden. *Pharm World Sci* 1995;17(5):152-7.
11. Bjerrum L, Andersen M, Petersen G, Kragstrup J. Exposure to potential drug interactions in primary health care. *Scand J Prim Health Care* 2003;21(3):153-8.
12. Bjorkman IK, Fastbom J, Schmidt IK, Bernsten CB. Drug-drug interactions in the elderly. *Ann Pharmacother* 2002;36(11):1675-81.
13. Braverman SE, Howard RS, Bryant PR, Belandres PV. Potential drug interactions in a physical medicine and rehabilitation clinic. *Am J Phys Med Rehabil* 1996;75(1):44-9.
14. Linnarsson R. Drug interactions in primary health care. A retrospective database study and its implications for the design of a computerized decision support system. *Scand J Prim Health Care* 1993;11(3):181-6.
15. 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.
16. van Roon EN, Flikweert S, le Comte M, et al. Clinical relevance of drug-drug interactions : a structured assessment procedure. *Drug Saf* 2005;28(12):1131-9.
17. WINAp. G-standaard. Den Haag: WINAp; 2006.
18. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999;341(10):709-17.
19. Juurlink DN, Mamdani MM, Lee DS, et al. Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. *N Engl J Med* 2004;351(6):543-51.
20. Stockley IH. Stockley's drug interactions : a source book of interactions, their mechanisms, clinical importance and management. London: Pharmaceutical Press; 2002.
21. Johnson AG. NSAIDs and increased blood pressure. What is the clinical significance? *Drug Saf* 1997;17(5):277-89.

22. Radley AS, Fitzpatrick RW. An evaluation of the potential interaction between enalapril and amiloride. *J Clin Pharm Ther* 1987;12(5):319-23.
23. Koffer H, Vlasses PH, Ferguson RK, Weis M, Adler AG. Captopril in diuretic-treated hypertensive patients. *JAMA* 1980;244(22):2532-5.

Chapter 2.2.

Potential determinants of drug-drug interaction associated dispensing in community pharmacies: a literature review

ABSTRACT

Background: Although the number of clinically relevant drug-drug interactions (DDIs) is probably low, DDIs may be responsible for a substantial number of hospital admissions. In some countries, the pharmacist is responsible for preventing the use of unsafe or non-effective drug regimens. Specifically they should avoid the dispensing of combinations of drugs that may cause serious DDIs. In order to assess the determinants related to community pharmacies and associated with these dispensings, a systematic literature review was conducted.
Methods: Medline and International Pharmaceutical Abstracts were searched for articles published in English between 1993 and 2003. Additional relevant articles were identified by screening the reference lists of relevant articles.
Results: Seven papers were located. The determinants described in the literature were divided into three groups. The first group focused on the relationship between the pharmacist and the prescriber. The number of prescribers is of importance as well as the number of dispensing pharmacies. Both a high number of primary care physicians and multiple dispensing pharmacies increased the risk of DDIs. The availability, quality and sensitivity of the medication surveillance software appeared to be a second important determinant. Both too many and too few signals increased the risk of dispensing interacting drugs. The third group of determinants was related to the pharmacist and pharmacy organization. Signals from the surveillance program are usually judged first by technicians and subsequently managed by the pharmacist. Consequently, knowledge, instructions and supervision are important determinants. A fourth group of determinants was identified in literature assessing interventions by pharmacists, including interventions for DDIs. A higher workload was associated with lower intervention rates, which indicated a higher risk of dispensing interacting drugs.
Conclusion: The determinants identified in this review can be used to develop strategies to minimize patient harm resulting from DDIs. Further assessment of the relation between these determinants and the dispensing of DDIs and of the relation between DDI-associated dispensing and patient harm is recommended.

INTRODUCTION

One of the consequences of multiple drug use is the risk of one drug influencing the effect of a second drug. This so called drug-drug interaction (DDI) is defined as a pharmacokinetic or pharmacodynamic influence of drugs on each other, which can result, besides desired effects, in reduced effectiveness or increased toxicity.[1] The seriousness and clinical relevance of DDIs vary considerably. Although DDIs are probably common, only 10-12% of the prescriptions involving a DDI have serious clinical consequences.[2-4] The seriousness of the DDI should be weighed against the benefit of both drug therapies and the availability of alternatives.

Previously, the tasks of the pharmacist focused on the production and dispensing of a limited number of drugs. With the growing number of available drugs and the increasing complexity of drug therapy, such as in the treatment of HIV-related diseases, the role of the pharmacist is changing rapidly from product-centered to patient-centered. In some countries, including the Netherlands, one of the present responsibilities of the pharmacist is to prevent the use of unsafe or non-effective drug regimens. In the Netherlands, every pharmacist is obliged to use a medication surveillance program for this task. One of their responsibilities is to prevent the dispensing of interacting drugs, which carries too much risk for patient harm. Studies assessing intervention by pharmacists show that the percentage of prescriptions that are intercepted ranges from 0.75 to 1.9%.[5-10] This variation may partly be attributable to variations in the definition of intervention. The percentage of intercepted prescriptions that prevent adverse clinical consequences ranges from 0.27% to 0.95%.[5-8] Only between 0.011% and 0.078% of the prescriptions are intercepted because of a DDI.[5,6,8] Although this low percentage suggests that DDIs are of no clinical significance, the adverse consequences may be substantial.[6] Different studies suggest that the number of hospital admissions due to DDIs is up to 3% of all admissions.[11-14] This could be an underestimation because of the inability of practitioners and pharmacists to identify a DDI as the cause of an adverse outcome. It is possible that a drug-related problem is ascribed to the last prescribed drug and not to an interaction of this drug with another one.

In this review we searched for process and structure characteristics that have a relationship with the dispensing of interacting drugs. Process and structure characteristics determine the outcome of care, which can be understood in terms of death, disease, disability, discomfort and dissatisfaction.[15] The dispensing of drugs involving a DDI is assessed as a proxy for the outcome of healthcare. There are three reasons for focusing on the determinants for DDI associated dispensing. First, DDIs are a clearly defined type of error and they have a relation with the present task of the pharmacist. Second, DDIs are considered to be an important cause of adverse events. Third, the dispensing of drugs that are part of a DDI can be traced in databases. In locations where the patient's medication history is filed, it is relatively easy to trace DDIs during observational assessments in the future.

The objective of the review was to investigate which determinants within community pharmacies are associated with a high frequency of DDI associated dispensing.

LITERATURE SEARCH METHODOLOGY

Determinants in this literature review were identified by searching Medline and International Pharmaceutical Abstracts (IPA) for articles published between January 1993 and December 2003. This timeframe was chosen because the tasks of the pharmacist have changed considerably in the last decade. Therefore, we assumed that literature written before 1993 would not apply to current daily practice.

In the Medline search the results of two search strategies were combined. The first one used the medical subject heading (MeSH) descriptors 'drug-interactions', 'drug-antagonism', 'drug-synergism' and 'medication-errors'. The second one used the MeSH descriptor 'community pharmacy services' or the keyword 'dispens*' for all fields. To exclude studies concerning dispensing in hospital pharmacies, papers with the keywords 'hospital pharmacy services' were omitted.

In the IPA search the results of a similar strategy were used. The first search used the terms 'drug interactions', 'medication errors', 'medication error?' and 'dispensing error?'. The latter two were searched for the title only. The second search used the terms 'community pharmacy serviced' and 'dispens?'. Studies with the terms 'hospital pharmacy services' or 'institutional hospital pharmacy' were excluded.

Papers from both literature searches were included if they were written in English, were applicable to community pharmacy services and to DDI-associated dispensing and described determinants involved in the dispensing of DDIs. Articles that matched the inclusion criteria were selected and additional relevant articles were identified by screening the reference lists of these articles. Papers looking at prescriber or patient characteristics as determinants were outside the scope of this review.

The Medline search yielded 134 articles and the IPA search yielded 357 articles. Reference tracking and verification as to whether the articles met the inclusion criteria resulted in the selection of seven articles on determinants of dispensing interacting drugs.[1,16-21] None of the articles discussed the entire range of determinants involved in DDI-associated dispensing.

DDI ASSOCIATED DETERMINANTS

The determinants for the dispensing of interacting drugs could be divided into three groups (table 1). The first group described the 'relationship with the prescriber' and the other groups ('medication surveillance program' and 'pharmacy organization') described determinants within the pharmacy.

Table 1 Determinants for drug-drug interaction (DDI) associated dispensing

Study	Independent variable	Effect	Dependent variable	Size of the effect
Relationship with prescriber				
Tamblyn et al.[21]	Single primary care physician	Lower	Receiving DDI	Cardiovascular drugs OR = 0.70 (99% CI 0.6, 0.8), psychotropic drugs OR = 0.79 (CI not given), NSAID OR = 0.94 (CI not given)
Tamblyn et al.[21]	Single dispensing pharmacy	Lower	Receiving DDI	Cardiovascular OR = 0.68 (99% CI 0.6, 0.8), psychotropic OR = 0.79 (CI not given), NSAID OR = 0.75 (CI not given)
Medication surveillance program				
Halkin et al.[17]	Introduction of medication surveillance program	Lower	Dispensing severe DDIs	OR=0.28 (95% CI 0.26, 0.30)
Tamblyn et al.[20]	Introduction of medication surveillance program	Higher	Discontinuation rate of prescriptions for DDIs	OR = 1.33 (95% CI 0.90, 1.95)
Hazlet et al.[18]	Software does not recognize interaction	Higher	Risk of DDI	NA
Schalekamp[1]	Software gives too many signals	Higher	Risk of DDI	NA
Bates & Leape[16]	Software does not sufficiently come up with 'red flag'	Higher	Risk of DDI	NA
Pharmacy organization				
Schalekamp[1] Heijboer-Vinks[19]	Management of medication surveillance signals	NA	NA	NA

NA: not available; OR: odds ratio; NSAID: non-steroid anti-inflammatory drug; CI: confidence interval.

Relationship with prescriber

Tamblyn et al.[21] assessed whether the risk of a DDI increased with the number of prescribers. Patients who had a single primary-care physician or a single dispensing pharmacy were less likely to be prescribed concomitant medications causing a DDI.

Medication surveillance program

In the study by Halkin et al.[17], the introduction of medication surveillance software for DDIs in the majority of community pharmacies and physician offices reduced the dispensing of prescriptions with severe interactions by 67.5%. Tamblyn et al.[20] also found that, although it was not significant, the introduction of medication surveillance programs by primary care physicians increased the discontinuation rate of prescriptions involving interacting drugs. On the other hand, discussion exists as to whether medication surveillance programs can prevent the dispensing of all relevant DDIs. In letters to the editor, both Cavuto et al.[22] and Kraft and Dore[23] reported that some of the pharmacists using a computer program were unable to prevent well documented DDIs. In their reply, Bates and Leape[16] discussed the

reasons why pharmacists failed to intervene in spite of the use of a medication surveillance program. First, the software may not be able to correctly identify clinically important DDIs because the software is not up-to-date or well documented DDIs are otherwise absent from the database.[18] Second, because of an overload of interaction signals, pharmacists may have grown accustomed to skipping through them rapidly. Too many warnings complicate medication surveillance because the identification of relevant signals becomes more difficult. They are most often caused by repeated warnings for the same patient, managed in an earlier dispensing.[1] The third reason why pharmacists may be unable to intervene in spite of use of medication surveillance programs is that the program does recognize certain drug combinations, but does not sufficiently alert the pharmacist or technician that a DDI is present and that the dispensing should be prevented.[16]

Pharmacy organization

The management of the signals generated by the medication surveillance program is important.[1,19] In the first place, the sensitivity of the software is an issue. Both ignoring signals that need to be managed and an overload of signals should be avoided. A signal must be judged on relevance and, if relevant, it must be followed by an appropriate action. In community pharmacies most signals will be noticed first by technicians. They should be instructed and supervised on how to judge and, if possible, how to manage these signals. The last issue is the knowledge of the pharmacist in managing DDIs and the ability of the pharmacist to judge the risk of DDIs.

DISCUSSION

The purpose of this literature review was to identify determinants of DDI-associated dispensing in community pharmacies. Determinants concerning the prescriber or the patient, for example interactions with over-the-counter drugs, were outside the scope of this review. Although the number of interventions related to DDIs is small, DDIs may be a major risk for hospital admission. Studies were identified that assessed the interventions by pharmacists and the number of hospital admissions caused by DDIs, but no studies were found that assessed the relationship between these interventions and hospital admissions. In some countries, pharmacists have a task to prevent serious DDIs. Focus on the determinants in the pharmacy may reduce the dispensing of drugs involving a DDI and improve patient outcome. The determinants of interest for surveillance of DDI-associated dispensing could be divided into three groups. These groups are 'relationship with the prescriber', 'medication surveillance program' and 'pharmacist and pharmacy organization'. Proper attention paid to these determinants can contribute to the prevention of the dispensing of interacting drugs.

In the first group, Tamblyn et al.[21] found that an increasing number of prescribers or pharmacists involved in the dispensing of drugs increases the risk of dispensing DDIs. The influence of the number and kind of prescribers was also described by studies assessing intervention by pharmacists, including interventions for DDIs (table 2). A high number of interventions suggest a high risk for DDI-associated dispensing as the risk of a DDI remaining unnoticed might increase. Although interventions for DDIs were only a small part of the total number of interventions, these studies give insight into what may go wrong during the process of drug dispensing. Pharmacists more often modified prescriptions from specialists and prescriptions from GPs other than the patient's own GP than prescriptions from the patient's own general practitioners (GP).[5] Westein et al.[9] also found that prescriptions from specialists had higher intervention rates than prescriptions from the patient's own GP, although this

Table 2 determinants for interventions including interventions for DDIs

Study	Independent variable	Effect	Dependent variable	Size of the effect
Relationship with prescriber				
Buurma et al.[5]	Prescriptions from specialists	Higher	Prescription interventions	OR = 1.82 (95% CI 1.57, 2.11) 27.5% in intervention sample versus 17.6% in control sample
Westein et al.[9]	Prescriptions from specialists	Higher	Prescription interventions	OR = 1.21 (95% CI 0.69, 1.72)
Buurma et al.[5]	GP not being patient's own GP	Higher	Prescription interventions	OR = 1.49 (95% CI 1.02, 2.17) 3.1% in intervention sample versus 2.4% in control sample
Westein et al.[9]	Drugs part of complex drug therapy	Higher	Prescription interventions	
	>3 prescribers			OR = 1.75 (95% CI 0.51, 2.99)
	>15 prescriptions in 3 months			OR = 1.60 (95% CI 0.80, 2.40)
	>3 different medications			OR = 1.48 (95% CI 0.98, 1.99)
Rupp et al.[8]	Direct prescription order transmission between GP and pharmacist	Lower	Prescription interventions	7.2% in intervention sample versus 18.9% in control sample
Buurma et al.[5]	Hand written prescriptions	Higher	Modification	OR = 3.30 (95% CI 2.90, 3.75)
Buurma et al.[5]	Physician has online access to actual patients medication record	Lower	Modification	OR = 1.61 (95% CI 1.33, 1.94)
Medication surveillance program				
Westein et al.[9]	Number of signals	No relationship	Interventions	
Pharmacy and pharmacy organization				
Currie et al.[24]	Pharmaceutical care training	Higher	Interventions	OR = 8.1 (95% CI 4.7, 14.2)
Westerlund et al.[10]	Work satisfaction	Higher	Drug related problem detection rate	R_c = 0.020 (95% CI –0.157, 0.197)
Rupp et al.[8]	Chain and independent pharmacies	No difference	Interventions	

GP: general practitioner; OR: odds ratio; R_c: slope.

result was not significant. The higher intervention rates for specialist prescriptions and for prescriptions from GPs other than the patient's own GP, show the importance of a central point for the drug therapy to be coordinated. A higher, but not significant, intervention rate was also found for drugs taken as part of a complex drug therapy.[9]

A direct prescription order communication between the prescriber and the pharmacist gave rise to less interventions than a prescription order communicated by the patient or a representative.[8] Intervention rates were higher for handwritten prescriptions and when the GP had no online access to the actual patient's medication record in the pharmacy computer.[5] Handwritten prescriptions require extra attention by the pharmacy because they can imply that no medication surveillance by computer took place during the prescribing process. In addition, misreading the prescription can lead to the wrong drug being dispensed.

The second group found that the medication surveillance program and its sensitivity is important. Differences exist between the degree of computerization and the availability of medication histories in community pharmacies, which are largely influenced by the environment. In the Netherlands all pharmacists are obliged to keep records of the drugs that are dispensed. In the first place, the availability of medication surveillance programs is of interest for reducing the dispensing of DDIs; in the second place, the way these programs are used is important. Hazlet et al.[18] assessed the differences between software programs in detecting a non-representative, but well documented group of interactions. Although they assessed differences between software programs only, they also found differences between users of the same software program, which emphasizes the importance of fine-tuning the sensitivity of the program. Different studies suggest that only some of the signals produced lead to interventions.[9,25,26] Westein et al.[9] did not find any association between the number of signals and the number of interventions. Therefore, it is important that the number of irrelevant signals is low, but that all relevant DDIs are detected and managed correctly. It is recommended that attention is paid to both the quality and sensitivity of the software.

The third group describes the influence of the pharmacist and pharmacy organization. These determinants may play an important role in avoiding DDI-associated dispensing. This group is influenced to a large extent by the environment, for example the contribution of technicians in the community pharmacy and the use of medication surveillance programs. Studies assessing interventions by pharmacists, including interventions for DDIs, reported that pharmaceutical care training[24] and higher work satisfaction[10] were associated with higher intervention rates. No differences in intervention rates was found between chain pharmacies and independently owned pharmacies.[8]

This literature review has some limitations. The ultimate purpose was to associate determinants of the dispensing of interacting drugs with the outcome of healthcare. In this review, these dispensings were assessed as a proxy for outcome. The relationship between the dispensing of interacting drugs and outcome can be assumed based on studies indicating that DDI-associated interventions prevent patient harm[6] and that DDIs are a cause of hospital ad-

missions.[11-14] The literature search was limited to the Medline and IPA databases and possibly caused publication bias and the exclusion of data that is published in journals not selected in Medline or IPA. Only a limited number of studies were found to have exclusively assessed the dispensing of concomitant medications that do interact. A number of studies assessed the interventions by pharmacists, including the interventions for DDIs. It can reasonably be expected that determinants described in these studies are also applicable to the dispensing of DDIs. An additional determinant was workload, with three of the four studies finding that an elevated number of dispensed prescriptions was significantly associated with a lower intervention rate and probably indicated a higher patient risk (table 3).[6-8,10]

Table 3 Influence of workload on community pharmacy services

Study	Country, year	Method	Study population	Independent variable	Dependent variable	Correlation	
Caleo, et al.[6]	Australia, 1996	Case series	580 pharmacy days	Prescriptions per pharmacy	Intervention rate	No	
Hawksworth et al.[7]	UK, 1999	Case series	840 pharmacy days	Prescriptions per pharmacy	Intervention rate	Yes	Correlation coefficient: -0.65
Rupp, et al.[8]	USA, 1992	Case-control	445 pharmacy days	Prescriptions per pharmacist per hour	Intervention rate	Yes	Regression coefficient: -0.40
Westerlund et al.[10]	Sweden, 1999	Case series	144 pharmacy professionals [a]	Weighted transactions	Drug-related problem detection rate	Yes	Regression coefficient: $5x10^{-6}$

[a] 34 pharmacists, 71 prescriptionists and 39 pharmacy technicians.

Most of the studies covered in this article were sensitive to bias, such as selection bias and bias because participants were aware that they were being observed. Most likely participating pharmacists were not afraid to show their shortcomings and probably had an increased level of attention during the observation period. Consequently, the number of actions taken by pharmacists may be overestimated and, thus, patient risk may be underestimated. Finally, none of the studies in the literature assessed the whole range of determinants for DDI-associated dispensing. Therefore, it cannot be guaranteed that no determinants were missed. Also, the definition of DDI used in the studies varied to a large extent. Because there is a wide range in the seriousness of DDIs, a drug combination could be considered as a DDI in one study, but not in another. Finally, the determinants identified in the studies for the dispensing of DDIs were influenced by the environment, for example legislation, the division of tasks between pharmacists and other personnel, and the healthcare system. Because most studies were performed in different countries, results may not be comparable to one another.

To conclude, there are three groups of determinants for the dispensing of DDIs in community pharmacy services. These groups are 'relationship with the prescriber', 'medication surveillance program' and 'pharmacy organization'. In studies assessing interventions by pharmacists, including the interception of prescriptions involving DDIs, determinants such

as workload were found. It can reasonably be expected that these determinants have a relationship with the dispensing of DDIs. To validate these results, further assessment of the relationship between DDI-associated dispensing and patient harm is necessary. The results of this review are used in an observational study on the association between the determinants and the dispensing of DDIs in community pharmacies.

REFERENCES

1. Schalekamp T. Omgaan met geneesmiddeleninteracties. *Geneesmiddelenbulletin* 1997;31(8):87-94.
2. Bergendal L, Friberg A, Schaffrath A. Potential drug-drug interactions in 5,125 mostly elderly outpatients in Gothenburg, Sweden. *Pharm World Sci* 1995;17(5):152-7.
3. Linnarsson R. Drug interactions in primary health care. A retrospective database study and its implications for the design of a computerized decision support system. *Scand J Prim Health Care* 1993;11(3):181-6.
4. Merlo J, Liedholm H, Lindblad U, et al. Prescriptions with potential drug interactions dispensed at Swedish pharmacies in January 1999: cross sectional study. *BMJ* 2001;323(7310):427-8.
5. Buurma H, de Smet PA, van den Hoff OP, Egberts AC. Nature, frequency and determinants of prescription modifications in Dutch community pharmacies. *Br J Clin Pharmacol* 2001;52(1):85-91.
6. Caleo S, Benrimoj S, Collins D. Clinical evaluation of community pharmacists' interventions. *Int J Pharm Pract* 1996(4):221-7.
7. Hawksworth GM, Corlett AJ, Wright DJ, Chrystyn H. Clinical pharmacy interventions by community pharmacists during the dispensing process. *Br J Clin Pharmacol* 1999;47(6):695-700.
8. Rupp MT, DeYoung M, Schondelmeyer SW. Prescribing problems and pharmacist interventions in community practice. *Med Care* 1992;30(10):926-40.
9. Westein MP, Herings RM, Leufkens HG. Determinants of pharmacists' interventions linked to prescription processing. *Pharm World Sci* 2001;23(3):98-101.
10. Westerlund T, Almarsdottir AB, Melander A. Factors influencing the detection rate of drug-related problems in community pharmacy. *Pharm World Sci* 1999;21(6):245-50.
11. Huic M, Mucolic V, Vrhovac B, Francetic I, Bakran I, Giljanovic S. Adverse drug reactions resulting in hospital admission. *Int J Clin Pharmacol Ther* 1994;32(12):675-82.
12. Jankel CA, Fitterman LK. Epidemiology of drug-drug interactions as a cause of hospital admissions. *Drug Saf* 1993;9(1):51-9.
13. McDonnell PJ, Jacobs MR. Hospital admissions resulting from preventable adverse drug reactions. *Ann Pharmacother* 2002;36(9):1331-6.
14. Peyriere H, Cassan S, Floutard E, et al. Adverse drug events associated with hospital admission. *Ann Pharmacother* 2003;37(1):5-11.
15. Donabedian A. The seven pillars of quality. *Arch Pathol Lab Med* 1990;114(11):1115-8.
16. Bates DW, Leape LL. Pharmacies and prevention of potentially fatal drug interactions. *JAMA* 1996;275(14):1086-7.
17. Halkin H, Katzir I, Kurman I, Jan J, Malkin BB. Preventing drug interactions by online prescription screening in community pharmacies and medical practices. *Clin Pharmacol Ther* 2001;69(4):260-5.
18. Hazlet TK, Lee TA, Hansten PD, Horn JR. Performance of community pharmacy drug interaction software. *J Am Pharm Assoc (Wash)* 2001;41(2):200-4.
19. Heijboer-Vinks IC. Kwaliteitszorg in de medicatiebewaking. The Hague: KNMP/WINAp; 1998.
20. Tamblyn R, Huang A, Perreault R, et al. The medical office of the 21st century (MOXXI): effectiveness of computerized decision-making support in reducing inappropriate prescribing in primary care. *CMAJ* 2003;169(6):549-56.
21. Tamblyn RM, McLeod PJ, Abrahamowicz M. Do too many cooks spoil the broth?: multiple physician involvement in medical management of elderly patients and potentially inappropriate drug combinations. *CMAJ* 1996;154(8):1177-84.

22. Cavuto NJ, Woosley RL, Sale M. Pharmacies and prevention of potentially fatal drug interactions. *JAMA* 1996;275(14):1086-7.
23. Kraft KE, Dore FH. Computerized drug interactions programs: how reliable? *JAMA* 1996;275(14):1087.
24. Currie JD, Chrischilles EA, Kuehl AK. Effect of a training program on community pharmacists' detection of and intervention in drug-related problems. *J Am Pharm Assoc* 1997;NS37(2):182-91.
25. Armstrong EP, Denemark CR. How pharmacists respond to on-line, real-time DUR alerts. *J Am Pharm Assoc* 1998;38(2):149-54.
26. van Mil JW, Dudok van Heel MC, Boersma M, Tromp TF. Interventions and documentation for drug-related problems in Dutch community pharmacies. *Am J Health Syst Pharm* 2001;58(15):1428-31.

Chapter 2.3.

Determinants of potential drug-drug interaction associated dispensing in community pharmacies in the Netherlands

ABSTRACT

Background: There are many drug-drug interactions (DDI) of which some may cause severe adverse patient outcomes. Dispensing interacting drug combinations should be avoided when the risks are higher than the benefits. The objective of this study was to identify determinants of dispensing undesirable interacting drug combinations by community pharmacies in the Netherlands.

Methods: A total of 256 Dutch community pharmacies were selected, based on the dispensing of eleven undesirable interacting drug combinations between January 1st 2001 and October 31st 2002. These pharmacies were sent a questionnaire by the Inspectorate for Health Care concerning their process and structure characteristics. We analyzed the association between the results from the questionnaire and the number of times the eleven undesirable interacting drug combinations were dispensed.

Results: 246 questionnaires (response rate 96,1%) were completed. Dispensing determinants were only found for the DDI between macrolide antibiotics and digoxin but not for the other ten DDIs. Pharmacies using different medication surveillance systems differed in the dispensing of this interacting drug combination, and pharmacies, which were part of a health care centre dispensed this interacting drug combination more often.

Conclusion: Medication surveillance in Dutch community pharmacies seems to be effective. Although for most DDIs no determinants were found, process and structure characteristics may have consequences for the dispensing of undesirable interacting drug combinations.

INTRODUCTION

Drug-drug interactions (DDIs) are responsible for many adverse patient outcomes. Different studies suggest that DDIs may cause up to three percent of all hospital admissions.[1-4] A DDI is defined as a pharmacokinetic or pharmacodynamic influence of drugs on each other, which may result in desired effects, in reduced efficacy and effectiveness or in increased toxicity.[5] Although many DDIs exist, only a small part of these DDIs is clinically relevant.[6-8] The potential benefits of drug combinations should be weighed against the seriousness of the DDI, taking into account the availability of alternatives. Only in cases that the risks associated with the DDI are higher than the benefits or if a better alternative is available, the DDI should be avoided.

In the Netherlands, one of the tasks of the pharmacist is to intervene in case of DDIs, which involve a high risk for the patient. Hereto, the pharmacist uses patient characteristics and the medication history. All prescriptions, which are submitted to the pharmacy, are screened on potential interactions with the help of medication surveillance software. These DDIs are evaluated by the pharmacist who intervenes if necessary. This task is important but cumbersome, and requires great attention from the pharmacist. The organizational aspects, such as the tuning of the medication surveillance software and instructions of technicians, should be managed by the pharmacist in such a way that in case of DDIs with a high risk the pharmacist intervenes. This is important for the prevention of adverse patient outcomes.[9]

The objective of this study was to assess process and structure characteristics associated with the dispensing of interacting drug combinations, which carry a high risk of adverse patient outcomes.

METHODS

Setting

The data for this study were retrieved from the Drug Information Project, a division of the Health Care Insurance Board. This is a database containing the reimbursement data from eight health care insurance companies in the Netherlands. The reimbursement data from January 1st 2001 until October 31st 2002 were analyzed. Eleven potential DDIs, that contained a high risk and could be substituted, because a good alternative was available, were selected and counted for each pharmacy in the database. These undesirable potential DDIs were mostly interactions between chronically used drugs which cannot be interrupted and short-term use of antibiotics or antimycotics, and were selected from the Dutch guidelines for the management of DDIs (table 1).[10, 11] A DDI was counted as such, when the chronically used drug was dispensed both in the period 150 days preceding and in the period 150 days after the dispensing of antibiotics or antimycotics for short-term use in the same pharmacy. Pharmacies with less than 5,000 dispensings in the database were excluded.

Table 1 Number of dispensings in the database of the individual drugs involved, the eleven potential DDIs and the calculated ratio

Drug-drug interaction			Number of dispensings drug A x 1,000 (range)		Number of dispensings drug B X 1,000 (range)		Number of DDIs counted (range)		Average ratio (range) [a]	
	Drug A	Drug B								
1	Erythromycin, clarithromycin, azithromycin, roxithromycin	Digoxin	440.8	(0-2754)	487.0	(0-3064)	3993	(0-41)	1.39	(0-18.52)
2	Itraconazole	Digoxin	88.7	(0-349)	487.0	(0-3064)	245	(0-7)	0.45	(0-21.69)
3	Ciprofloxacin	Theophylline	105.4	(0-769)	100.9	(0-756)	944	(0-14)	6.39	(0-534.38)
4	Miconazole, oral gel	Acenocoumarol, fenprocoumon	44.6	(0-233)	608.2	(5-3156)	154	(0-3)	0.38	(0-21.30)
5	Erythromycin	Carbamazepine	49.7	(0-531)	193.6	(0-871)	35	(0-4)	0.24	(0-40.92)
6	Erythromycin, clarithromycin, azithromycin	Disopyramide	426.6	(0-2754)	9.4	(0-151)	61	(0-4)	-	
7	Erythromycin, clarithromycin	Pimozide	274.4	(0-2004)	57.4	(0-394)	70	(0-15)	0.46	(0-46.12)
8	Propranolol, oxprenolol, pindolol	Beta2-mimetics, inhalation corticosteroids	250.6	(1-1075)	2546.9	(27-10504)	5127	(0-94)	0.54	(0-12.98)
9	Erythromycin, clarithromycin	Cisapride	274.4	(0-2004)	127.5	(0-821)	586	(0-11)	1.16	(0-40.45)
10	Itraconazole, fluconazole, ketoconazole	Cisapride	199.9	(0-727)	127.5	(0-821)	347	(0-12)	0.95	(0-57.10)
11	Acenocoumarol, fenprocoumon	Azapropazon	608.2	(5-3156)	8.4	(0-164)	32	(0-19)	-	

[a] Calculated with formula 1.

Procedure

For each pharmacy, we calculated the dispensing-ratios for the eleven potential DDIs with formula 1. This formula was used because the risk of dispensing a DDI between drug A and drug B is dependent on the number of times each drug is dispensed. The more drug A or drug B are dispensed, the higher the risk that these drugs are combined on the basis of chance alone. In case the dispensing of drug A is independent from the dispensing of drug B and the DDI is never intervened, the ratio will on average be one. The number of times this ratio was above one was calculated, because a ratio above one might indicate that medication surveillance fails. In this calculation, there were 342 DDIs between norfloxacin and theophylline, which were excluded in the analysis because the guidelines concerning the management of this DDI were inconsistent. Two groups of pharmacies were selected, and the pharmacists were asked in August 2003 by the Inspectorate for Health Care (IHC) to fill in a questionnaire. The first group included pharmacies with a high risk of dispensing these DDIs, while the

Formula 1

$$\text{Ratio 1} = \frac{\frac{k_{i,ab}}{N_i}}{\frac{k_{i,a}}{N_i} \bullet \frac{k_{i,b}}{N_i}}$$

Formula 2

$$\text{Ratio 2} = \frac{\frac{k_{i,ab}}{N_i \bullet \sum_i k_{i,ab} / N_{tot}}}{\frac{k_{i,a}}{N_i \bullet \sum_i k_{i,a} / N_{tot}} \bullet \frac{k_{i,b}}{N_i \bullet \sum_i k_{i,b} / N_{tot}}}$$

with

- $k_{i,ab}$: number of dispensings of interacting drug combination AB in pharmacy i
- $k_{i,a}$: number of dispensings of drug A in pharmacy i
- $k_{i,b}$: number of dispensings of drug B in pharmacy i
- N_i: total number of dispensed drugs known in the database in pharmacy i
- N_{tot}: total number of dispensed drugs known in the database in all pharmacies

second group consisted of a random sample from the remaining pharmacies. These groups were equal in size. The selection criteria are described in figure 1. The selection criterion for receiving a questionnaire (≥ 4 times a ratio >1) was chosen on pragmatic reasons to have enough power for statistical analysis with manageable numbers. A concept questionnaire was composed on basis of a literature search and interviews with experts.[9] The questions concerned process and structure characteristics of several quality aspects and those questions were selected that could discriminate between high and low quality pharmacies. Mostly questions with objective answers were included, for example about written instructions for technicians, filing of data, tuning of the software (which signals were shown and which not) and personnel. The concept questionnaire was tested in three pharmacies and some questions were amended on the basis of their comments. The final questionnaire contained 183 questions, divided into twelve subjects (table 2). The questionnaire was accessible via the Internet. Pharmacies who had no access to the Internet received the questionnaire by post. Pharmacies who failed to fill in the questionnaire received reminders at regular intervals. A sample from both groups was visited by the IHC (figure 1). Also here, the selection criterion (≥ 5 times a ratio >1) was chosen to have enough power with manageable numbers. Thirty-

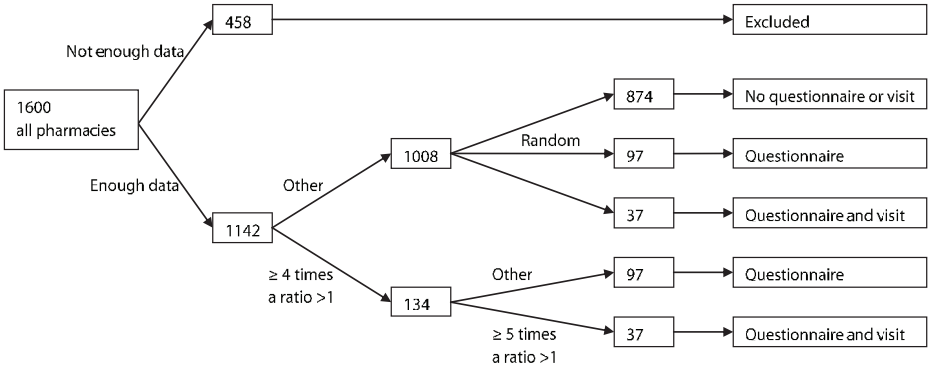


Figure 1 The selection of the pharmacies receiving a questionnaire and IHC visit

seven questions from the questionnaire were selected and during the visits these questions were verified. The pharmacies were informed in advance that a selection of the pharmacies would be visited. The selected pharmacies were acquainted after completing the questionnaire. The visiting inspectors were blinded to the number of interacting drug combinations.

Table 2 The subjects and number of questions in the questionnaire

Chapter	Subject (number of questions)
General pharmacy data	Ownership of the pharmacy (1), cooperation with other pharmacies (1), cooperation with general practitioners (1), electronic submission of prescriptions (4)
Facilities	Alterations (2)
Quality policy	Setting up and implementing a quality system (4), certification (2), attitude towards quality management (12)
Quality measurement	Measurement of errors (2), complaints (1), patient satisfaction (2), interventions (3), and participation in mystery guest investigations (2)
Receipt procedure	Number of personnel involved in dispensing a receipt (2), checks in dispensing a receipt (3)
Medication surveillance – tuning software	Medication surveillance system used (1), tuning of the system e.g. which signals are showed and which are regarded as irrelevant (55[a]), surveillance of pharmacy preparations (2)
Medication surveillance – organization	The way technicians are instructed to manage medication surveillance signals (5), the way this is supervised (2), number of interventions (1), use of resources (2), participation in courses (4), management of the DDI between carbamazepine and erythromycin (5) and between Sulfamethoxazole/trimethoprim and Acenocoumarol (7)
Medication surveillance – recording management	The way the management of signals is recorded (4)
Pharmacy preparations	The way instructions for pharmacy preparations are recorded (1), the way pharmacy preparations are supervised (3), the number of pharmacy preparations (2), the policy regarding analyzing pharmacy preparations (3)
Personnel and workload	Subjective workload (3), absence through illness (1), number of receipts dispensed per technician (2), personnel and experience of personnel (18)
Patient care	Information given to patients (6), information exchange with hospitals (4), participation in health care projects (4)
Pharmacotherapeutic consultation groups	Participation in pharmacotherapeutic consultation groups (3), agreements made (3)

[a] As the questions for the four systems (Pharmacom, Aposys, Euroned, others) differed, pharmacists had to fill in only a quarter of these questions.

Statistical analysis

For each pharmacy, dispensing-ratios for the DDIs, comparable to the standardized mortality ratio, were calculated using formula 2. With this formula, we standardize for the total number of dispensings per pharmacy in the database. In case all pharmacies dispense the DDIs in equal numbers, the ratio will be one for all pharmacies, and therefore the ratios have a better comparability. Pharmacies, which have only a small number of dispensings in the database, will have extremely high numbers in case they dispense one or a small number of DDIs. Therefore, the results were equalized with Bayesian statistics to prevent extreme ups and downs due to low numbers of dispensings.[12] The pharmacies were divided into two sets. One set was used for the analyses and contained two-third of the pharmacies, the other set

was used for the validation of the results obtained in the analyses. In the univariate analysis, correlations were searched between the answers in the questionnaire and these ratios. Correlations are only given if in both sets a correlation was found ($p<0.01$). In the multivariate analysis, models were composed using the analysis set, predicting the dispensing of the interacting drug combinations. The models were validated using the validation set. The number of questions was too large for the multivariate analysis, and only a limited number of questions were selected. From every chapter, those questions were selected that correlated with the other questions and that could discriminate between pharmacies.

RESULTS

The database contained a total of 100,295,311 dispensings in the selected study period. 1,142 pharmacies were recorded in the database with 5,000 or more dispensings. The number of dispensings per pharmacy varied from 5,019 to 264,631. Because pharmacies receive reimbursements from several health care insurance companies and because not all health care insurance companies were included in the database, these numbers do not correspond with the total number of dispensings per pharmacy. The eleven potential DDIs were dispensed 11,594 times. In five percent more than one pharmacy was involved. As these DDIs could not be assigned to a single pharmacy, they were excluded from further analyses. The number of dispensings and DDIs are shown in table 1. Disopyramide (DDI number 6) and azapropazon (DDI number 11) were not dispensed by 44% and 46% of the pharmacies, respectively. Therefore, a ratio could not be calculated for these pharmacies and these DDIs were excluded from the analyses.

The number of times a ratio above one was found was calculated (table 1) and pharmacies were selected as shown in figure 1. Two hundred and sixty-eight pharmacies were selected to receive a questionnaire and 74 pharmacies were selected for a visit by the IHC. For several reasons, such as recent visitations and duplications in the database, twelve pharmacies were excluded. Eventually, 256 pharmacies received a questionnaire and 62 pharmacies were selected for a visit. Two hundred and forty-six questionnaires were filled in (response rate 96.1%) and 58 (93.5%) pharmacies were visited after the questionnaire was completed. The judgments during the visits by the IHC were compared with the answers by the pharmacists. In 33 of the 37 verified questions, the IHCs judgment matched in more than 90% the answer of the pharmacist. Except four questions, the judgment by the IHC was equally more positive and more negative than the answers by the pharmacist.

In the univariate analysis, all combinations between the questions and DDIs were searched for significant correlations. Two correlations were found with DDI number 1 between macrolide antibiotics and digoxin (table 3). Pharmacies, which are part of a health care centre, dispensed this interacting drug combination more often than other pharmacies. A correlation with the type of medication surveillance system was also found. Pharmacies using the

Table 3 Significant univariate correlations between the questionnaire and the number of dispensings of the DDIs between macrolide antibiotics and digoxin (number 1)

Question	Correlation	p-value
Is the pharmacy part of a health care centre? (1 yes, 2 no) (yes n=18, no n=228)	-0.165	0.009
Which medication surveillance system is used in the pharmacy?		
• Pharmacom (1 yes, 0 other) (n=81)	-0.261	0.000
• Aposys (1 yes, 0 other) (n=62)	0.088	0.170
• Euroned (1 yes, 0 other) (n=89)	0.197	0.002

Euroned system dispensed this interacting drug combination more often, while pharmacies using the Pharmacom system dispensed this interacting drug combination less often.

For the multivariate analysis, 32 variables were selected, representative of the whole range of questions. These variables were used in the analysis-set to compose models. The adjusted explained variance ranged from 2.6% to 28.9% (table 4). The model explaining the DDI between macrolide antibiotics and digoxin had by far the highest adjusted explained variance. The models were validated in the validation-set, calculating the unexplained variance (table 4). The six variables in this model explaining the DDI between macrolide antibiotics and digoxin are shown in table 5.

Table 4 Predictability of the models (%) composed in the multivariate analysis

DDI	Adjusted explained variance (r^2) (analysis-set)	Unexplained variance [a] (validation-set)
1	28.9	0.61
2	12.8	-0.22
3	17.3	31.5
4	7.0	-0.18
5	14.4	-0.41
7	6.5	6.4
8	16.1	0.68
9	14.0	-0.43
10	2.6	0.90

[a] An unexplained variance of zero means that the predictability found in the validation set equals the predictability in the analysis-set. The higher the unexplained variance, the worse the predictability in the validation-set compared to the analysis-set.

DISCUSSION

In this study, we investigated determinants for the dispensing of eleven undesirable interacting drug combinations. In general, our results are in line with the expectation that the medication surveillance system plays an important role in medication surveillance. Although the eleven potential DDIs were counted 11,594 times which suggests that a considerable number of patients is exposed to potential and avoidable adverse patient outcomes, these results should be judged against a background of approximately 100 million dispensings.

Table 5 The questions in the multivariate model predicting the dispensing of the DDI between macrolide antibiotics and digoxin (number 1)

Variable	Answer (coding)	Direction coefficient
Constant		3.37
Is the pharmacy part of a health care centre? (yes n=18, no n=228)	yes (0) versus no (1)	-2.27
Co-trimoxazole – acenocoumarol: no appointments were made with the GPs. The drug will be dispensed. (8 options of choice; option 1 'with all GPs', and option 8 'with no GPs')	option 1 'with all GPs' (1) versus other option (0) (n=11)	ref.
	option 2 (1) versus other option (0) (n=10)	1.03
	option 3 (1) versus other option (0) (n=4)	0.379
	option 4 (1) versus other option (0) (n=4)	-0.454
	option 5 (1) versus other option (0) (n=3)	0.903
	option 6 (1) versus other option (0) (n=2)	-0.510
	option 7 (1) versus other option (0) (n=4)	-0.191
	option 8 'with no GPs' (1) versus other option (1) (n=202)	0.0886
Are separate signal texts in the medication surveillance program adjusted to the situation in the pharmacy? (yes n=72, no n=165)	yes (0) versus no (1)	0.179
Is the management of signals traceably recorded on the receipt? (yes n=211, no n=35)	yes (0) on the receipt, no not on the receipt (1)	0.269
The supervision on management of signals takes place on the basis of signal lists (yes n=158, no n=86)	yes (0) on the basis of signal lists, no (1) not on the basis of signal lists	0.0723
How many receipts are dispensed per year divided by the number of FTE technicians		$< 10^{-4}$

GP: general practitioner; FTE: full-time equivalent.

It is possible that in these cases due to particular circumstances any other option, such as substituting or not dispensing one of the drugs, is a less favorable choice than dispensing the DDI. In five percent of the total number of DDIs more than one pharmacy was involved, indicating the importance of communication. For the DDI between macrolide antibiotics and digoxin, two determinants were found. Although the type of medication surveillance system was a determinant, this does not mean that the differences are determined by the quality of the system itself because they may also correlate with the attitude of the pharmacists using the systems. The three medications surveillance systems differ in the extent to which communication with other healthcare providers is possible and developments were made in recent years. The Pharmacom system has the most advanced communication possibilities and compared to the other systems, new developments to the Euroned system were modest. Unexpectedly, pharmacies part of a health care centre dispensed this DDI more often than other pharmacies. In health care centers the communication lines between pharmacists and general practitioners are much shorter, suggesting that intervening undesirable DDIs will be easier. Possibly, pharmacies, which are part of a health care centre, oppose the opinions from

the general practitioners less often, to avoid harming the cooperation within the health care centre but, of course, there may be several other reasons.

For the other eight assessed DDIs no determinants were found in the univariate analysis, neither did the models in the multivariate analysis have a good predictability. A possible explanation is that the quality of medication surveillance in community pharmacies in the Netherlands is high. Therefore, the number of pharmacies dispensing high-risk DDIs seems to be small.

Our study has some potential limitations. First, because we used strict inclusion criteria to prevent false-positive results, it is likely that the number of dispensings of undesirable interacting drug combinations in this study is an underestimation and it is possible that important determinants were not recognized or difficult to assess. In the univariate analyses only those questions are given which had a significant ($p<0.01$) correlation in two independent sets. Although we included 183 questions and nine DDIs in the univariate analysis, the possibility of including a significant correlation by chance was small (on average 0.16 question). Second, the reimbursement data from eight health care insurance companies were used. In the Netherlands, these companies work mostly regionally. It is nevertheless not to be expected that the determinants of dispensing interacting drugs differ per region or that pharmacies differ in their management of DDIs between patients of different health care insurance companies. Third, from all potential DDIs, only eleven (but highly clinically relevant ones) were selected for this study. According to the Dutch guidelines, for all eleven combinations the dispensing of an alternative was strongly advised as a good alternative was available. Nevertheless, it is possible that these dispensings were not an error because any other option was not possible. For example, when a patient is hypersensitive to the alternative drug recommended in the guidelines or when the alternative drug is not effective. In these cases the benefit of both drug therapies should be weighed against the potential risks of the DDI. The potential risks can partly be avoided by taking appropriate measures such as monitoring of drug levels. In this study, we could not retrieve why the pharmacist had dispensed the interacting drug combination, and whether the dispensing was erroneous or not.

Fourth, the questionnaire was composed on the basis of a literature search and interviews with experts. It is possible that not all characteristics correlating with the dispensing of undesirable interacting drug combinations were disclosed, such as differences in population characteristics between pharmacies. For example, pharmacies with an elderly population using more drugs simultaneously have a higher risk of dispensing interacting drug combinations than pharmacies with a younger population. Also, it is possible that in areas with many general practitioners who use a medication surveillance system for prescribing, the background chance of a DDI is much smaller. Fifth, it is possible that the differences between pharmacies were too small compared with the power of this study to distinguish determinants.

All associations found in this study were directly related to the management of signals. In our questionnaire we also included other topics, such as pharmacy preparations and patient

care. Future research should focus on the management of a larger variety of signals than the ones in our study and on how DDI associated dispensing could be further reduced.

In conclusion, both medication surveillance systems and being part of a health care centre may play an important role in the management of DDIs and the avoidance of adverse patient outcomes. Pharmacies in a healthcare centre dispensed DDIs more often. For most DDIs no determinants were found possibly indicating that the quality of medication surveillance in the Netherlands is high.

REFERENCES

1. Huic M, Mucolic V, Vrhovac B, Francetic I, Bakran I, Giljanovic S. Adverse drug reactions resulting in hospital admission. *Int J Clin Pharmacol Ther* 1994;32(12):675-82.
2. Jankel CA, Fitterman LK. Epidemiology of drug-drug interactions as a cause of hospital admissions. *Drug Saf* 1993;9(1):51-9.
3. McDonnell PJ, Jacobs MR. Hospital admissions resulting from preventable adverse drug reactions. *Ann Pharmacother* 2002;36(9):1331-6.
4. Peyriere H, Cassan S, Floutard E, et al. Adverse drug events associated with hospital admission. *Ann Pharmacother* 2003;37(1):5-11.
5. Schalekamp T. Omgaan met geneesmiddeleninteracties. *Geneesmiddelenbulletin* 1997;31(8):87-94.
6. Merlo J, Liedholm H, Lindblad U, et al. Prescriptions with potential drug interactions dispensed at Swedish pharmacies in January 1999: cross sectional study. *BMJ* 2001;323(7310):427-8.
7. Bergendal L, Friberg A, Schaffrath A. Potential drug-drug interactions in 5,125 mostly elderly outpatients in Gothenburg, Sweden. *PharmWorld Sci* 1995;17(5):152-7.
8. Linnarsson R. Drug interactions in primary health care. A retrospective database study and its implications for the design of a computerized decision support system. *Scand J Prim Health Care* 1993;11(3):181-6.
9. Becker ML, Kallewaard M, Caspers PW, Schalekamp T, Stricker BH. Potential determinants of drug-drug interaction associated dispensing in community pharmacies. *Drug Saf* 2005;28(5):371-8.
10. Stichting Health Base (2001) Commentaren Medicatiebewaking - Pharmacom Medicom. Houten: Stichting Health Base.
11. WINAp. G-standaard. Den Haag: WINAp; 2001.
12. Clayton D, Kaldor J. Empirical bayes estimates of age-standardized relative risks for use in disease mapping. *Biometrics* 1987;43:671-8

Chapter 2.4.

Hospitalizations and emergency department visits due to drug-drug interactions: a literature review

ABSTRACT

Background: Our objective was to evaluate the incidence of adverse patient outcomes due to drug-drug interactions (DDIs), the type of drugs involved and the underlying reason. As a proxy for adverse patient outcomes, emergency department (ED) visits, hospital admissions and re-hospitalizations were assessed.

Methods: A literature search in the Medline and Embase database (1990-2006) was performed and references were tracked. An overall cumulative incidence was estimated by dividing the sum of the cases by the sum of the study populations.

Results: Twenty-three studies were found assessing the relationship between DDIs and ED-visits, hospitalizations or re-hospitalizations. The studies with a large study size showed low incidences and vice versa. DDIs were held responsible for 0.054% of the ED-visits, 0.57% of the hospital admissions and 0.12% of the re-hospitalizations. In the elderly population, DDIs were held responsible for 4.8% of the admissions. Drugs most often involved were NSAIDs and cardiovascular drugs. The reasons for admissions or ED-visits, which were most often found, were GI-tract bleeding, hyper- or hypotension and cardiac rhythm disturbances.

Conclusion: This review provides information on the overall incidence of DDIs as a cause of adverse patient outcomes, although there is still uncertainty about the impact of DDIs on adverse patient outcomes. Our results suggest that a limited number of drugs is involved in the majority of cases and that the number of reasons for admission as a consequence of DDIs seems to be modest.

INTRODUCTION

The use of two or more drugs has the potential risk of a drug-drug interaction (DDI). DDIs can contribute to drug induced illnesses that may result in hospitalizations and deaths.[1-3] However, few studies have paid attention to the quantitative share of DDIs in adverse patient outcomes. Lack of information in this area can easily result in over, as well as underestimation of the clinical consequences of DDIs. Better knowledge of the incidence of DDIs and the drugs most frequently involved, can be helpful in a more accurate assessment of their overall clinical importance. A DDI is defined as a pharmacokinetic or pharmacodynamic influence of drugs on each other, which can result in reduced effectiveness or increased toxicity.[4] DDIs do occur frequently in normal drug therapy. The percentage of patients in primary or secondary health care that receives interacting drugs ranges from 7 to 22.[5-8] In the elderly, this percentage ranges from 22 to 31.[7,9-11] Although these high percentages suggest a serious health hazard, the consequences seem to be limited. The seriousness of DDIs varies considerably, and only a part of them has potential clinical consequences. One to three percent of these patients in primary or secondary health care is at risk for a DDI which might have major clinical significance.[6,8,9,11]

Little is known about the actual contribution that DDIs have on adverse drug reactions. Some authors suggest that their contribution is limited,[12,13] while others suggest that DDIs are a major cause of adverse drug reactions.[14-16] Whether two interacting drugs can be used at the same time without serious consequences depends on the question whether the benefit of both drug therapies outweighs the risk of the DDI, taking into account the availability of alternatives. In this review we assessed the risk of adverse patient outcomes as a consequence of DDIs for the total population. As a proxy for adverse patient outcomes, emergency department (ED) visits, hospital admissions and re-hospitalizations were assessed. Since we were interested in the contribution of all DDIs to adverse patient outcomes, and not in the contribution of individual DDIs or of a group of DDIs, we searched for studies assessing adverse patient outcomes caused by DDIs in general and not by individual DDIs or a group of DDIs. We conducted a literature review concerning the incidence of these adverse patient outcomes as a consequence of DDIs, the types of drugs involved and the underlying reason for admission or ED-visit.

METHODS

Articles describing adverse patient outcomes due to DDIs were searched using Medline and Embase (period January 1990 – April 2006) and by reference tracking. This period was chosen, because pharmacy practice before 1990 is not comparable with nowadays practice and a review was performed in 1993.[17] In the Medline search, the results of two search strategies

were combined. In the first search, all articles with the medical subject heading (MeSH) descriptor "drug interactions" or with the keyword "drug-interaction" or the keyword "drug" near the keyword "interaction" were selected. In the second search, articles were selected with the MeSH descriptors "hospitals", "hospitalization", "emergency service hospital" or "patient admission", or with the keyword "adverse" in the title. These search terms were chosen because they were closest to the research question of this study and included all articles found in an initial screening. Articles that appeared in both literature searches were screened to judge whether they met the inclusion criteria. A comparable search was performed in Embase. Inclusion criteria were assessments on ED-visits, hospital admissions or re-hospitalizations that paid attention to DDIs and that described or quantified the association. Papers not written in English, papers that assessed a subgroup of DDIs instead of all DDIs and papers that did not assess adverse patient outcomes as a direct consequence of DDIs but for example consequences on a theoretical base, were excluded. We searched in the references for additional articles meeting the inclusion criteria.

In each of the articles, the incidence of one or more proxies was reported. The 95% confidence intervals, based on a Poisson distribution, were calculated around the incidences, depending on the height of the incidence and the study size. For each outcome, the overall cumulative incidence was estimated by dividing the sum of the cases by the sum of the study populations.

RESULTS

The Medline search yielded 601, and the Embase search 713 articles of potential use. The results of the literature search are summarized in figure 1. After applying the inclusion criteria, fifteen articles were left. A major part was excluded because they assessed only a limited number of drugs or described case reports. Eight additional articles were found by reference tracking. Six articles assessed ED visits, fourteen articles assessed hospitalizations and three articles assessed re-hospitalizations. The studies on ED-visits and hospitalizations assessed the medication use retrospectively, the studies on re-hospitalization prospectively. The study by McDonnell et al.[15] was the only study identifying outcomes by voluntary reporting and review of the ICD codes. The other studies identified outcomes by review of the medical records. The main differences between the studies are given in table 1. The 23 studies comprised 148,236 patients. The study sizes ranged from 150 to 62,216 patients and the incidence of adverse patient outcome ranged from 0 to 6.2%. In 405 patients the ED-visit, hospitalization or re-hospitalization was attributed to a DDI (table 2).

The incidence of adverse patient outcome was plotted against the study size (figure 2). The studies with a large study size showed low incidences and studies with a small study size showed high incidences, irrespective of the type of adverse patient outcome. The incidences of adverse patient outcomes attributed to DDIs are described below and are summarized in table 3.

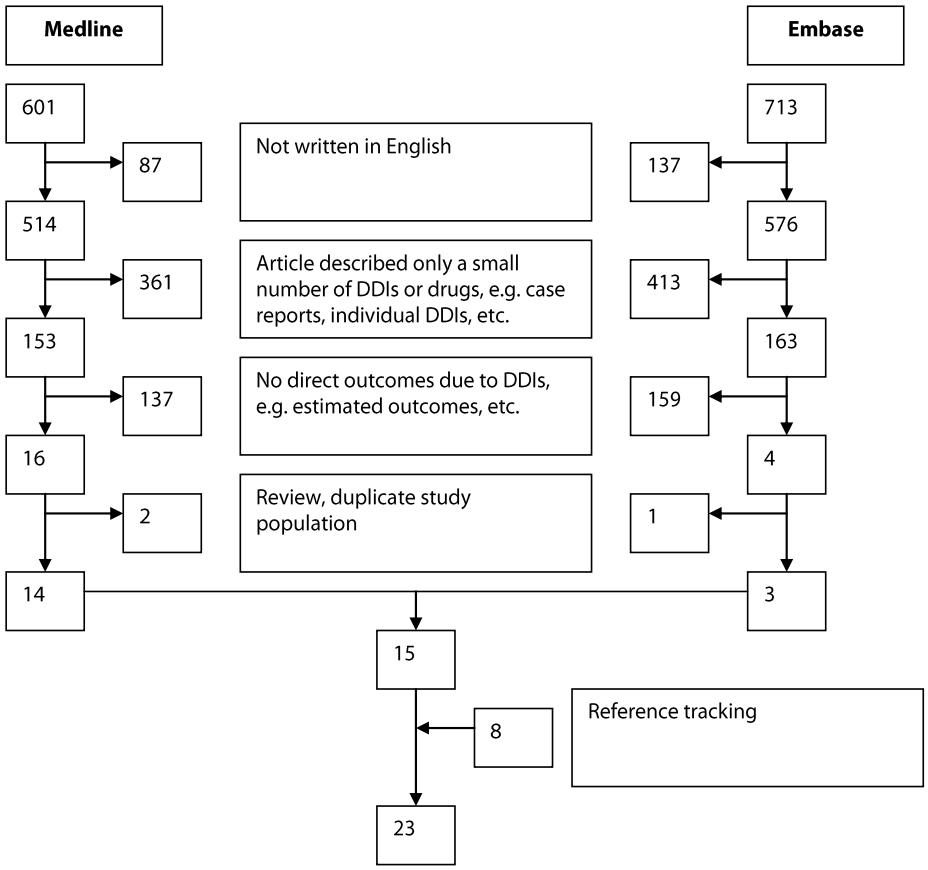


Figure 1 Results of the literature search

Emergency department visits

Five of the six studies focusing on ED-visits assessed all patients visiting the ED.[16,18-21] They comprised 83,921 ED-visits, and 45 cases were reported, giving an overall cumulative incidence of 0.054% (95% CI 0.039, 0.072%). The percentages reported ranged considerably, with the larger studies finding a lower percentage (figure 3). One study[12] included 282 elderly patients, but did not find any case.

Hospital admission

Fourteen studies assessed 62,487 hospital admissions, 358 (0.57%; 95% CI 0.52, 0.64%) of which were attributed to a DDI (figure 4). Ten studies[15,22-30] included all patients admitted to a hospital instead of a subpopulation. A total of 49,357 admissions were assessed with 282 admissions (0.57%; 95% CI 0.51, 0.64%) attributed to a DDI. The percentages ranged from 0.10 to 2.6. In the elderly population (65 or 70 years and older), 75 of 1,566 admissions were attributed to a DDI.[13,14,31] The percentages reported were 0.67, 2.9 and 6.2, with an overall in-

Table 1 Main differences between the studies

Author	Study focus	Drug use assessment	Identification DDI	Assessment of relationship	
Dennehy[18]	DRA	Medical record	-	Strand	(identifiable, probable)
Malhotra[19]	ADE	Medical record, interview	Stockley	Naranjo	(definite, probable, possible, contributing factor)
Prince[20]	DRA	Medical record	-	-	-
Raschetti[16]	ADE	Medical record	Itialian Pharmaceutical Repertory, Micromedex	-	-
Schneitman-McIntire[21]	ADE	Medical record, interview	-	-	-
Hohl[12]	ADE	Medical record	Computer program Pharm Vigilance	Karch and Lasagna	(definite, probable, possible)
Bhalla[29]	DRA	Medical record, interview	British National Formulary	Hallas	(definite, probable, possible)
Dormann[22]	ADR	Medical record	European Physicians' Drug Index	Naranjo	(definite, probable, possible)
Hallas[24]	ADR and TF	Medical record, interview	-	Karch (modified)	(definite, probable, possible)
Hallas[23]	ADR and TF	Medical record, interview	-	Karch (modified)	(definite, probable, possible)
Huic[25]	ADR	Medical record	Hansten and Horn	Karch and Lasagna	(definite, probable, possible)
McDonnell[15]	ADR	Medical record	-	Naranjo	(highly probable, probable)
Mok[26]	DDI	Medical record	Stockley	-	-
Peyriere[27]	ADE	Medical record	-	Begaud	-
Pirmohamed[30]	ADR	Medical record, interview	British National Formulary	Naranjo and Jones	(definite/highly probable, probable, possible)
Stanton[28]	DRA	Interview	-	Hallas	(definite, probable)
Easton[32]	DRA	Medical record, other healthcare providers	-	Easton	(definite, probable, possible)
Courtman[31]	DRA	Medical record	-	Hallas	(major reason, contributing, not contributing)
Doucet[14]	DDI	Interview	Vidal Dictionary DDI guide	Grymonpre and Karch	(probable)
Lindley[13]	ADR	Medical record	British National Formulary	-	-
Herr[36]	DDI	Interview	Hansten computer program	-	-
Egger[34]	DDI	Medical record	Drug-Reax (Micromedex)	-	-
Bero[35]	DRA	Medical record	Tatro	-	-

ADR: adverse drug reaction; ADE: adverse drug event; DRA: drug related admission; TF: therapeutic failure.

cidence of 4.8% (95% CI 3.8, 6.0%). In a pediatric population (younger than 18 years) one study assessed 11,564 admissions, one being attributed to a DDI (0.009%; 95% CI 0.0001, 0.048%).[32]

Three studies reported whether patients who had visited the ED due to DDIs were subsequently hospitalized.[16,19,20] Of the nineteen patients, seven patients were hospitalized. Ra-

Table 2 Adverse patient outcome due to DDIs

Author	Year	Outcome	Population	Size	Cases	Incidence	95% CI	Country	DDI described
Dennehy[18]	1996	ED-visit		1,260	0	0 %	-	USA	-
Malhotra[19]	2001	ED-visit		4,764	8	0.17 %	0.072, 0.33 %	India	N
Prince[20]	1992	ED-visit		10,184	2	0.020 %	0.0022, 0.071 %	USA	Y/N [a]
Raschetti[16]	1999	ED-visit		5,497	9	0.16 %	0.075, 0.31 %	Italy	N
Schneitman-McIntire[21]	1996	ED-visit		62,216	26	0.042 %	0.027, 0.061 %	USA	Y/N [a]
Hohl[12]	2001	ED-visit	≥ 65 yr.	282	0	0 %	-	Canada	-
Bhalla[29]	2003	Admission		840	2	0.24 %	0.027, 0.86 %	UK	N
Dormann[22]	2003	Admission		915	5	0.55 %	0.18, 1.3 %	Germany	N
Hallas[24]	1992	Admission		1,999	2	0.10 %	0.011, 0.36 %	Denmark	Y
Hallas[23]	1990	Admission		333	4	1.2 %	0.32, 3.1 %	Denmark	Y
Huic[25]	1994	Admission		5,237	31	0.59 %	0.40, 0.84 %	Croatia	Y
McDonnell[15]	2002	Admission		20,166	25	0.12 %	0.080, 0.18 %	USA	N
Mok[26]	1991	Admission		200	3	1.5 %	0.30, 4.4 %	Ireland	Y
Peyriere[27]	2003	Admission		156	4	2.6 %	0.69, 6.6 %	France	N
Pirmohamed[30]	2004	Admission		18,820	203	1.1 %	0.94, 1.2 %	UK	N
Stanton[28]	1994	Admission		691	3	0.43 %	0.087, 1.3 %	Australia	Y
Easton[32]	2004	Admission	< 18 yr.	11,564	1	0.009 %	0.0001, 0.048 %	Australia	Y/N [a]
Courtman[31]	1995	Admission	≥ 65 yr.	150	1	0.67 %	0.0087, 3.7 %	Canada	N
Doucet[14]	1996	Admission	≥ 70 yr	1,000	62	6.2 %	4.8, 7.9 %	France	Y/N [a]
Lindley[13]	1992	Admission	≥ 65 yr	416	12	2.9 %	1.5, 5.0 %	UK	Y
Herr[36]	1992	Re-hospitalization (2 months)[b]		340	0	0 %	-	USA	-
Egger[34]	2003	Re-hospitalization (6 months)	≥2 drugs	500	1	0.20 %	0.0026, 1.1 %	Switzerland	Y
Bero[35]	1991	Re-hospitalization (4 weeks)	≥ 65 yr, ≥3 drugs	706	1	0.14 %	0.0019, 0.79 %	USA	N

[a] Only part of the DDIs described or only one of the two drugs involved described. [b] Re-hospitalization after ED-treatment.

Table 3 The average percentage of adverse patient outcomes in the included studies

Proxy	General					Elderly				
	n	N	%	95% CI	Studies	n	N	%	95% CI	Studies
ED-visit	45	83,921	0.054	0.039, 0.072	5	0	282	0	-	1
Admission	282	49,357	0.57	0.51, 0.64	10	75	1,566	4.8	3.8, 6.0	3
Re-hospitalization	1	840	0.12	0.0016, 0.66	2	1	706	0.14	0.0019, 0.79	1

n: sum of the number of adverse patient outcomes caused by DDIs; N: sum of the study populations.

schetti et al.[16] followed 1,833 patients who were hospitalized after ED-visit. The death of one patient (0.055%; 95% CI 0.00071, 0.30%) was attributed to a DDI. On the other hand, Juntti Patinen et al.[33] studied 1,511 deaths in a hospital with 141,484 admissions in that period, and found that five deaths (0.0035%; 95% CI 0.0011, 0.0082%) could be attributed to DDIs.

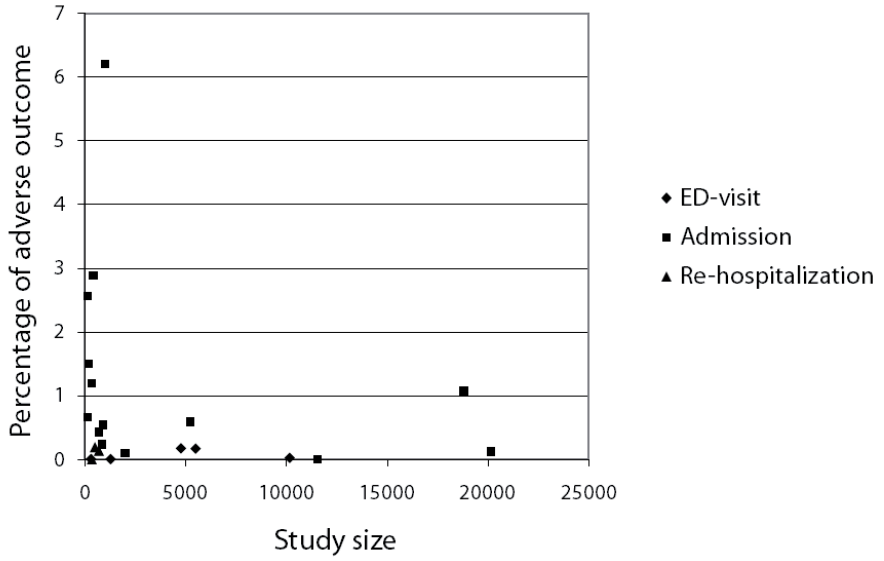


Figure 2 Relation between study size and incidence of adverse outcome (the study by Schneitman-McIntire is omitted, because it is out of range)

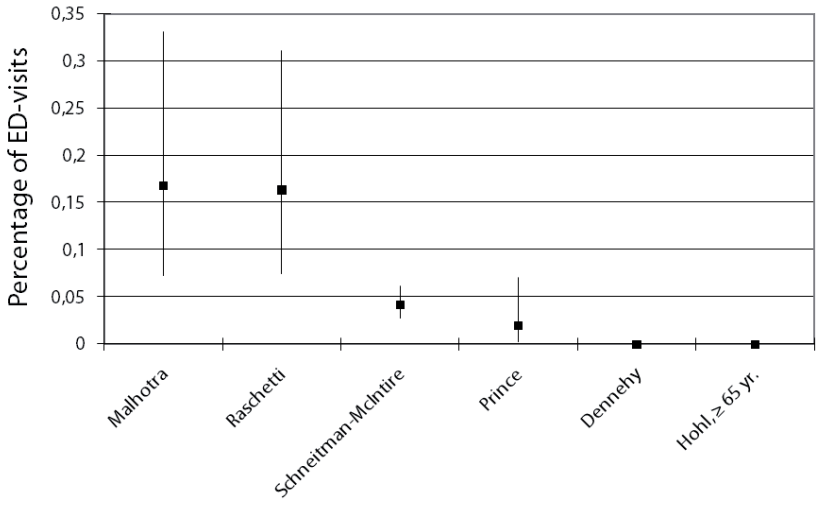


Figure 3 Emergency department-visits attributed to DDIs (95% CI)

Re-hospitalization

The risk of being re-hospitalized due to a DDI after discharge from a hospital was assessed in three studies. Egger et al.[34] followed 500 patients for two months and found one patient (0.20%; 95% CI 0.0026, 1.1%) whose re-hospitalization was attributed to a DDI. Bero et al.[35] found in a geriatric population of 706 patients one re-hospitalization (0.14%; 95% CI

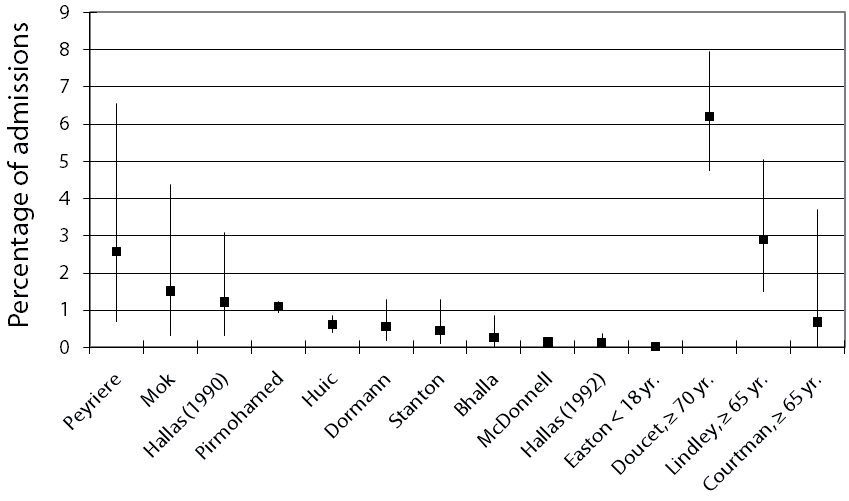


Figure 4 Hospital admissions attributed to DDIs (95% CI)

0.0019, 0.79%) within six months attributed to a DDI. Herr et al.[36] assessed the incidence of re-hospitalization within four weeks after ED-treatment. None of the 340 patients included in the study was re-hospitalized.

Drugs involved and reason for admission or visit

In 61 of the 405 cases (15.1%) of an adverse patient outcome attributed to DDIs, the two or more drugs involved were described as well as the reason for admission or visit. In 57 cases the DDI could be assigned to two drugs. Three or more drugs were involved in the remaining four DDIs (table 4). NSAIDs were involved in 28 (45.9%) of the 61 cases, in 16 cases (26.2%) interacting with another NSAID, followed by diuretics (15 cases, 24.6%), heart glycosides (13 cases, 21.3%) and Ca-channel blockers (10 cases, 16.4%). The diagnoses or symptoms that most often occurred (table 5) were GI-tract bleeding (20 cases, 32.8%), hypertension or hypotension (11 cases, 18.0%) and cardiac rhythm disturbances (11 cases, 18.0%). Interactions between or with NSAIDs, anticoagulants and corticosteroids were responsible for all cases of GI-tract bleeding. All cases of hypertension or hypotension were caused by interactions between diuretics or Ca-channel blockers and another drug. Heart glycosides interacting with another drug were the cause of all cases of cardiac rhythm disturbances.

DISCUSSION

DDIs are a common event in current pharmacotherapy but the risk involved seems mostly acceptable. Usually, DDIs have the attention of health care providers in daily practice. Only a

Table 4 DDIs responsible for the adverse patient outcome, divided per drug group

	Number of times involved	NSAIDs	ACE-inhibitors	Beta-blockers	Ca-channel blockers	Diuretics	Nitrates	Heart glycosides	Anti-arrhytmics	Anticoagulants	Antibiotics	Corticosteroids	Immunosuppressives	Anti-rheumatics	Tricyclic antidepressives	Benzodiazepines	Parasympaticolytics	Lipid modifying drugs	Insulin	Oncolytics	Anti-epileptics
NSAIDs	28	16																			
ACE-inhibitors	2																				
Beta-blockers	3																				
Ca-channel blockers	10																				
Diuretics	15	6	2			1															
Nitrates	2				2																
Heart glycosides	13			1	7	2															
Anti-arrhytmics	5			1	1			3													
Anticoagulants	3	3																			
Antibiotics	1										1										
Corticosteroids	2	1				1															
Immunosuppressives	1																				
Anti-rheumatics	1	1																			
Tricyclic antidepressives	3					2															
Benzodiazepines	1					1															
Parasympaticolytics	1														1						
Lipid modifying drugs	1																	1			
Insulin	1			1																	
Oncolytics	1												1								
Anti-epileptics	1	1																			

DDIs involving three or more drugs, not described in the above mentioned table:

	drug A	drug B	drug C	drug D
1.	Methyldopa	Furosemide	Atenolol	
2.	Glibenclamide	Furosemide	Prochorperazine	
3.	Glibenclamide	Phenformin	Furosemide	ACE-inhibitor
4.	Glibenclamide	Phenformin	Aspirin	Captopril

limited number of DDIs comprises a risk of adverse patient outcomes which is too high. This review was performed to assess the population risk of DDIs. We focused on ED-visits, hospitalizations and re-hospitalizations. As far as we know, no studies assessed other adverse outcomes due to DDIs, such as visits to family physicians. DDIs were held responsible for 0.054% of the ED-visits, 0.57% of the admissions and 0.12% of the re-hospitalizations. Although the percentages are modest, the number of adverse outcomes due to DDIs is substantial because of the large numbers of ED-visits and (re-)hospitalizations. Drugs most often involved were NSAIDs and cardiovascular drugs, and the reason for admission or ED-visit most often found were GI-tract bleeding, hypertension or hypotension and cardiac rhythm disturbances.

Table 5 Diagnosis or symptom of adverse patient outcomes by DDIs

Symptom/diagnosis	Frequency	Percentage
GI-tract bleeding	20	32.8
Hypertension / hypotension	11	18.0
Cardiac rhythm disturbances	11	18.0
Hyperglycemia / hypoglycemia	4	6.6
Hyperkalemia / hypokalemia	4	6.6
Digitalis intoxication [a]	2	3.3
Renal dysfunctioning	2	3.3
Arthritis	1	1.6
Bradycardia	1	1.6
Headache	1	1.6
Pneumonitis	1	1.6
Rhabdomyolysis	1	1.6
Raised phenytoin plasma concentration [b]	1	1.6
Anticholinergic effect	1	1.6

[a] Most common features of digitalis intoxication are anorexia, nausea and arrhythmia. [b] Most common features of phenytoin intoxication are nystagmus, ataxia and dysarthria

The present review has some limitations. Studies assessing adverse patient outcomes due to DDIs were searched using the Medline and Embase database. This possibly causes bias because unpublished literature and literature published in journals not selected in Medline and Embase were missed. The studies used in this review, differed in their methods. Differences existed in the way DDIs were searched and study populations were assessed, and there were differences in the degree of certainty with which adverse patient outcome were attributed to a DDI. Certain articles included an adverse patient outcome if it was possibly caused by a DDI, other articles included only cases with probable or definite causal relationships. As a consequence of the differences in study methods, there was a substantial variation in results between the studies. Due to the limited numbers of articles, other subgroup analysis than type of outcome and age were not possible.

The studies with a larger sample size showed low incidences and studies with a smaller size showed high incidences. This is remarkable because one might expect that the incidence should be independent from the study size. There may be three potential reasons for this variation. First, results from studies with a smaller study size will have a larger standard error, and outliers to higher numbers occur more often, wrongly assuming a higher incidence. However, a variation around the average is to be expected while most smaller studies showed percentages above the average. Second, the variation in results might be explained by publication bias because studies with a smaller study size are published only when they report a high incidence. However, many studies did not focus on DDIs as a cause for the ED-visit or (re-)hospitalization, but on adverse drug reactions or adverse drug events in general. Third, it is possible that in the smaller studies medication histories were studied in more detail than in the larger ones, and were therefore more readily able to recognize adverse patient outcomes

due to DDIs. If that is the case, this may indicate that the percentages found in the larger studies are an underestimation of the true risk.

On the other hand, the percentages in this review may also be an underestimation, if medical practitioners or pharmacists did not recognize adverse patient outcomes caused by DDIs as such. It is possible that the adverse patient outcome was attributed in many instances to the last drug prescribed and not to a potential interaction between two drugs. In this respect, the low incidence of DDIs found in some studies might also be an indication of a lack of knowledge, understanding and recognition of DDIs in general. It seems plausible that complex, rare DDIs could be easily missed as a cause of an adverse patient outcome.[37] The percentage of ED-visits due to DDIs is lower than the percentage of admissions and re-hospitalizations due to DDIs. This may indicate that the adverse patient outcomes due to DDIs belong to the more serious cases and lead to hospitalization more frequently.

Two groups of drugs, NSAIDs and cardiovascular drugs, were involved in a majority of the adverse patient outcomes attributed to DDIs. These percentages are not adjusted for differences in the number of users. The group of cardiovascular drugs comprises a large number of different drugs, including some drugs that interact frequently with other drugs. An explanation may be that NSAIDs and cardiovascular drugs have a higher risk of an adverse patient outcome. Another explanation may be that these DDIs are more well-known and therefore more easily recognized as the cause of the adverse patient outcome. The diagnoses or symptoms of the adverse patient outcomes caused by DDIs were most often GI-tract bleeding, hypertension or hypotension and cardiac rhythm disturbances.

One previous review was found on hospital admissions due to DDIs, published in 1993.[17] The reported incidences ranged from 0 to 2.8% and cardiovascular drugs were most often involved. These results are largely similar to ours, although the involvement of NSAIDs in adverse patient outcomes was not found in the former review. Doucet et al.[14] report that the number of adverse effects did not differ significantly between the group of contraindicated DDIs and the group of DDIs that require precautionary use.

In conclusion, we can say that there is great uncertainty about the impact of DDIs on adverse patient outcomes. Our results suggest that approximately 0.05% of the ED-visits, 0.6% of the hospital admissions and 0.1% of the re-hospitalizations are caused by DDIs, but it is possible that these figures are an underestimation. A limited number of drugs are involved in a majority of the adverse patient outcomes due to DDIs. These drugs include NSAIDs, diuretics, heart glycosides and Ca-channel blockers. More cautious use of these drugs with interacting drugs may result in a decrease of the number of adverse patient outcomes. This more cautious use is particularly favorable for the elderly population. Special attention should be paid to patients treated for GI-tract bleeding, hypertension or hypotension and cardiac rhythm disturbances, because these events are relatively commonly the consequence of a DDI. Further assessment of the association between the use of interacting drugs and clinically relevant adverse patient outcomes is recommended.

REFERENCES

1. Leape LL, Bates DW, Cullen DJ, et al. Systems analysis of adverse drug events. ADE Prevention Study Group. *JAMA* 1995;274(1):35-43.
2. Beijer HJ, de Blaey CJ. Hospitalisations caused by adverse drug reactions (ADR): a meta-analysis of observational studies. *Pharm World Sci* 2002;24(2):46-54.
3. Kohn LT, Corrigan JM, Donaldson MS. To Err Is Human. Washington: National Academy Press; 2003.
4. Schalekamp T. Omgaan met geneesmiddeleninteracties. *Geneesmiddelenbulletin* 1997;31(8):87-94.
5. Braverman SE, Howard RS, Bryant PR, Belandres PV. Potential drug interactions in a physical medicine and rehabilitation clinic. *Am J Phys Med Rehabil* 1996;75(1):44-9.
6. Gronroos PE, Irjala KM, Huupponen RK, Scheinin H, Forsstrom J, Forsstrom JJ. A medication database-a tool for detecting drug interactions in hospital. *Eur J Clin Pharmacol* 1997;53(1):13-7.
7. Linnarsson R. Drug interactions in primary health care. A retrospective database study and its implications for the design of a computerized decision support system. *Scand J Prim Health Care* 1993;11(3):181-6.
8. Merlo J, Liedholm H, Lindblad U, et al. Prescriptions with potential drug interactions dispensed at Swedish pharmacies in January 1999: cross sectional study. *BMJ* 2001;323(7310):427-8.
9. Bergendal L, Friberg A, Schaffrath A. Potential drug-drug interactions in 5,125 mostly elderly outpatients in Gothenburg, Sweden. *PharmWorld Sci* 1995;17(5):152-7.
10. Bjorkman IK, Fastbom J, Schmidt IK, Bernsten CB. Drug-drug interactions in the elderly. *Ann Pharmacother* 2002;36(11):1675-81.
11. Costa AJ. Potential drug interactions in an ambulatory geriatric population. *Fam Pract* 1991;8(3):234-6.
12. Hohl CM, Dankoff J, Colacone A, Afilalo M. Polypharmacy, adverse drug-related events, and potential adverse drug interactions in elderly patients presenting to an emergency department. *Ann Emerg Med* 2001;38(6):666-71.
13. Lindley CM, Tully MP, Paramsothy V, Tallis RC. Inappropriate medication is a major cause of adverse drug reactions in elderly patients. *Age Ageing* 1992;21(4):294-300.
14. Doucet J, Chassagne P, Trivalle C, et al. Drug-drug interactions related to hospital admissions in older adults: a prospective study of 1000 patients. *J Am Geriatr Soc* 1996;44(8):944-8.
15. McDonnell PJ, Jacobs MR. Hospital admissions resulting from preventable adverse drug reactions. *Ann Pharmacother* 2002;36(9):1331-6.
16. Raschetti R, Morgutti M, Menniti-Ippolito F, et al. Suspected adverse drug events requiring emergency department visits or hospital admissions. *Eur J Clin Pharmacol* 1999;54(12):959-63.
17. Jankel CA, Fitterman LK. Epidemiology of drug-drug interactions as a cause of hospital admissions. *Drug Saf* 1993;9(1):51-9.
18. Dennehy CE, Kishi DT, Louie C. Drug-related illness in emergency department patients. *Am J Health Syst Pharm* 1996;53(12):1422-6.
19. Malhotra S, Jain S, Pandhi P. Drug-related visits to the medical emergency department: a prospective study from India. *Int J Clin Pharmacol Ther* 2001;39(1):12-8.
20. Prince BS, Goetz CM, Rihn TL, Olsky M. Drug-related emergency department visits and hospital admissions. *Am J Hosp Pharm* 1992;49(7):1696-700.

21. Schneitman-McIntire O, Farnen TA, Gordon N, Chan J, Toy WA. Medication misadventures resulting in emergency department visits at and HMO medical center. *Am J Health Syst Pharm* 1996;53(12):1416-22.
22. Dormann H, Criegee-Rieck M, Neubert A, et al. Lack of awareness of community-acquired adverse drug reactions upon hospital admission: dimensions and consequences of a dilemma. *Drug Saf* 2003;26(5):353-62.
23. Hallas J, Harvald B, Gram LF, et al. Drug related hospital admissions: the role of definitions and intensity of data collection, and the possibility of prevention. *J Intern Med* 1990;228(2):83-90.
24. Hallas J, Gram LF, Grodum E, et al. Drug related admissions to medical wards: a population based survey. *Br J Clin Pharmacol* 1992;33(1):61-8.
25. Huic M, Mucolic V, Vrhovac B, Francetic I, Bakran I, Giljanovic S. Adverse drug reactions resulting in hospital admission. *Int J Clin Pharmacol Ther* 1994;32(12):675-82.
26. Mok H, Mulpeter K, O'Connor P, Feely J. Drug-drug interactions in hospital. *Ir Med J* 1991;84(1):26.
27. Peyriere H, Cassan S, Floutard E, et al. Adverse drug events associated with hospital admission. *Ann Pharmacother* 2003;37(1):5-11.
28. Stanton LA, Peterson GM, Rumble RH, Cooper GM, Polack AE. Drug-related admissions to an Australian hospital. *J Clin Pharm Ther* 1994;19(6):341-7.
29. Bhalla N, Duggan C, Dhillon S. The incidence and nature of drug-related admissions to hospital. *Pharmaceutical J* 2003;270:583-6.
30. Pirmohamed M, James S, Meakin S, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ* 2004;329(7456):15-9.
31. Courtman BJ, Stallings SB. Characterization of drug-related problems in elderly patients on admission to a medical ward. *Can J Hosp Pharm* 1995;48(3):161-6.
32. Easton KL, Chapman CB, Brien JA. Frequency and characteristics of hospital admissions associated with drug-related problems in paediatrics. *Br J Clin Pharmacol* 2004;57(5):611-5.
33. Juntti-Patinen L, Neuvonen PJ. Drug-related deaths in a university central hospital. *Eur J Clin Pharmacol* 2002;58(7):479-82.
34. Egger SS, Drewe J, Schlienger RG. Potential drug-drug interactions in the medication of medical patients at hospital discharge. *Eur J Clin Pharmacol* 2003;58(11):773-8.
35. Bero LA, Lipton HL, Bird JA. Characterization of geriatric drug-related hospital readmissions. *Med Care* 1991;29(10):989-1003.
36. Herr RD, Caravati EM, Tyler LS, Iorg E, Linscott MS. Prospective evaluation of adverse drug interactions in the emergency department. *Ann Emerg Med* 1992;21(11):1331-6.
37. Juurlink DN, Mamdani M, Kopp A, Laupacis A, Redelmeier DA. Drug-drug interactions among elderly patients hospitalized for drug toxicity. *JAMA* 2003;289(13):1652-8.

Chapter 3.

Genetic factors affecting pharmaco-therapy for type 2 diabetes mellitus

Chapter 3.1.

Cytochrome P450 2C9 *2 and *3 polymorphisms and the dose and effect of sulfonylurea in type 2 diabetes mellitus

ABSTRACT

Background: Sulfonylurea hypoglycemics are mainly metabolized by the cytochrome P450 2C9 (CYP2C9) enzyme. The CYP2C9*2 and *3 polymorphisms encode proteins with less enzymatic activity and are correlated with elevated serum levels of sulfonylurea, as demonstrated in healthy volunteers. In this study, the effect of these variants is described for patients with diabetes mellitus treated with sulfonylurea.

Methods: Associations between CYP2C9 polymorphisms, prescribed doses of sulfonylurea, and change in glucose levels after start of sulfonylurea therapy were assessed in all patients with incident diabetes mellitus starting on sulfonylurea therapy in the Rotterdam Study, a population based cohort study of 7,983 elderly people.

Results: In CYP2C9*3 allele carriers using tolbutamide, the prescribed dose was lower compared to patients with the wild-type CYP2C9 genotype. No differences in the prescribed dose were found in tolbutamide users with the CYP2C9*1/*2 or CYP2C9*2/*2 genotype compared to wild-type patients or in patients using other sulfonylurea. In CYP2C9*3 allele carriers, the mean decrease in fasting serum glucose levels after start of tolbutamide therapy was larger than in patients with the wild-type genotype, although not statistically significant.

Conclusion: Patients with diabetes mellitus who are carrier of a CYP2C9*3 allele require lower doses of tolbutamide to regulate their serum glucose levels compared to patients with the wild-type genotype.

INTRODUCTION

Type 2 ('maturity-onset') diabetes mellitus affects more than 150 million people worldwide, and the prevalence is still increasing.[1] This form of diabetes mellitus is treated with oral hypoglycemic drugs or, in a more progressive disease stage, with insulin. Both undertreatment and overtreatment are associated with adverse outcomes. Undertreatment will lead to long-term microvascular and macrovascular complications such as coronary artery disease and nephropathy, whereas overtreatment will lead to hypoglycemia. Sulfonylurea have been used in diabetes mellitus since decades and are the most widely used oral hypoglycemic drugs.[2-4] Tolbutamide, glibenclamide (glyburide), gliclazide and glimepiride are the main representatives of this group.[5] Sulfonylurea stimulate the secretion of insulin from pancreatic β-cells by closing potassium channels.[2] Type 2 diabetes mellitus is a progressive disease in which the impairment of insulin secretion worsens. Consequently, dosages need to be increased over time.[5] Adding a second oral hypoglycemic agent that targets a different pathophysiological process, such as metformin, rosiglitazone or pioglitazone is often indicated. If all oral hypoglycemic drugs fail, adding or switching to insulin therapy is necessary.

Sulfonylurea are mainly metabolized by the cytochrome P450 2C9 (CYP2C9) enzyme. Allelic variants of the CYP2C9 gene, CYP2C9*2 ($Arg^{144}Cys$, rs1799853) and CYP2C9*3 ($Ile^{359}Leu$, rs1057910), encode proteins with less enzymatic activity for the metabolism of several substrates than the wild-type allele CYP2C9*1 (Arg^{144}/Ile^{359}). In Caucasian populations, approximately 23% carry a CYP2C9*2 allele and 13% a CYP2C9*3 allele.[6,7] Both in vitro and in vivo studies showed a modest reduction of the enzyme activity in people with the CYP2C9*2 polymorphism and a strong reduction in people with the CYP2C9*3 polymorphism. Compared with the CYP2C9*1/*1 genotype, the tolbutamide clearance in people with the CYP2C9*2/*2 genotype was reduced by 25% and in people with the CYP2C9*3/*3 genotype by 84%.[8] For glibenclamide, the reductions in clearance were 25% and 57%, respectively.[9] In healthy volunteers, using glibenclamide or glimepiride, drug exposure was 1.3- to 2.8-fold increased for people with a CYP2C9*3 allele compared to people with the CYP2C9*1/*1 genotype.[10-12]

None of these pharmacokinetic studies assessed the clinical relevance of the differences, because all were performed in healthy volunteers. The aim of this population-based cohort study was to evaluate the effect of the CYP2C9*2 and CYP2C9*3 polymorphisms on the prescribed sulfonylurea doses and on serum glucose levels in incident type 2 diabetes mellitus patients starting with sulfonylurea therapy.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study in 7,983 people aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[13] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until July 1st 2005 was available and included the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[14]

For this study, we used the glucose assessments from the stichting trombosedienst en artsenlaboratorium rijnmond (STAR), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam with a potential source population of more than 1 million inhabitants. Hereby, we obtained all outpatient glucose assessments from all participants of the Rotterdam area between April 1st 1997, the time at which a new computer system was introduced at STAR, and November 30th 2004.

Cohort definition

The study cohort consisted of all subjects in the Rotterdam Study, who received a first prescription of sulfonylurea between July 1st 1991 and July 1st 2005, and who had not been treated with hypoglycemic drugs in the period of at least six months before. Subjects were followed until the first prescription of another oral hypoglycemic drug than the patient started on, death, or end of the study period, whichever came first.

A subset of this cohort was used for the analyses of blood glucose levels. All patients with glucose measurements between April 1st 1997 and November 30th 2004, who had one or more glucose measurements both in the period of 90 days before and 180 days after the start of sulfonylurea therapy, were selected for this analysis.

Outcomes

We used two types of study outcome, the prescribed daily dose of sulfonylurea and the change in fasting serum glucose assessments.

First, for every prescription of a sulfonylurea, the change in prescribed daily dose compared to the first prescription of the sulfonylurea was calculated. The influence of the CYP2C9*2

and CYP2C9*3 polymorphisms on the change in prescribed daily dose, between the first and tenth prescription of the sulfonylurea, was analyzed.

Second, the subset of the cohort with blood glucose measurement, both in the period of 90 days before and 180 days after start of sulfonylurea therapy, was selected to analyze the change of fasting serum glucose levels after starting sulfonylurea therapy. The change in fasting glucose levels between the last measurement before start of sulfonylurea therapy and the first measurement after start of sulfonylurea therapy was analyzed. Patients who had stopped using sulfonylurea the day before the first measurement after the start of sulfonylurea therapy were excluded. Differences in the change of fasting glucose levels per genotype were analyzed.

Cofactors

The following patient characteristics were considered as potential determinants for affecting the change of daily dose of sulfonylurea after start: age, sex and renal function. Determinants potentially affecting change in fasting glucose levels after start of sulfonylurea therapy were age, sex, the glucose level before start, and the daily dose of sulfonylurea the day before the second measurement. These determinants were entered into the regression model.

Genotyping

Genotyping for the CYP2C9*2 and CYP2C9*3 allele variants was performed by using polymerase chain reaction followed by restriction enzyme digestion analysis (PCR-RFLP), as described previously.[15] Approximately 5 ng of genomic DNA was amplified in 35 cycles of PCR: 1 min 94°C, 1 min 60°C (CYP2C9*2) or 1 min 62°C (CYP2C9*3) and 1 min 72°C, in a total volume of 10 μl, using primers P141 (5'-CACTGGCTGAAAGAGCTAACAGAG-3') and P142 (5'-GTGATATGGAGTAGGGTCACCCAC-3') for CYP2C9*2, or P143 (5'-AGGAAGAGATTGAACGTGTGA- 3') and P144 (5'-GGCAGGCTGGTGGGGAGAAGG**C**CAA-3') for CYP2C9*3 (the bold and underlined nucleotide represents a mismatch to the genomic sequence). The PCR product was digested with Sau96 (CYP2C9*2) or StyI (CYP2C9*3), and analyzed on a 3% TBE/agarose gel with ethidium bromide staining. A random sample of five percent was re-analyzed, all with the same result as the original measurement. All CYP2C9*2 and CYP2C9*3 heterozygote and homozygote variants detected were reanalyzed to confirm the genotype. Patients in whom neither CYP2C9*2 nor CYP2C9*3 alleles were identified were regarded as wild-type.

Statistical analysis

A χ^2-test was used to test for deviation from Hardy-Weinberg equilibrium. One-way analysis of variance was used to test for differences in starting dose between genotypes. For the tenth prescription of sulfonylurea in the cohort, multivariate linear regression was used to analyze the difference per genotype in change of prescribed daily dose compared with the prescribed daily dose of the first prescription. Multivariate linear regression was used to assess differences per genotype in change of glucose levels after start of sulfonylurea therapy. These

analyses were performed with SPSS software (version 11.0.1; SPSS, Chicago, IL). Additionally, we used unbalanced repeated measurements analysis to analyze change of prescribed daily dose compared with the prescribed daily dose of the first prescription, in series of consecutive prescriptions for the same patient with the Proc Mixed module of SAS (version 8.2; SAS, Cary, NC). For two reasons, we grouped patients with the CYP2C9*1/*2 and CYP2C9*2/*2 genotype and CYP2C9*3 carriers (CYP2C9*1/*3, CYP2C9*2/*3 and CYP2C9*3/*3) in the analysis. First, because the number of patients with two variant polymorphisms is too small to analyze them separately, and, second, because the effect of the *2 polymorphism on sulfonylurea clearance is limited compared to the wild-type genotype. For example, the sulfonylurea clearance in patients with the CYP2C9*1/*2 genotype will be similar to the clearance in patients with the CYP2C9*1/*1 genotype.

RESULTS

During the study period, 571 patients started on sulfonylurea therapy; 86 patients were excluded because blood samples were not available, and ten patients were excluded because of difficulties in genotyping (due to suboptimal quality of the long-term storage of DNA of some samples). Consequently, 475 patients were available for the analysis. Baseline characteristics of these patients are given in table 1. The population was in Hardy-Weinberg equilibrium ($\chi^2 = 2.22$, p=0.53), indicating that no selection or errors in genotyping had occurred. Most patients started with tolbutamide (62.3%). Other patients started with glibenclamide (16.2%), glimepiride (16.0%), or gliclazide (5.5%). The average prescribed starting dose was 6.1 mg for glibenclamide, 613 mg for tolbutamide and 1.38 mg for glimepiride. No differences in starting dose were found between genotypes. Patients were followed on average 2.6 years (median 1.9 years) while on monotherapy and did receive 14 prescriptions (median 11 prescriptions) of sulfonylurea during that period. The average duration of one prescription was 69 days (range 2-180 days).

Table 1 Baseline characteristics of the study population

		CYP2C9*1/*1	CYP2C9*1/*2 or CYP2C9*2/*2 [a]	CYP2C9*1/*3 or CYP2C9*2/*3 or CYP2C9*3/*3 [b]
N		321	103	51
Gender (%)	Male	152 (47%)	46 (45%)	20 (39%)
Age	Average	75.1 years	74.7 years	74.5 years
Caucasian origin		321 (100%)	103 (100%)	51 (100%)
Follow-up time	Mean	2.6 years	2.4 years	2.8 years
Body mass index		27.9 kg/m^2 (n=307)	28.4 kg/m^2 (n=100)	28.4 kg/m^2 (n=51)
Serum creatinine		86.8 µmol/l (n=250)	82.7 µmol/l (n=74)	86.3 µmol/l (n=39)

[a] 11 patients had the CYP2C9*2/*2 genotype. [b] 6 patients had the CYP2C9*2/*3 genotype and 2 patients had the CYP2C9*3/*3 genotype.

The changes in prescribed daily doses between the first and tenth prescription are given in table 2. Differences in prescribed doses were only found for tolbutamide (figure 1). The prescribed daily dose in patients with the wild-type genotype increased with 279 mg from the first until the tenth prescription, whereas in patients with a *3 allele the increase was only 12 mg. Patients with the *1/*2 or *2*2 genotype had an increase of 265 mg. The difference between CYP2C9*3 carriers and patients with the wild-type genotype was statistically significant ($p<0.05$) from the sixth until the twentieth prescription. No differences were found between patients with the *1/*2 or *2/*2 genotype and patients with the wild-type genotype. Twenty patients with a *3 allele received at least ten prescriptions of tolbutamide. In fifteen patients (75%) the prescribed dose of the tenth prescription was the same as the dose of the first prescription. In two patients (10%), the prescribed dose was lower; in two patients (10%), the dose was increased with 500 mg or less, and in one patient (5%), the dose was increased with more than 500 mg. One hundred and seventeen patients with the wild-type genotype received at least ten prescriptions of tolbutamide. In 62 patients (53%), the prescribed dose was not changed; in five patients (4%), the prescribed dose was lower; in 29 patients (25%), the prescribed dose was increased with 500 mg or less; and in 21 patients (18%), the prescribed dose was increased with 500 mg or more. Adjusting for renal function did not change the results.

Table 2 Difference in change of daily prescribed dose between the first and tenth prescription of sulfonylurea per genotype

Genotype	Patients	Change [a,b]	Difference in change compared to wild-type [a,b]	95% CI	p-value
Glibenclamide					
*1/*1	20	2.7	ref.		
*1/*2 *2/*2	8	0.5	-2.0	(-6.2, 2.2)	0.35
*1/*3 *2/*3 [c]	6	0.9	-1.3	(-4.7, 2.1)	0.47
Tolbutamide					
*1/*1	117	279	ref.		
*1/*2 *2/*2	35	265	-14	(-182, 155)	0.87
*1/*3 *2/*3 [c]	20	12	**-269**	**(-469, -69)**	**0.009**
Glimepiride					
*1/*1	27	0.49	ref.		
*1/*2 *2/*2	12	0.40	-0.07	(-0.65, 0.65)	0.84
*1/*3 *2/*3 [c]	3	1.61	1.1	(-0.43, 2.62)	0.17

[a] In mg, the defined daily dose is 10 mg for glibenclamide, 1500 mg for tolbutamide and 2 mg for glimepiride. [b] Adjusted for age and sex. [c] No tenth prescriptions were dispensed in patients with the *3/*3 genotype.

In an additional analysis, the change in doses, from the sixth until the twentieth prescription was compared with the first prescription by repeated measurements, in which we adjusted for prescriptions in the same patient. In patients with the CYP2C9*3 polymorphism using tolbutamide, the difference in prescribed daily dose between prescription six and twenty, compared to the first prescription, was 316 mg lower (95% CI -497, -135; p=0.0008) than in patients with the wild-type genotype. In patients with the CYP2C9*1/*2 or CYP2C9*2/*2

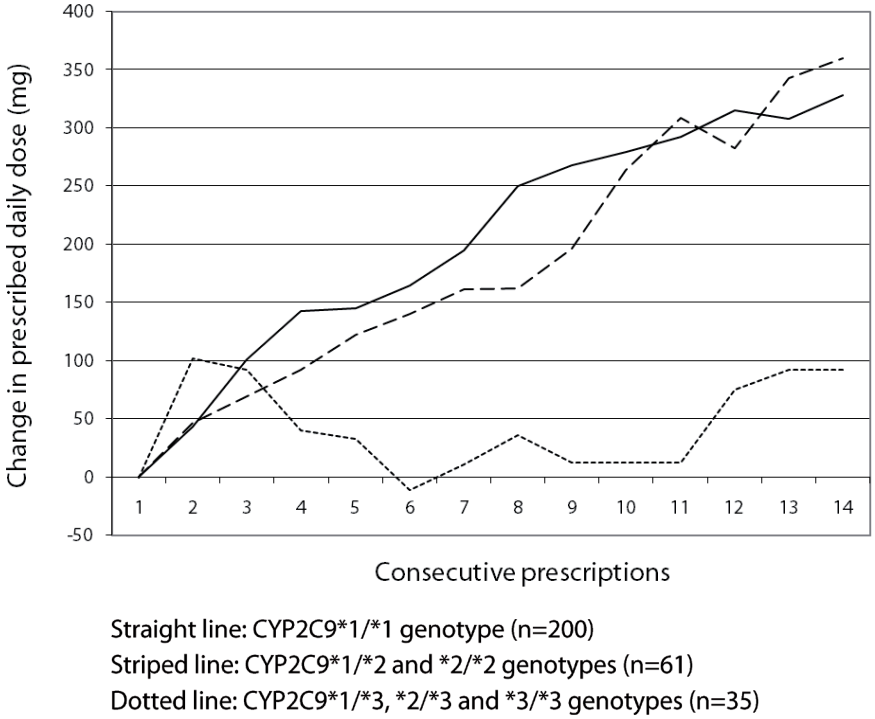


Figure 1 Average change in prescribed doses of tolbutamide compared to the first prescribed dose per genotype for each consecutive prescription

genotype using tolbutamide, the prescribed daily dose was 27 mg lower (95% CI -175, 121; p=0.72) than in patients with the wild-type genotype.

In 79 patients, fasting serum glucose levels were measured both in the period 90 days prior to start of sulfonylurea therapy and in the period 180 days after start (table 3). None of these patients switched in the period until the first measurement of fasting serum glucose levels or received a second hypoglycemic drug. In six patients, the dose of sulfonylurea changed between start and the first measurement. In two patients, the dose decreased (both CYP2C9*1/*1) and in four patients the dose increased (three patients with CYP2C9*1/*1, one patient with CYP2C9*1/*2). Sixty-five patients, in whom fasting serum glucose levels were

Table 3 Change in glucose level after start of tolbutamide therapy

	Patients	Average before start [a]	Average after start [a]	Change [a,b]	Average dose (range) [c]		Difference in glucose level change [d]	95% CI	p-value
*1/*1	45	11.0	8.9	-2.3	572	(250-1000)	ref.		
*1/*2 *2/*2	13	11.8	8.9	-3.0	577	(500-1000)	-0.28	(-1.25, 0.69)	0.57
*1/*3 *2/*3 [e]	7	11.8	7.5	-3.7	607	(250-1000)	-1.24	(-2.75, 0.27)	0.11

[a] In mmol/l. [b] Adjusted for age and sex. [c] In mg, the day before measurement. [d] In mmol/l, adjusted for the last measured glucose level before start, the dose the day before the measurement after start, age and sex. [e] No glucose measurements were done in patients with the *3/*3 genotype.

measured, were using tolbutamide. In these patients, the adjusted decrease in fasting serum glucose levels in patients with the CYP2C9*1/*2 or CYP2C9*2/*2 genotype was 0.3 mmol/l larger than in patients with the wild-type genotype and 1.2 mmol/l larger in patients with the CYP2C9*1/*3 or CYP2C9*2/*3 genotype than in patients with the wild-type genotype, although these differences did not reach statistical significance.

DISCUSSION

In this population based cohort study, CYP2C9*3 carriers who started on tolbutamide received significantly lower doses of tolbutamide on the tenth prescription than patients with the wild-type genotype. The tenth consecutive prescription was chosen for the analyses, because the majority of patients did receive at least this number of prescriptions during the study period. Differences in daily dose between patients with different genotypes will not establish immediately but will become visible only after several prescriptions as a consequence of downwards titration on the basis of serum glucose levels. Therefore, differences in metabolism of sulfonylurea were analyzed on the short-term as differences in glucose levels and on the long-term as differences in prescribed doses. The differences between CYP2C9*3 carriers and patients with the wild-type genotype were significantly different from the sixth until the twentieth prescription. As sulfonylurea doses are changed according to measured glucose levels, it is likely that the differences in doses reflect a difference in glucose levels. For the other sulfonylurea, no significant differences were found. Post hoc power analyses revealed that the power to detect a difference, the same as in tolbutamide users, was 0.05 for glibenclamide users with the CYP2C9*1/*2 or CYP2C9*2/*2 genotype and 0.58 for CYP2C9*3 carriers. In glimepiride users, these numbers were 0.05 and 0.11. Particularly in glimepiride users, the power of our study was too small to detect differences that were the same in size as the differences found in tolbutamide users.

Most patients who had fasting serum glucose measurements both before and after start of sulfonylurea therapy were using tolbutamide. The decrease in glucose levels was 0.3 mmol/l larger for patients with the CYP2C9*1/*2 or CYP2C9*2/*2 genotype and 1.2 mmol/l for CYP2C9*3 carriers compared to patients with the wild-type genotype. These differences did not reach the level of statistical significance but are in line with the aforementioned findings of this study. It is likely that the power of this study was too small to detect significant differences in change of glucose levels, because in only seven CYP2C9*3 carriers, glucose levels both before and after start of sulfonylurea therapy were available. Analyses were also performed comparing patients carrying the wild-type genotype with patients carrying the CYP2C9*1/*2 or CYP2C9*1/*3 genotype and patients carrying the wild-type genotype with patients carrying the CYP2C9*2/*2 or CYP2C9*3/*3 genotype. These analyses did not add much to the analyses described in this article.

In this study, clinically relevant differences in sulfonylurea response between patients with different CYP2C9 polymorphisms were only found for tolbutamide and not for glibenclamide, gliclazide, and glimepiride. Tolbutamide is the most regularly used sulfonylurea in this study. As the number of users of the non-tolbutamide sulfonylurea is small, it is likely that these numbers are too small to detect differences for these drugs in this study. This is demonstrated by the post-hoc power analysis. This does not, however, prove that differences in prescribed daily doses between the genotypes do not exist, but merely that we cannot draw a conclusion on the non-tolbutamide sulfonylurea. There are differences in metabolism between tolbutamide and the other sulfonylurea. Although CYP2C9 is the main metabolizing route for sulfonylurea, other routes are also involved.[5,16,17] It is possible that in patients with decreased CYP2C9 enzyme activity, these alternative metabolic routes compensate the decreased functioning, making the influence of the polymorphism less clinically relevant. For example, up to fifteen percent of gliclazide is excreted unchanged by the kidney.[18-20] In patients with decreased CYP2C9 enzyme functioning it is possible that an increased renal excretion partly compensates for the decreased CYP2C9 enzyme functioning. However, the pharmacokinetic studies in healthy volunteers showed differences in drug exposure related to the CYP2C9 polymorphism.

Three studies assessed the differences in glucose tolerance in healthy volunteers using tolbutamide or glibenclamide.[8,9,21] Only the study by Shon et al. found lower levels of serum glucose in individuals with the CYP2C9*1/*3 genotype after using tolbutamide, whereas the studies by Kirchheiner and co-workers found no significant differences after using glibenclamide or tolbutamide. In our study, we demonstrated that CYP2C9*3 carriers with diabetes mellitus require lower doses of tolbutamide, which strongly suggests an increased response and possibly a higher risk of hypoglycemia.

In population-based studies, bias may affect the obtained results. We believe that bias in our study is minimal. Selection bias was probably negligible, because we identified all patients starting on sulfonylurea in a population based cohort study, and absences of blood samples and difficulties with genotyping were probably random. Moreover, the study population was in Hardy-Weinberg equilibrium, suggesting that no selection bias among genotypes has occurred, which could have explained the observed association. The prescription and glucose measurement data in this study were collected prospectively without prior knowledge of the study hypothesis, making information bias unlikely. It is also unlikely that confounding has influenced the results of our study, because physician's decisions about the prescribed drug and initial dose are made on the basis of product information and not on the mostly unknown presence of CYP2C9 variant allele carriership of a patient. Consequently, differences between patients in the different CYP2C9 genotype groups at the start of sulfonylurea therapy, such as the fasting serum glucose levels before start and the starting dose, are due to chance and therefore random.

In conclusion, this is the first population-based study assessing the clinical relevance of CYP2C9 polymorphism in diabetes mellitus patients. It shows that diabetes mellitus patients with the CYP2C9*3 polymorphism treated with tolbutamide require lower doses of tolbutamide to regulate serum glucose. This knowledge is clinically important, because it may mean that such patients have a higher risk of hypoglycemia after starting treatment according to a standard dose scheme.

REFERENCES

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 2005;365(9467):1333-46.
2. Bell DS. Practical considerations and guidelines for dosing sulfonylureas as monotherapy or combination therapy. *Clin Ther* 2004;26(11):1714-27.
3. Kirchheiner J, Roots I, Goldammer M, Rosenkranz B, Brockmoller J. Effect of genetic polymorphisms in cytochrome p450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet* 2005;44(12):1209-25.
4. Drug Information Project. Health Care Insurance Board Web site. Available from http://www.gipdatabank.nl. Accessed 30 december 2006.
5. Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs* 2005;65(3):385-411.
6. Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002;12(3):251-63.
7. Schwarz UI. Clinical relevance of genetic polymorphisms in the human CYP2C9 gene. *Eur J Clin Invest* 2003;33 Suppl 2:23-30.
8. Kirchheiner J, Bauer S, Meineke I, et al. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002;12(2):101-9.
9. Kirchheiner J, Brockmoller J, Meineke I, et al. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002;71(4):286-96.
10. Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002;72(3):326-32.
11. Wang R, Chen K, Wen SY, Li J, Wang SQ. Pharmacokinetics of glimepiride and cytochrome P450 2C9 genetic polymorphisms. *Clin Pharmacol Ther* 2005;78(1):90-2.
12. Suzuki K, Yanagawa T, Shibasaki T, Kaniwa N, Hasegawa R, Tohkin M. Effect of CYP2C9 genetic polymorphisms on the efficacy and pharmacokinetics of glimepiride in subjects with type 2 diabetes. *Diabetes Res Clin Pract* 2006;72(2):148-54.
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. Complete ATC index 2006. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 30 December 2006.
15. Aynacioglu AS, Brockmoller J, Bauer S, et al. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. *Br J Clin Pharmacol* 1999;48(3):409-15.
16. Rendell M. The role of sulphonylureas in the management of type 2 diabetes mellitus. *Drugs* 2004;64(12):1339-58.
17. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998;45(6):525-38.
18. Campbell DB, Lavielle R, Nathan C. The mode of action and clinical pharmacology of gliclazide: a review. *Diabetes Res Clin Pract* 1991;14 Suppl 2:S21-36.
19. Holmes B, Heel RC, Brogden RN, Speight TM, Avery GS. Gliclazide. A preliminary review of its pharmacodynamic properties and therapeutic efficacy in diabetes mellitus. *Drugs* 1984;27(4):301-27.

20. Oida T, Yoshida K, Kagemoto A, Sekine Y, Higashijima T. The metabolism of gliclazide in man. *Xenobiotica* 1985;15(1):87-96.
21. Shon JH, Yoon YR, Kim KA, et al. Effects of CYP2C19 and CYP2C9 genetic polymorphisms on the disposition of and blood glucose lowering response to tolbutamide in humans. *Pharmacogenetics* 2002;12(2):111-9.

Chapter 3.2.

Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with type 2 diabetes mellitus

ABSTRACT

Background: The organic cation transporter 1, encoded by the *SLC22A1* gene, is responsible for the uptake of the antihyperglycemic drug metformin in the hepatocyte. We assessed whether genetic variation in the *SLC22A1* gene is associated with the glucose lowering effect of metformin.

Methods: Incident metformin users in the Rotterdam Study, with HbA1c measurements available, were identified. Associations between eleven tagging SNPs in the *SLC22A1* gene and change in HbA1c level were analyzed.

Results: One hundred and two incident metformin users were included in the study sample. Except for the rs622342 A>C polymorphism, no significant differences in metformin response were observed. For each minor C allele at rs622342, the reduction in HbA1c levels was 0.28% less (95% CI 0.09, 0.47; p=0.005). After Bonferroni correction the p-value was 0.050.

Conclusion: Genetic variation at rs622342 in the *SLC22A1* gene was associated with the glucose lowering effect of metformin in patients with diabetes mellitus.

INTRODUCTION

Metformin is an oral antihyperglycemic drug, widely used in the treatment of type 2 diabetes mellitus. The major mode of action is to reduce hepatic glucose production, although the exact pharmacological action has not yet been fully determined.[1,2] Besides, metformin also increases insulin responsiveness of skeletal muscles.[3] The main route of elimination is through tubular renal secretion.

Metformin is actively transported across membranes. The organic cation transporter 1 (OCT1) is responsible for the uptake in hepatocytes, which is an essential step in reducing hepatic glucose production.[4] In OCT1 gene knockout mice, the liver concentration of metformin was 30 times lower than in mice with normal functioning OCT1 transporters and metformin blood concentrations were higher while the glucose lowering effect was decreased.[5-7]

In humans, OCT1 is encoded by the *SLC22A1* gene located at chromosome 6q25.3.[8] Controversy exists as to whether polymorphisms in this gene are associated with the glucose lowering effect of metformin.[9] In a study of 20 healthy Caucasian volunteers differences in metformin blood concentrations and glucose levels after an oral glucose tolerance test were found between individuals with a reduced function allele (coding for the amino acid changes R61C, G401S, M420del and G465R) in the *SLC22A1* gene and individuals without.[5,10] However, in a study with 33 Japanese diabetes mellitus patients comparing responders and non-responders to metformin, no differences in allele frequencies were found.[11]

In this prospective population-based cohort study, we studied the association between tagging single nucleotide polymorphisms (SNPs) in the *SLC22A1* gene and metformin response in Caucasian patients with diabetes mellitus.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[12,13] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included

the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[14]

For this study, we used the HbA1c assessments from the Stichting trombosedienst en artsenlaboratorium rijnmond – medisch diagnostisch centrum (STAR-MDC), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam with a potential source population of more than 1 million inhabitants. Hereby, we obtained all outpatient HbA1c assessments from all participants between April 1st 1997, the time at which a new computer system was introduced at STAR-MDC, and January 1st 2008.

Study Sample

All participants in the Rotterdam Study, who were incident metformin users in the period between April 1st 1997 and January 1st 2008, were included in this analysis. Incident metformin use was defined as a first dispensed prescription for metformin in the database, which included all prescriptions from January 1st 1991 onwards. The study sample consisted of all incident metformin users who had both a measurement of HbA1c in the period of 30 days before the first prescription of metformin and in the period between 14 and 100 days following the first prescription of metformin. Patients who discontinued metformin therapy before the first measurement after start were excluded. We also excluded patients who were co-prescribed acarbose, rosiglitazone, pioglitazone or insulin at the time of one of the two HbA1c measurements, because defined daily doses (DDD) for these drugs are not comparable to each other, and these patients most likely differ in their severity of disease. Patients using sulfonylurea were not excluded.

Outcomes

The goal of antihyperglycemic therapy is to reduce plasma glucose levels. The HbA1c level is the percentage of hemoglobin in the blood that is glycosylated and represents the average glucose level in the preceding period of time. Since the HbA1c level is a more stable measurement of glycemic control than plasma glucose levels, HbA1c levels are used more frequently for long-term therapeutic purposes. We analyzed the association between genetic variation in the *SLC22A1* gene and difference in HbA1c level between the last HbA1c measurement before start of metformin therapy and the first HbA1c measurement after start. The target level for diabetes mellitus patients is an HbA1c level below 7%.[15]

Cofactors

Characteristics considered as potential determinants affecting the change in HbA1c level were age, gender, the HbA1c level at the last measurement before start of metformin, the daily prescribed dose of metformin at the time of the first measurement after start and the change in daily prescribed dose of sulfonylurea. To make the prescribed doses of different sulfonylurea comparable to each other, we divided the prescribed daily dose by the DDD.[14]

The DDD is a standardized dosing measure representing the recommended daily dose for the main indication in an adult.

Genotyping

In this study we used a selection of tagging SNPs on the Illumina 550k SNP array (Illumina Inc, San Diego, CA) for genotyping according to the manufacturer's instruction. Quality controls and results of the genotyping were previously described.[16] The tagging SNPs on the array were selected using an algorithm with which in a Caucasian population ninety percent of all Phase I and II Hapmap SNPs are covered by at least one SNP on the array.[8,17,18] This coverage arises because genetic variation is transmitted in blocks, in which haplotype alleles exist. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. For this study we selected the tagging SNPs in the *SLC22A1* gene that were on the array. SNPs with a minor allele frequency lower than 0.05 were excluded, because the power of this study was too low to found significant associations for these SNPs.

Statistical Analysis

Deviations from Hardy-Weinberg equilibrium and differences in genotypes between patients who continued and discontinued metformin therapy were analyzed using χ^2-tests, and differences in baseline HbA1c levels, prescribed doses of metformin and change in prescribed doses of sulfonylurea were analyzed using one-way ANOVA. Multivariate linear regression was used to analyze differences in HbA1c change between genotypes. For each polymorphism we calculated the association between the number of variant alleles and the difference in HbA1c change. For polymorphisms significant in this analysis, we calculated the difference in HbA1c change between *Aa* and *AA* and between *aa* and *AA*, in which *A* represents the more common allele and *a* the minor allele. These analyses were performed with SPSS software (version 15.0; SPSS, Chicago, IL).

RESULTS

In the Rotterdam Study, we identified 152 patients with diabetes mellitus who had a first prescription for metformin between April 1st 1997 and January 1st 2008 and for whom an HbA1c measurement both in the period of 30 days before and in the period between 14 and 100 days after start of metformin therapy was available. Eight patients were excluded because they were prescribed rosiglitazone (one patient), pioglitazone (one patient) or insulin (six patients) at the time of the HbA1c measurement before start. In 24 patients no blood sample was available for genotyping. Eighteen patients discontinued metformin therapy before the

first HbA1c measurement after start (sixteen patients) or started acarbose (one patient) or rosiglitazone (one patient) therapy. Eventually, we could analyze the change in HbA1c level in 102 participants starting on metformin therapy (table 1).

Table 1 Baseline characteristics of the study population (n=102)

Characteristic		
Gender	Male	40 (39 %)
	Female	62 (61 %)
Age (SD)		76.5 (6.7) year
HbA1c level (SD) [a]		8.3 (1.2) %
Body-mass index (SD) [b]		28.0 (3.4) kg/m^2
Creatinine level (SD) [b]	(n=78)	83.7 (15.2) µmol/l
Sulfonylurea use [a]	Glibenclamide	16 (15.7 %)
	Tolbutamide	26 (25.5 %)
	Gliclazide	6 (5.9 %)
	Glimepiride	13 (12.7 %)

[a] At the time of the last HbA1c measurement before start of metformin therapy. [b] At the time of entrance in the Rotterdam Study.

The average HbA1c level decreased from 8.3% (SD 1.2%) before start of metformin therapy to 7.9% (SD 1.3%) after start. The average time between the last HbA1c measurement before start, and the start of metformin therapy was 8 days (SD 6 days), and 52 days (SD 23 days) between start of metformin therapy and the first HbA1c measurement after start. The average prescribed daily dose of metformin was 677 mg (SD 303 mg) or 0.34 DDD. In 12 of the 102 patients (11.8%) the prescribed daily dose was changed between the first prescription and the first measurement of HbA1c. In 11 patients the prescribed daily dose was increased and in one patient it was decreased. Sulfonylurea had been prescribed in 60 participants before start of metformin therapy (average 1.29 DDD), and in 49 participants after start of metformin therapy (average 1.33 DDD).

Twelve tagging polymorphisms in the *SLC22A1* gene were analyzed (table 2). All genotype distributions were in Hardy-Weinberg equilibrium. The SNP rs3798168 was excluded from the analyses, because the minor allele frequency was 0.02. The SNPs, rs1443844 and rs2297374, were in linkage disequilibrium (r^2=0.89, D'=1.00), the other SNPs were not in linkage disequilibrium (r^2<0.8). A statistically significant association (p=0.005) was found between SNP rs622342 and change in HbA1c level, leading to an average of 0.28% less decrease in HbA1c levels for each minor C allele (95% CI 0.09, 0.47; p=0.0050) (table 3). After Bonferroni correction for multiple testing, the p-value for this association was 0.050. For the other tagging SNPs, no significant associations with change in HbA1c level were found. Participants with

Table 2 Genotyped polymorphisms in the *SLC22A1* gene [a]

Genotype		AA	Aa	aa	MAF	HWE (p-value)
rs3798174	C>T	91	11	0	0.05	0.56
rs6937722	G>A	89	13	0	0.06	0.49
rs3798168	C>A	97	5	0	0.02	0.80
rs628031	G>A	35	53	14	0.40	0.39
rs9457843	C>T	71	29	2	0.16	0.63
rs3798167	G>T	66	34	2	0.19	0.31
rs2197296	G>A	52	46	4	0.26	0.11
rs622342	A>C	38	48	13	0.37	0.72
rs1443844	A>G	30	53	19	0.45	0.60
rs2297374	C>T	34	48	17	0.41	0.99
rs1564348	T>C	70	30	2	0.17	0.55
rs622591	C>T	68	31	3	0.18	0.81

[a] Genotyping failed in some participants. Therefore, not all numbers add up to 102. A: variant allele with the major allele frequency; a: with minor allele frequency. MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium.

Table 3 Difference in change of HbA1c after start of metformin therapy per genotype

Genotype	Difference in HbA1c change (%) [a]	p-value	p-value after Bonferroni correction [b]
rs3798174	0.15	0.49	1.00
rs6937722	-0.17	0.40	1.00
rs628031	0.02	0.87	1.00
rs9457843	-0.11	0.40	1.00
rs3798167	0.17	0.20	1.00
rs2197296	0.06	0.61	1.00
rs622342	**0.28**	**0.0050**	**0.050**
rs1443844	-0.13	0.18	1.00
rs2297374	-0.14	0.15	1.00
rs1564348	0.05	0.71	1.00
rs622591	-0.16	0.19	1.00

[a] Additive model (number of variant allele – dose effect), adjusted for: age, gender, HbA1c level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea. [b] We corrected for ten independent tests, because the minor allele frequency of one tagging SNP was below 0.05 and two tagging SNPs were in linkage disequilibrium.

the AA genotype at rs622342 had an average decrease of 0.53% in HbA1c level, while in participants with the AC genotype the average decrease was 0.32% and in participants with the CC genotype the HbA1c level increased on average with 0.02% (table 4). After adjustment for the cofactors, the difference in HbA1c decrease between patients with AC and patients with the AA genotype was 0.29% (95% CI 0.002, 0.58; p=0.049). The difference between patients with the CC and patients with the AA genotype was 0.58% (95% CI 0.22, 0.93; p=0.002). No differences were found between rs622342 genotypes in baseline HbA1c levels (p=0.58), prescribed doses of metformin (p=0.41) or changes in prescribed doses of sulfonylurea (p=0.59). The rs622342 genotypes did not differ significantly in frequency between patients who con-

tinued metformin and those who discontinued metformin or started acarbose, rosiglitazone, pioglitazone or insulin therapy (χ^2=3.51, p=0.17).

Table 4 Difference in change of HbA1c after start of metformin therapy for polymorphism rs622342

rs622342	N [a]	Average change in HbA1c (%)	Difference in HbA1c change (%) [b]	95% CI	p-value
AA	38	-0.53	ref.		
AC	48	-0.32	**0.29**	**(0.002, 0.58)**	**0.049**
CC	13	0.02	**0.58**	**(0.22, 0.93)**	**0.002**
Additive model [c]			**0.28**	**(0.09, 0.47)**	**0.0050**

[a] In three participants genotyping for rs622342 failed. [b] Adjusted for: age, gender, HbA1c level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea. [c] Number of variant alleles.

DISCUSSION

In this population-based cohort study, the SNP rs622342 was associated with differences in HbA1c reduction in diabetes mellitus patients using metformin. The HbA1c levels represent glycemic control in the preceding period of time, and are therefore a stable measurement of metformin response and a better outcome measure in a population-based setting than serum glucose. The decrease in HbA1c level in patients with the AC genotype starting on metformin therapy was 0.29% less than in patients with the AA genotype and in patients with the CC genotype the decrease in HbA1c level was 0.58% less than in patients with the AA genotype. In patients with the CC genotype the HbA1c levels increased by 0.02% after start of metformin therapy. Most likely, patients with the AC or CC genotype have less OCT1 transporter activity and their capacity to transport metformin into hepatocytes is reduced. As a consequence the glucose and HbA1c lowering effect of metformin is impaired.

The HbA1c level is expressed as the proportion of haemoglobin that is glycosylated and is a marker for the average glucose levels in the preceding period of time. The average life-span of erythrocytes, incorporating haemoglobin, is 90 days and the HbA1c level represents the average glucose level in the preceding 90 days, although it mainly reflects the preceding two to four weeks before measurement. In this study we choose to include all HbA1c levels from 14 days after start of metformin therapy. Physicians measured the HbA1c levels between 14 and 30 days after start of metformin therapy in 21 of the 102 participants in this study. Although the effect of metformin therapy is not completely established at that time, the HbA1c level gives an indication of the change in glucose level and most likely physicians adjust the therapy according to these results. Not including this first measurement will probably introduce bias, due to the changes in therapy such as discontinuing metformin therapy or switching to other antidiabetic drugs. If we selected the first HbA1c measurements in the

time period between 30 and 120 days after start of metformin therapy, we found a tendency towards more discontinuations of metformin and switches to acarbose, pioglitazone, rosiglitazone or insulin in users with the A allele. In the group of incident users with the AA genotype, 23% (n=14) discontinued metformin therapy or switched to acarbose, pioglitazone, rosiglitazone or insulin, versus 15% (n=9) in users with the AC genotype and 0% in users with the CC genotype (χ^2=4.94, p=0.085). In the patient files of the general practitioners, we were able to find back the reason for stopping or switching in seven of the twenty-three cases. In five cases the reason for stopping or switching was an adverse drug reaction, in one case a sufficient regulation without drug therapy and in one case insufficient regulation with oral antidiabetic drugs. The adverse drug reactions identified were malaise, nausea, itching, decreased appetite and diarrhoea. These results may suggest that incident metformin users with the AA genotype have more problems with adverse effects due to metformin therapy.

The average decrease in HbA1c level (0.4%) is rather lower. A possible explanation is that after on average 52 days, the decrease in HbA1c is not completely established. Another explanation is that the average prescribed dose of metformin (677 mg) is lower than recommended in guidelines. The guidelines recommend an initial daily dose of 1,500 to 2,000 mg and this dose may be increased after 10 to 15 days to at most 3,000 mg a day. The reason for the low doses of metformin used in this study may be that the average age of the study population is 77 years, and physicians are cautious when they prescribe high doses of metformin in this elderly population because of fear of potential adverse effects.

In our study we used twelve tagging SNPs, different from the SNPs used in the study by Shu et al.[5,10] As we are not aware of studies genotyping both the coding SNPs and the tagging SNPs, we do not know whether these SNPs are in linkage disequilibrium with each other. The SNP rs622342, associated in this study with the glucose lowering effect of metformin, is located between exon 8 and exon 9 (figure 1). The SNPs studied by Shu et al. were all situated in exons resulting in amino acid changes and were identified in *in vitro* studies. With the use of tagging SNPs in this study, we could analyze both SNPs in introns and in exons, not necessarily resulting in amino acid changes. Beside changes in amino acid sequence, SNPs may also affect gene expression, resulting in increased or decreased transporter functioning. With the use of tagging SNPs we could identify other SNPs associated with metformin response.

In population-based studies, bias may affect results. We believe that bias in our study is minimal. The HbA1c measurements in this study were part of regular daily practice. Bias may have occurred if discontinuation of metformin therapy was associated with the genotype. For the rs622342 polymorphism, no differences in genotype frequency were found between patients who continued or discontinued metformin therapy in the time period used in this study. Participants in this study were co-prescribed sulfonylurea before and after start of metformin therapy. As the polymorphisms in the OCT1 gene do not affect sulfonylurea therapy, the changes in prescribed doses are random. Moreover, we adjusted in our analyses for the change in prescribed dose of sulfonylurea. If there were differences in prescribed doses,

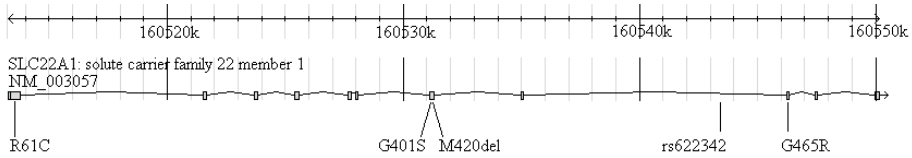


Figure 1 Polymorphisms in the *SLC22A1* gene associated with metformin response

patient with a low response to metformin would receive higher prescribed doses of sulfonylurea and this would underestimate the effects of the OCT1 polymorphism. No differences in time to the first HbA1c measurement after start were seen, and therefore it is unlikely that there were differences in frequency of HbA1c measurement between genotypes, influencing the effect size found in this study. We identified all incident metformin users in the Rotterdam Study and information was collected prospectively, without prior knowledge of the study hypothesis. The permission of patients to take blood and isolate DNA for scientific research was most likely independent from the genotype we studied. In this study we analyzed eleven SNPs in the *SLC22A1* gene and therefore multiple testing may play a role. To cope with this, we adjusted the cut-off for ten independent SNPs using Bonferroni correction, which gave a p-value of 0.050. Two SNPs were in strong linkage disequilibrium and therefore counted as one independent test. The Bonferroni test assumes independence between the SNPs. In our study, there was some linkage disequilibrium between many SNPs and therefore the Bonferroni test is a conservative test, underestimating the significance of the association. Nevertheless, replication of our results in a prospective observational study or trial is warranted.

To conclude, in this population-based cohort study we found an association between genetic variation in the gene encoding the OCT1 transporter protein and glucose reduction by metformin in diabetes mellitus patients. Metformin therapy is less effective in reducing glucose and HbA1c levels in diabetes mellitus patients carrying the minor C allele at SNP rs622342 compared to wildtype AA patients. This information could be clinically relevant to predict the glucose lowering effect of metformin before start of therapy.

REFERENCES

1. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med* 2002;137(1):25-33.
2. Hundal RS, Krssak M, Dufour S, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 2000;49(12):2063-9.
3. Borst SE, Snellen HG. Metformin, but not exercise training, increases insulin responsiveness in skeletal muscle of Sprague-Dawley rats. *Life Sci* 2001;69(13):1497-507.
4. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics* 2008;9(4):415-22.
5. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;117(5):1422-31.
6. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* 2002;302(2):510-5.
7. Wang DS, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol Pharmacol* 2003;63(4):844-8.
8. The international Hapmap consortium. The international Hapmap project. *Nature* 2003;426(6968):789-96.
9. Shu Y, Leabman MK, Feng B, et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc Natl Acad Sci U.S.A.* 2003;100(10):5902-7.
10. Shu Y, Brown C, Castro RA, et al. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
11. Shikata E, Yamamoto R, Takane H, et al. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *J Hum Genet* 2007;52(2):117-22.
12. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
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. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 28 April 2008.
15. Standards of medical care in diabetes-2008. Diabetes Care 2008;31 Suppl 1:S12-54.
16. Richards JB, Rivadeneira F, Inouye M, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008;371(9623):1505-12.
17. Sentrix® HumanHap550 Genotyping Beadchip. Illumina Inc. Available from http://www.illumina.com/downloads/HUMANHAP550_DataSheet.pdf. Accessed 29 february 2008
18. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74(1):106-20.

Chapter 3.3.

Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose lowering effect of metformin in patients with type 2 diabetes mellitus

ABSTRACT

Background: Metformin, an oral glucose-lowering drug, is taken up in hepatocytes by the organic cation transporter (OCT) 1 and in renal epithelium by OCT2. In these cells, the multidrug and toxin extrusion (MATE) 1 protein, encoded by the *SLC47A1* gene, is responsible for the excretion of metformin into the bile and urine, respectively. We studied the effect of single nucleotide polymorphisms (SNPs) in the *SLC47A1* gene on the HbA1c lowering effect of metformin.

Methods: We identified all incident metformin users in the Rotterdam Study, a population-based cohort study. Associations between twelve tagging SNPs in the *SLC47A1* gene and change in HbA1c level were analyzed.

Results: One-hundred and sixteen incident metformin users were included in the study sample. The rs2289669 G>A SNP was significantly associated with metformin response. For the other SNPs, no associations were found. For each minor A allele at rs2289669, the HbA1c reduction was 0.30% (95% CI -0.51, -0.10; p=0.005) larger. After Bonferroni correction for multiple testing, the p-value was 0.045.

Conclusion: The rs2289669 G>A SNP is associated with a reduction in HbA1c level, consistent with a reduction in MATE1 transporter activity. These results suggest that the transporter MATE1, encoded by *SLC47A1*, may have an important role in the pharmacokinetics of metformin, although replication is necessary.

INTRODUCTION

Metformin is an oral glucose-lowering drug, widely used for the treatment of type 2 diabetes mellitus.[1] The molecular mechanism of the glucose-lowering effect is not fully understood, although it is known that inhibition of the hepatic gluconeogenesis has an important role.[2] Metformin is mainly eliminated by tubular secretion, and hepatic metabolism has a minor role.

Several drug transporters are involved in the distribution and excretion of metformin.[3] The role of two organic cation transporters (OCTs), OCT1 and OCT2, is assumed. OCT1 and OCT2 are members of the solute carrier (SLC) 22 family and encoded by the *SLC22A1* and *SLC22A2* gene, respectively, with gene-location 6q25.3. OCT1 is expressed in the basolateral membrane of hepatocytes and the uptake of metformin in the hepatocytes by OCT1 is an essential step for the glucose-lowering effect.[4-6] In OCT1 gene knock out mice, the metformin liver concentrations were lower and the glucose-lowering effect impaired.[4,7] Genetic variations in the *SLC22A1* gene (R61C, G401S, M420del and G465R) are associated with differences in metformin plasma levels and glucose concentrations after an oral glucose tolerance test in healthy volunteers.[4,7] OCT2 is expressed in the basolateral membrane of the renal epithelium, and transportation of metformin over this membrane may be the first step to tubular secretion.[8,9] Genetic variations in *SLC22A2* (T199I, T201M and A270S) are associated with decreased renal excretion and increased plasma concentrations of metformin.[10,11]

Recently, a multidrug and toxin extrusion (MATE) transporter protein family was identified, assigned as the SLC 47 family.[12,13] The *SLC47A1* gene with gene location 17p11.2, encodes the MATE1 transporter. Metformin is one of the substrates of this transporter.[14] MATE1 is located in the bile canalicular membrane in the hepatocyte and in the brush border of the renal epithelium and is responsible for the final step of metformin excretion through the bile and urine.[12] Another transporter in this family is MATE2-K, encoded by *SLC47A2*. MATE2-K is located in the brush border of the renal epithelium and may also be involved in metformin excretion.[14]

The co-localization of OCT1 and MATE1 in the hepatocyte and OCT2 and MATE1 in the renal epithelium suggests that MATE1 may have an important influence on the pharmacokinetics of metformin. The intrahepatic uptake of metformin by OCT1 is an essential step in the glucose-lowering effect, while the excretion out of the hepatocyte into the bile by MATE1 probably averts this. The uptake in the renal epithelium by OCT2 and subsequent excretion by MATE1 are two consecutive steps in the tubular secretion of metformin.

Little is known about the effect of genetic variation in the *SLC47A1* gene on the glucose-lowering effect of metformin. In this prospective, population-based cohort study, we assessed the association between tagging single nucleotide polymorphisms (SNPs) in the *SLC47A1* gene and metformin response in Caucasian incident metformin users.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. The rationale, ethical approval, and design of this study have been described before.[15,16] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[17]

For this study, we used the HbA1c assessments from the stichting trombosedienst en artsenlaboratorium rijnmond – medisch diagnostisch centrum (STAR-MDC), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam. Hereby, we obtained all outpatient HbA1c assessments from all participants between April 1st 1997, the time at which a new computer system was introduced at STAR-MDC, and January 1st 2008. The HbA1c levels were measured by high-performance liquid chromatography on a BiaRad Variant and from October 2004 onwards on a Menarini HA8160, according to professional standards and quality. The STAR-MDC is a CCKL certified laboratory and the quality is continuously monitored by internal and external quality-assurance programs.

Study Sample

All participants in the Rotterdam Study, who were incident metformin users in the period between April 1st 1997 and January 1st 2008, were included in this analysis. Incident metformin use was defined as a first dispensed prescription for metformin in the database, which included all prescriptions from January 1st 1991 onwards. The study sample consisted of all incident metformin users who had both a measurement of HbA1c in the period of 30 days before the first prescription of metformin and in the period between 30 and 120 days following the first prescription of metformin. Patients who discontinued metformin therapy before the first measurement after 30 days were excluded. We also excluded patients who were coprescribed acarbose, rosiglitazone, pioglitazone or insulin at the time of one of the two HbA1c measurements, because defined daily doses (DDD) for these drugs are not similar, and these patients most likely differ in their severity of disease. Patients using sulfonylurea were not excluded.

Outcomes

The aim of antihyperglycemic therapy is to reduce plasma glucose levels. The HbA1c level is the percentage of hemoglobin in the blood that is glycosylated and represents the average glucose level in the preceding period of time. Since the HbA1c level is a more stable measurement of glycemic control than plasma glucose levels, HbA1c levels are used more frequently for long-term therapeutic purposes. We analyzed the association between genetic variation in the *SLC47A1* gene and difference in HbA1c level between the last HbA1c measurement before start of metformin therapy and the first HbA1c measurement after 30 days of metformin therapy. The target level for diabetic patients is an HbA1c level below 7%.[18]

Cofactors

Characteristics considered as potential determinants affecting the change in HbA1c level were age, gender, the HbA1c level at the last measurement before start of metformin, the daily prescribed dose of metformin at the time of the first measurement after start, the change in daily prescribed dose of sulfonylurea, the time from diabetes mellitus diagnosis to start of metformin therapy and the estimated glomerular filtration rate (eGFR). To make the prescribed doses of different sulfonylurea comparable with each other, we divided the prescribed daily dose by the DDD.[17] The DDD is a standardized dosing measure representing the recommended daily dose for the main indication in an adult. For the diabetes diagnosis, the World Health Organization definition was used.[19] If patients were diagnosed with diabetes before entrance in the Rotterdam Study, the date of entrance was used. The eGFR was estimated from the serum creatinine level at baseline with the Cockcroft-Gault formula.

Genotyping

Participants were genotyped using the Illumina 550k SNP array according to the manufacturer's instruction. Quality controls and results of the genotyping were previously described.[20] The tagging SNPs on the array were selected using an algorithm with which, in a Caucasian population, ninety percent of all Phase I and II Hapmap SNPs are covered by at least one SNP on the array.[21-23] This coverage arises because genetic variation is transmitted in blocks, in which haplotype alleles exist. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. For this study we selected the tagging SNPs in the *SLC47A1* gene, including the tagging SNPs within ten kilobasepairs (kbp) of the gene that were on the array.

Statistical Analysis

Deviations from Hardy-Weinberg equilibrium and differences in genotypes between patients who continued and discontinued metformin therapy were analyzed using χ^2-tests. We used one-way ANOVA to test for differences in average time between the last HbA1c measurement

and start of metformin therapy, and in the average time between metformin start and the first HbA1c measurement after start. Linear regression was used to analyze differences in HbA1c change between genotypes. For each polymorphism we calculated the association between the number of variant alleles and the difference in HbA1c change. We adjusted for multiple testing with the Bonferroni correction, multiplying the p-value with the number of independent tests. Two or more SNPs that were in strong linkage disequilibrium (r^2>0.80) were counted as one independent test. For the associations that were statistically significant after Bonferroni correction, we calculated separately the difference between patients with one variant allele and those with the wild type genotype, and the difference between patients with two variant alleles and those with the wild type genotype. The analyses were performed with SPSS software (version 11.0.1; SPSS, Chicago, IL).

RESULTS

One hundred and eighty-one participants of the Rotterdam Study were incident metformin users between April 1st 1997 and January 1st 2008 and had an HbA1c measurement both in the period of 30 days before start and in the period between 30 and 120 days after start of metformin therapy. Seven patients were excluded because they were prescribed insulin at the time of one of the HbA1c measurements, and six patients were excluded because they were prescribed acarbose (n=1), rosiglitazone (n=3) or pioglitazone (n=2). Blood samples for genotyping were not available for 34 patients and 18 patients discontinued metformin therapy before the first HbA1c measurement in the period between 30 and 120 days after start. Eventually, we included 116 incident metformin users in the analysis, for whom the change in HbA1c levels was available (table 1). The average initial starting dose was 648 mg metformin (SD 310 mg). At the time of the first HbA1c measurement after start, the participants were prescribed on average 741 mg metformin (SD 358 mg)

The average time from the last HbA1c measurement before start and start of metformin therapy was 12 days (SD 16 days) and the average time from start of metformin therapy to the first measurement after start was 66 days (SD 25 days). These times did not differ significantly between genotypes. The average HbA1c level before start of metformin therapy was 8.3% (SD 1.2 %) and decreased to 7.7% (SD 1.1 %) after start of metformin therapy.

We identified nine tagging SNPs in the *SLC47A1* gene and three tagging SNPs (rs2453594, rs2453589, rs2165894) in the ten kbp downstream region (table 2). There were no tagging SNPs in the ten kbp upstream region. For the SNP rs16960201, no genetic variation was found in the study population. The SNPs rs2441054 and rs2453568 (r^2=0.84, D'=0.97), and the SNPs rs2441055 and 1961669 (r^2=0.85, D'=0.96) were in linkage disequilibrium. For the other SNPs, no linkage disequilibrium was found (r^2<0.8). The genotype distributions of the eleven tagging SNPs were in Hardy-Weinberg equilibrium. In the Caucasian sample of Hapmap, the

Table 1 Baseline characteristics of the study population (n=116)

Characteristic		
Gender	Male	47 (41 %)
	Female	69 (59 %)
Age (SD)		76.8 (6.7) year
HbA1c level (SD) [a]		8.3 (1.2) %
Body-mass index (SD) [b]	(n=114)	28.3 (3.7) kg/m^2
Creatinine level (SD) [b]	(n=88)	82.5 (14.4) µmol/l
Sulfonylurea use [a]	Glibenclamide	17 (14.7 %)
	Tolbutamide	31 (26.7 %)
	Gliclazide	7 (6.0 %)
	Glimepiride	17 (14.7 %)

[a] At the time of the last HbA1c measurement before start of metformin therapy. [b] At the time of entrance in the Rotterdam Study.

Table 2 Genotyped polymorphisms in the *SLC47A1* gene [a]

SNP		AA	Aa	aa	MAF	HWE (p-value)
rs894680	G>A	43	58	15	0.38	0.51
rs2018675	C>T	43	57	16	0.38	0.67
rs2440154	G>A	50	52	14	0.34	0.93
rs2440155	T>C	77	35	4	0.19	0.99
rs16960201	-	116	0	0	0	-
rs2453568	C>T	58	45	13	0.31	0.35
rs2244280	G>A	73	36	7	0.22	0.38
rs2289669	G>A	36	58	21	0.43	0.78
rs1961669	A>G	79	32	4	0.17	0.73
rs2453594	T>C	73	36	7	0.22	0.38
rs2453589	A>G	41	56	19	0.38	0.91
rs2165894	A>G	68	39	9	0.25	0.32

[a] Genotyping failed in some participants. Therefore, not all numbers add up to 116. A: variant allele with the major allele frequency; a: with minor allele frequency; MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium.

eleven tagging SNPs cover 25 of the 32 (78%) Hapmap SNPs (r^2>0.80) in the selected gene region.[22]

The SNP rs2289669 G>A, with a minor allele frequency of 0.43, was significantly associated with a decrease in HbA1c level after start of metformin therapy (table 3). For each minor A allele the decrease in HbA1c level was 0.30 % (95% CI -0.51, -0.10; p=0.005) more (table 4). For the other tagging SNPs, no significant associations were found. After Bonferroni correction for multiple testing, this association remained significant (p=0.045).

The rs2289669 genotype distributions did not differ significantly between patients who continued metformin therapy and those who discontinued at the time of the HbA1c

Table 3 Difference in change of HbA1c after start of metformin therapy per genotype

SNP	Adjusted difference in HbA1c change (%) [a]	p-value	p-value after Bonferroni correction [b]
rs894680	-0.15	0.19	1.00
rs2018675	0.029	0.80	1.00
rs2440154	0.11	0.35	1.00
rs2440155	0.23	0.10	0.90
rs16960201	-		
rs2453568	0.09	0.42	1.00
rs2244280	0.23	0.062	0.56
rs2289669	**-0.30**	**0.005**	**0.045**
rs1961669	0.16	0.27	1.00
rs2453594	0.26	0.036	0.32
rs2453589	0.12	0.28	1.00
rs2165894	0.28	0.019	0.17

[a] Additive model (number of variant allele – dose effect), adjusted for: age, gender, HbA1c level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea, time from diagnosis of diabetes mellitus to start of metformin therapy and eGFR. [b] We corrected for nine independent tests, because one tagging SNP had no genetic variation and two times two tagging SNPs were in linkage disequilibrium.

Table 4 Difference in change of HbA1c after start of metformin therapy for polymorphism rs2289669

rs2289669	N [a]	Unadjusted average change in HbA1c (%)	Adjusted difference in HbA1c change (%) [b]	95% CI	p-value
GG	36	-0.28	ref.		
GA	58	-0.59	-0.32	(-0.65, 0.01)	0.055
AA	21	-0.87	**-0.66**	**(-1.19, -0.14)**	**0.015**
Additive model [c]			**-0.30**	**(-0.51, -0.10)**	**0.005**

[a] In one participant genotyping for rs2289669 failed. [b] Adjusted for: age, gender, HbA1c level before start, prescribed dose of metformin, change in prescribed doses of sulfonylurea, time from diagnosis of diabetes mellitus to start of metformin therapy and eGFR. [c] Number of variant alleles.

measurement after start (χ^2=1.61, p=0.45). There was a trend that in patients with the AA genotype the decrease in dose of co-prescribed sulfonylurea was larger than in patients with the GG genotype (table 5), although this association was not significant (p=0.08).

Table 5 Cofactors by the rs2289669 polymorphism

rs2289669 genotype	GG	GA	AA
Gender (male)	18 (50%)	22 (38%)	7 (33%)
Age (SD)	75.3 (7.0) year	77.9 (6.5) year	75.6 (6.1) year
HbA1c level before start (SD)	8.3 (0.9) %	8.3 (1.4) %	8.4 (1.1) %
Prescribed metformin dose (SD)	853 (476) mg	662 (262) mg	757 (320) mg
Sulfonylurea use	22 (61%)	33 (57%)	13 (62%)
Change in sulfonylurea dose [a]	-0.01 (0.53) DDD	-0.17 (0.61) DDD	-0.27 (0.52) DDD
Time from diabetes mellitus diagnosis (SD)	5.5 (4.4) year	5.6 (4.8) year	4.7 (3.7) year
eGFR (SD)	74 (19) ml/min	68 (17) ml/min	68 (14) ml/min
BMI (SD)	28.9 (3.9) kg/m^2	28.1 (3.8) kg/m^2	27.6 (3.2) kg/m^2

[a] p=0.08 for trend.

DISCUSSION

This population-based cohort study in diabetic patients is the first one in which the role of MATE1 in the glucose-lowering effect of metformin was assessed. We identified that the SNP rs2289669 was associated with the HbA1c lowering effect of metformin. The decrease in HbA1c level was 0.3% larger per copy of the A allele. These results suggest that polymorphisms in MATE1 may have a role in the pharmacokinetics of metformin and accordingly with the glucose-lowering effect. As metformin is recommended as first line treatment for type 2 diabetes mellitus, these results may be valuable for daily clinical practice.[18]

The average prescribed daily dose of metformin at the time of the first HbA1c measurement after start was 741 mg. The guidelines recommend an initial daily dose of 1,500 to 2,000 mg and this dose may be increased after 10 to 15 days to at most 3,000 mg a day. The reason for the low doses of metformin used in this study may be that the average age of the study population is 77 years, and physicians are prudent to prescribe high doses of metformin in this elderly population because of potential adverse effects. The average decrease in HbA1c level (0.6%) is less than what would be expected when recommended doses are prescribed, and this may explain why the decrease in HbA1c level in patients with the GG genotype was near zero and did not differ significantly from zero.

A reduced efflux of metformin in the renal brush border due to an impaired MATE1 transporter will lead to an increase in metformin plasma levels and possibly to a larger decrease in glucose levels. Similarly, a reduced efflux from the hepatocyte will lead to higher metformin levels in the hepatocyte and a stronger inhibition of the gluconeogenesis, resulting in lower glucose levels. The rs2289669 G>A polymorphism was associated with an increased glucose-lowering effect, implying that the gene with the A allele encodes a MATE1 efflux transporter less effective in transporting metformin. This SNP is located in an intron, not coding for an amino acid change. Most likely, the SNP rs2289669 is in linkage disequilibrium with a SNP causing the reduced MATE1 functioning, although we cannot exclude that it has a direct effect, for example, by affecting gene expression.

One previous study assessed the effect of a SNP in the *SLC47A1* gene on MATE1 expression.[24] The authors identified a SNP in the promoter region (G-32A) that downregulates the basal promoter activity. Whether this SNP affects metformin efflux is unknown. Four glutamate amino acids in MATE1 were found to have an important role in substrate recognition, although genetic variation in the nucleotides encoding these amino acids has not been described.[25]

In population-based studies, bias may affect the obtained results. At the time of the first HbA1c measurement after start, there was a trend towards lower doses of co-prescribed sulfonylurea in patients with the AA genotype. This is in line with the results of our study. The glucose-lowering effect of metformin was stronger in patients with the AA genotype, and these patients require less antidiabetic drugs to reach their target levels. In our analyses, we adjusted for these changes in prescribed doses of sulfonylurea. The HbA1c measurements

in this study were done in regular clinical practice. If discontinuation of metformin therapy and measurement of HbA1c levels were dependent on the genotype, bias might have occurred. However, no differences in genotype frequency were found for rs2289669 between patients who continued metformin until the first HbA1c measurement and patients who discontinued. Bias may also have occurred if there were differences in frequency of HbA1c level measurements. However, the time from start of metformin therapy until the first HbA1c measurement did not differ between genotypes and both the prescribing physician and the patient were not aware of the genetic variation in the *SLC47A1* gene. Selection bias is unlikely, because we identified all incident metformin users in the Rotterdam study and we collected information prospectively, without prior knowledge of the study hypothesis. The permission of patients to take blood and isolate DNA for scientific research was most likely independent from the genetic variation in the *SLC47A1* gene.

The Rotterdam Study is a population-based cohort study on chronic diseases and not primarily designed to assess the effects of metformin therapy. We identified 116 patients who started metformin treatment during follow-up. This limited sample size may result in both false negative results and chance findings. The SNP rs2289669 was the SNP with the highest minor allele frequency. Post-hoc power analyses with α=0.00556 (0.05 divided by nine independent tests) and β=0.8 revealed that this sample size could identify changes in HbA1c levels for the other SNPs ranging from 0.44 to 0.56%, dependent on the minor allele frequency. Therefore, it is possible that we had false negative results. We avoided chance findings by adjusting for multiple testing with the Bonferroni correction. Replication of these results in a prospective observational study or trial is necessary.

To conclude, we found an association between the SNP rs2289669 in the *SLC47A1* gene, encoding the MATE1 transporter, and the glucose-lowering effect of metformin. In incident metformin users the decrease in HbA1c level was 0.30% larger per copy of the A allele. These results suggest that MATE1 may have an important role in the pharmacokinetics and pharmacodynamics of metformin. This is the first epidemiological study assessing the role of MATE1 in metformin response and replication of these results is necessary.

REFERENCES

1. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern* Med 2002;137(1):25-33.
2. Hundal RS, Krssak M, Dufour S, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 2000;49(12):2063-9.
3. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics* 2008;9(4):415-22.
4. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;117(5):1422-31.
5. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* 2002;302(2):510-5.
6. Wang DS, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. *Mol Pharmacol* 2003;63(4):844-8.
7. Shu Y, Brown C, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
8. Kimura N, Okuda M, Inui K. Metformin transport by renal basolateral organic cation transporter hOCT2. *Pharm Res* 2005;22(2):255-9.
9. Kimura N, Masuda S, Tanihara Y, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab Pharmacokinet* 2005;20(5):379-86.
10. Song IS, Shin HJ, Shim EJ, et al. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin Pharmacol Ther* 2008;84(5):559-62.
11. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet Genomics* 2008;18(7):637-45.
12. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci U.S.A.* 2005;102(50):17923-8.
13. Terada T, Inui K. Physiological and pharmacokinetic roles of H+/organic cation antiporters (MATE/SLC47A). *Biochem Pharmacol* 2008;75(9):1689-96.
14. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 2007;74(2):359-71.
15. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
16. 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.
17. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 8 December 2008.
18. Standards of medical care in diabetes-2008. *Diabetes Care* 2008;31 Suppl 1:S12-54.
19. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1. Geneva; World Health Organization. 1999.
20. Richards JB, Rivadeneira F, Inouye M, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008;371(9623):1505-12.
21. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74(1):106-20.

22. The International HapMap Project. *Nature* 2003;426(6968):789-96.
23. Sentrix® HumanHap550 Genotyping Beadchip. Illumina Inc. Available from http://www.illumina.com/downloads/HUMANHAP550_DataSheet.pdf. Accessed 29 february 2008
24. Kajiwara M, Terada T, Asaka J, et al. Critical roles of Sp1 in gene expression of human and rat H+/organic cation antiporter MATE1. *Am J Physiol Renal Physiol* 2007;293(5):F1564-70.
25. Matsumoto T, Kanamoto T, Otsuka M, Omote H, Moriyama Y. Role of glutamate residues in substrate recognition by human MATE1 polyspecific H+/organic cation exporter. *Am J Physiol Cell Physiol* 2008;294(4):C1074-8.

Chapter 3.4.

Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response

ABSTRACT

Background: Metformin is transported into the hepatocyte by OCT1, and out of the hepatocyte by MATE1. Recently, we discovered that the polymorphisms rs622342 A>C in the *SLC22A1* gene, coding for OCT1, and rs2289669 G>A in the *SLC47A1* gene, coding for MATE1, were associated with the glucose lowering effect of metformin. In this study, we assessed whether there is interaction between these two polymorphisms.

Methods: We identified all incident metformin users in the Rotterdam Study, a population-based cohort study of 7,983 elderly people. Multiplicative interaction between the two polymorphisms and the change in HbA1c levels was analyzed.

Results: In incident metformin users with the rs622342 AA genotype, genetic variation in the rs2289669 polymorphism was not associated with change in HbA1c levels (–0.10%; 95% CI –0.35, 0.14; p=0.39). In users with the rs622342 AC genotype, there was a tendency between rs2289669 polymorphisms and change in HbA1c (–0.31 %; 95% CI –0.65, 0.03; p=0.070) and in users with the rs622342 CC genotype there was a significant association (–0.68 %; 95% CI –1.06, –0.30; p=0.005). The multiplicative interaction between these two genotypes was statistically significant (–0.52%; 95% CI –0.94, –0.11; p=0.015).

Conclusion: The glucose lowering effect of metformin is impaired in patients with both a reduced functioning OCT1 influx transporter, encoded by the rs622342 C allele, and a normal functioning MATE1 efflux transporter, encoded by the rs2289669 G allele. In patients with a normal functioning OCT1 influx transporter, the rs2289669 polymorphism does not affect the glucose lowering effect of metformin.

INTRODUCTION

Metformin is a drug widely used for the treatment of type 2 diabetes mellitus.[1] The pharmacologic basis for the glucose lowering effect of metformin is not completely clarified, although inhibition of hepatic gluconeogenesis has a key role.[2] Drug transporters play a major role in the distribution of metformin over tissues and elimination of metformin through renal excretion. Metformin is not metabolized by hepatic enzymes but excreted unchanged by the kidneys.

There are three transporters known to be involved in metformin transport in humans. The organic cation transporter 1 (OCT1) and organic cation transporter 2 (OCT2) are expressed in the basolateral membrane of hepatocytes and renal epithelium, respectively.[3-6] These transporters are members of the solute carrier (SLC) 22 family and encoded by the *SLC22A1* and *SLC22A2* gene with gene location 6q25.3. They are involved in the intracellular uptake of metformin. The uptake of metformin in the hepatocytes by OCT1 is an essential step for the inhibition of hepatic gluconeogenesis and the glucose lowering effect of metformin.[3] Genetic variation in the *SLC22A1* gene (R61C, G401S, M420del, G465R), coding for the OCT1 transporter enzyme, is associated with differences in metformin blood levels and glucose levels after an oral glucose tolerance test in health volunteers.[3,7] Genetic variation in *SLC22A2* (T199I, T201M and A270S), coding for OCT2, is associated with differences in metformin blood levels and renal excretion.[8]

The third transporter involved in the distribution of metformin is the multidrug and toxin extrusion 1 (MATE1), encoded by the *SLC47A1* gene with gene location 17p11.2.[9,10] MATE1 is co-located with OCT1 and OCT2 in the hepatocytes and renal epithelium. MATE1 is involved in transportation of metformin out of the cell into the bile and urine.[11]

Recently, we identified a polymorphism in the *SLC22A1* gene, rs622342 A>C, and a polymorphism in the *SLC47A1* gene, rs2289669 G>A, which were associated with the glucose lowering effect of metformin.[12,13] In incident metformin users, the number of rs622342 minor C alleles was associated with a reduced glucose lowering effect, suggesting that this gene encodes an OCT1 less effective in transporting metformin into the hepatocytes, or in reduced transcription rates and less OCT1 expression resulting in a decreased transport of metformin into the hepatocytes. The number of rs2289669 minor A alleles was associated with an increased glucose lowering effect. Possibly, the gene with a minor A allele encodes a less effective MATE1 enzyme or lower numbers of MATE1, resulting in a reduced efflux out of the hepatocytes and higher intracellular metformin levels.

Although non-significant, the rs622342 C variant allele was associated with less metformin discontinuations. After tracking the general practitioner patient files, the most likely explanation was a lower incidence of adverse drug reactions. The rs2289669 A variant allele was associated with lower doses of co-prescribed sulfonylurea. Since both transporters are located in the hepatocyte, transporting metformin into and out of the hepatocyte, we

assessed whether there is interaction between the polymorphism rs622342 in the *SLC22A1* gene and rs2289669 in the *SLC47A1* gene and the response to metformin therapy in incident metformin users.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[14,15] The seven pharmacies in Ommoord dispense the prescriptions of more than 99 % of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[16]

For this study, we used the HbA1c assessments from the stichting trombosedienst en artsenlaboratorium rijnmond – medisch diagnostisch centrum (STAR-MDC), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam with a potential source population of more than 1 million inhabitants. Hereby, we obtained all outpatient HbA1c assessments from the participants between April 1st 1997, the time at which a new computer system was introduced at STAR-MDC, and January 1st 2008.

Study Sample

All participants in the Rotterdam Study, who were incident metformin users in the period between April 1st 1997 and January 1st 2008, were included in this analysis. Incident metformin use was defined as a first dispensed prescription for metformin in the database. The study sample consisted of all incident metformin users who had both a measurement of HbA1c in the period of 30 days before the first prescription of metformin and in the period between 14 and 100 days following the first prescription of metformin. Patients who discontinued metformin therapy before the first measurement after start were excluded. We also excluded patients who were co-prescribed acarbose, rosiglitazone, pioglitazone or insulin at the time of one of the two HbA1c measurements, because defined daily doses (DDD) for these drugs are not comparable to each other, and these patients most likely differ in their severity of disease. Patients using sulfonylurea were not excluded.

Outcomes

The goal of antihyperglycemic therapy is to reduce plasma glucose levels. The HbA1c level is the percentage of hemoglobin in the blood that is glycosylated and represents the average glucose level in the preceding period of time. Since the HbA1c level is a more stable measurement of glycemic control than plasma glucose levels, HbA1c levels are used more frequently for long-term therapeutic purposes. We analyzed the interaction between the polymorphism rs622342 and rs2289669 and difference in HbA1c level between the last HbA1c measurement before start of metformin therapy and the first HbA1c measurement after start. The goal of antidiabetic therapy is an HbA1c level below seven percent.[17]

Cofactors

Characteristics considered as potential determinants affecting the change in HbA1c level were age, gender, the HbA1c level at the last measurement before start of metformin, the daily prescribed dose of metformin at the time of the first measurement after start and the change in daily prescribed dose of sulfonylurea. To make the prescribed doses of different sulfonylurea comparable to each other, we divided the prescribed daily dose by the DDD. The DDD is a standardized dosing measure representing the recommended daily dose for the main indication in an adult.[16]

Genotyping

All participants were genotyped using the tagging single nucleotide polymorphisms (SNP) on the Illumina 550k SNP array for genotyping according to the manufacturer's instruction. The tagging SNPs on the array were selected using an algorithm with which in a Caucasian population ninety percent of the Hapmap SNPs are covered by at least one SNP on the array.[18-20] This coverage arises because genetic variation is transmitted in blocks, in which haplotype alleles exist. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. For this study we used the tagging SNPs rs622342 in the *SLC22A1* gene and rs2289669 in the *SLC47A1* gene, as previously described.[12,13]

Statistical Analysis

Deviations from Hardy-Weinberg equilibrium and differences in genotypes between patients who continued and discontinued metformin therapy were analyzed using χ^2-tests. Differences between genotypes in average time between the last HbA1c measurement before start and start of metformin therapy, and the average time between metformin start and the first HbA1c measurement between 14 and 100 days after start was tested using one-way ANOVA. Multivariate linear regression was used to analyze differences in HbA1c change between genotypes. We calculated the association between the number of variant alleles

and the difference in HbA1c change. The analysis for the rs622342 genotype was stratified for the rs2289669 genotype and vice versa. We tested for multiplicative interaction between the rs622342 and rs2289669 genotype in the multivariate linear regression model. These analyses were performed with SPSS software (version 15.0; SPSS, Chicago, IL).

RESULTS

In the Rotterdam Study, we identified 152 incident metformin users between April 1st 1997 and January 1st 2008, who had an HbA1c measurement available both in the period of 30 days before and in the period between 14 and 100 days after start of metformin therapy. We excluded 10 patients, because they were using acarbose (n=1) rosiglitazone (n=2), pioglitazone (n=1) or insulin (n=6) at the time of the HbA1c measurements. Twenty patients discontinued metformin therapy before the first HbA1c measurement after start of metformin therapy. Blood samples for genotyping were not available for twenty patients and genotyping for the SNP rs622342 or rs2289669 failed in four patients. Eventually, we included 98 incident metformin users in the analyses (table 1). The minor allele frequency was 0.37

Table 1 Baseline characteristics of the study population (n=98)

Characteristic		
Gender	Male	38 (39 %)
	Female	60 (61 %)
Age (SD)		76.3 (6.7) year
HbA1c level (SD) [a]		8.2 (1.2) %
Body-mass index (SD) [b]		28.1 (3.4) kg/m^2
Serum creatinine level (SD) [b]	(n=74)	83.0 (14.9) µmol/l
Sulfonylurea use [a]	Glibenclamide	14 (14 %)
	Tolbutamide	25 (26 %)
	Gliclazide	5 (5 %)
	Glimepiride	12 (12 %)
rs622342 genotype (OCT1)	AA	38 (39 %)
	AC	47 (48 %)
	CC	13 (13 %)
rs2289669 genotype (MATE1)	GG	31 (32 %)
	GA	48 (49 %)
	AA	19 (19 %)

[a] At the time of the last HbA1c measurement before start of metformin therapy. [b] At the time of entrance in the Rotterdam Study.

for the SNP rs622342 and 0.44 for the SNP rs2289669. Both genotype distributions were in Hardy-Weinberg equilibrium (rs622342 p=0.40; rs2289669 p=0.48). Genotype distributions did not differ between patients who continued metformin use until the first HbA1c measurement after start and those who discontinued (rs622342 p=0.34; rs2289669 p=0.36).

The average HbA1c level before start of metformin therapy was 8.2% (SD 1.2%). At the time of the first HbA1c measurement in the period between 14 and 100 days after start, the HbA1c level on average decreased by 0.36% (SD 0.70%) to 7.9% (SD 1.3%). The average time from the last HbA1c measurement before start and start of metformin therapy was 8 days (SD 5.8 days) and the average time from start of metformin therapy and the first measurement in the period between 14 and 100 days after start was 52 days (SD 23 days). These times did not differ significantly between genotypes.

In table 2, the average change in HbA1c level is given per genotype. The decrease in HbA1c levels was larger for each rs622342 A allele and rs2289669 A allele. In incident metformin users with the rs622342 AA and rs2289669 AA genotype the average decrease in HbA1c level was largest (-0.91 %; SD 0.78 %), while in users with the rs622342 CC and rs2289669 GG genotype the HbA1c levels increased (0.48%; SD 0.30 %). The change in HbA1c levels is visually presented in figure 1. In users with the rs622342 AA and AC genotype, the effect of the rs2289669 genotype is smaller than in users with the rs622342 CC genotype.

Table 2 The number of participants and the average change in HbA1c level (in %) per genotype

			rs2289669 (MATE1)			Overall
			GG	GA	AA	
rs622342 (OCT1)	AA	n	11	19	8	38
		Delta HbA1c (SD)	-0.46 (0.48)	-0.42 (0.58)	-0.91 (0.78)	-0.53 (0.62)
	AC	N	15	25	7	47
		Delta HbA1c (SD)	-0.11 (0.94)	-0.38 (0.68)	-0.60 (0.53)	-0.33 (0.76)
	CC	N	5	4	4	13
		Delta HbA1c (SD)	0.48 (0.30)	0.03 (0.28)	-0.58 (0.51)	0.02 (0.57)
Overall		n	31	48	19	98
		Delta HbA1c (SD)	-0.14 (0.78)	-0.36 (0.62)	-0.73 (0.64)	-0.36 (0.70)

The association between the number of variant alleles in the rs2289669 and the change in HbA1c level, stratified for the rs622342 genotypes, is given in table 3. In patients with the rs622342 AA genotype, the HbA1c level was on average 0.10 % lower (95% CI –0.35 , 0.14; p=0.39) with each rs2289669 minor A allele, while in patients with the rs622342 CC genotype the HbA1c level was on average 0.68% lower (95% CI –1.06 , –0.30; p=0.005). Testing for interaction between the rs622342 and rs2289669 genotype, revealed that the change in HbA1c level for the number of rs2289669 minor A alleles, differed significantly between patients

with the rs622342 AA and rs622342 CC genotype (–0.52 %; 95% CI –0.94 , –0.11; p=0.015). Analyzing the effect of the rs622342 genotype stratified for the rs2289669 genotype, did not add much to the presented analyses.

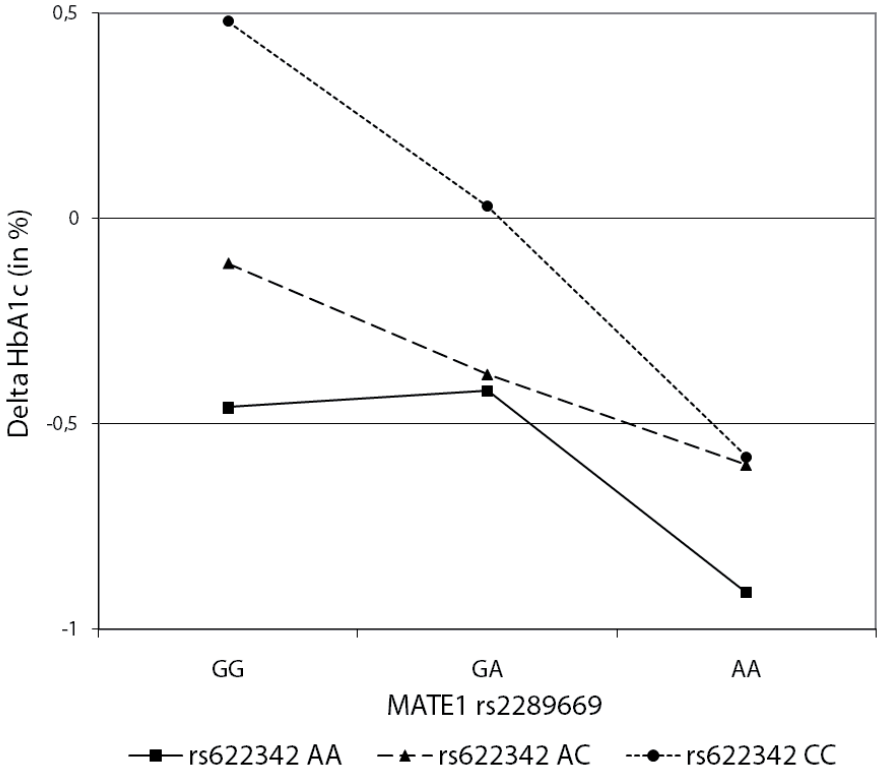


Figure 1 The average change in HbA1c level per genotype

Table 3 The average change in HbA1c level (in %) for the rs2289669 genotype (MATE1) stratified for the rs622342 genotype (OCT1)

OCT1 genotype	Change in HbA1c	95% CI	p-value	Interaction [a]	95% CI	p-value
rs622342 AA	-0.10	(-0.35, 0.14)	0.39	ref.		
rs622342 AC	-0.31	(-0.65, 0.03)	0.070	-0.11	(-0.53, 0.31)	0.60
rs622342 CC	**-0.68**	**(-1.06, -0.30)**	**0.005**	**-0.52**	**(-0.94, -0.11)**	**0.015**

[a] Multiplicative interaction; difference in slope, with the slope of the rs622342 AA genotype as the reference.

DISCUSSION

In two previous publications, we identified an association between polymorphisms in the gene coding for OCT1 (rs622342 A>C)[12] and MATE1 (rs2289669 G>A)[13], and response to metformin treatment. Most likely, the rs622342 C allele was associated with a crippled OCT1

influx transporter, either quantitative or qualitative, in the hepatocyte, and the rs2289669 A allele was associated with a crippled MATE1 efflux transporter. In the present study, we describe an interaction between these two SNPs. In patients homozygous for a normal functioning OCT1 influx transporter, genetic variation in the gene coding for the MATE1 efflux transporter did not affect the glucose lowering effect of metformin. On the other hand, in patients homozygous for a crippled functioning OCT1 influx transporter, genetic variation in the gene coding for the MATE1 efflux transporter has a substantial impact on the glucose lowering effect of metformin.

MATE1 is found both in the hepatocytes and in the renal epithelium. Probably, the hepatocytes are the major site of action of genetic variation in the gene coding for MATE1, because they interact with genetic variation in the gene coding for OCT1 that is particularly found in the hepatocytes. In incident metformin users homozygous for a normal functioning OCT1 influx transporter, the effect of genetic variation in the gene coding for the MATE1 efflux transporter is minimal. The normal functioning OCT1 influx transporter probably outperforms the MATE1 efflux transporter, irrespective of the functioning of the MATE1 efflux transporter, and the intracellular metformin levels in the hepatocyte will be high enough to reduce gluconeogenesis and to lower blood glucose levels. In patients homozygous for a crippled OCT1 influx transporter, two genes coding for a normal MATE1 efflux transporter outperform the influx of metformin, resulting in lower intracellular metformin levels and an impaired glucose lowering effect. However, if the patient is homozygous for both a crippled OCT1 influx transporter and a crippled MATE1 efflux transporter, the OCT1 influx transporter still outperforms the MATE1 efflux transporter, and the intracellular metformin levels in the hepatocyte are high enough to lower glucose levels. Therefore, the glucose lowering effect of metformin will be most impaired in patients with the rs622342 CC genotype, encoding a crippled OCT1 influx transporter and with the rs2289669 GG genotype, encoding a normal functioning MATE1 efflux transporter. Patients heterozygous for one of these genes will have a glucose lowering effects somewhere in between.

In this study we included all incident metformin users with an HbA1c measurement in the period of 30 days before start and between 14 and 100 days after start. The HbA1c level represents the glucose level in the preceding 90 days, although it mainly reflects the preceding two to four weeks before measurement. In the selected time period after start of metformin therapy the effect on HbA1c levels will not be completely established. However, the longer the time period the more changes in therapy will be made, possibly introducing bias. This is especially the case for the rs622342 polymorphism. The rs622342 A allele was associated with more metformin discontinuations and switches to acarbose, thiazolidinediones and insulin, although the differences did not reach statistical significance. Probably, this is due to a higher incidence of adverse drug reactions in patients with the rs622342 A allele. The results from the analyses of the first HbA1c measurement between 30 and 120 days after start of metformin did not differ much from the results presented in this article. However, the average reduction

in HbA1c in incident users with the rs622342 CC genotype was stronger than in the analyses presented here, while in incident users with the rs622342 AA or AC genotype the average decrease was similar. A possible explanation is the lower percentage of discontinuations due to adverse drug reactions in the users with the rs622342 CC genotype.

In population-based cohort studies, bias may affect the results. The HbA1c measurements were done in routine clinical practice. The time from start of metformin therapy until the HbA1c measurement did not differ between genotypes and therefore it is unlikely that there were differences in frequency of HbA1c measurements. In the selected time periods, no differences were found in genotype frequencies between patients who continued metformin therapy until the HbA1c measurement after start and those who discontinued metformin therapy. We identified all incident metformin users in the Rotterdam Study and information was collected prospectively without prior knowledge of the study hypothesis. The absence of blood samples for genotyping was most likely independent from the genetic variation analyzed in this study.

To conclude, the effect of the polymorphism rs2289669 in the gene coding for the MATE1 efflux transporter on the glucose lowering effect in incident metformin users is larger in patients with the rs622342 CC polymorphism in the gene coding for the OCT influx transporter, than in patients with the AA genotype. In patients with the AC genotype the effect of the rs2289669 polymorphism is in between. This interaction is most likely due to the OCT1 transporter transporting metformin into the hepatocyte and MATE1 transporting metformin out of the hepatocyte into the bile. The intracellular metformin concentrations will be sufficiently high to lower glucose levels in most individuals, except in those who have an impaired influx due to reduced functioning OCT1 influx transporter and a normal efflux with a normal functioning MATE1 efflux transporter.

REFERENCES

1. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med* 2002;137(1):25-33.
2. Hundal RS, Krssak M, Dufour S, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 2000;49(12):2063-9.
3. Shu Y, Brown C, Castro RA, et al. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
4. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* 2002;302(2):510-5.
5. Kimura N, Masuda S, Tanihara Y, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab Pharmacokinet* 2005;20(5):379-86.
6. Kimura N, Okuda M, Inui K. Metformin transport by renal basolateral organic cation transporter hOCT2. *Pharm Res* 2005;22(2):255-9.
7. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;117(5):1422-31.
8. Song I, Shin H, Shim E, et al. Genetic Variants of the Organic Cation Transporter 2 Influence the Disposition of Metformin. *Clin Pharmacol Ther* 2008:84(5):559-62.
9. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci U.S.A.* 2005;102(50):17923-8.
10. Terada T, Inui K. Physiological and pharmacokinetic roles of H+/organic cation antiporters (MATE/SLC47A). *Biochem Pharmacol* 2008;75(9):1689-96.
11. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 2007;74(2):359-71.
12. Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the Organic Cation Transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J*. Accepted 2009.
13. Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose lowering effect of metformin in patients with diabetes mellitus: a preliminary study. *Diabetes* 2009;58(3):745-9.
14. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
15. 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.
16. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 28 April 2008.
17. Standards of medical care in diabetes-2008. *Diabetes Care* 2008;31 Suppl 1:S12-54.
18. Sentrix® HumanHap550 Genotyping Beadchip. Illumina Inc. Available from http://www.illumina.com/downloads/HUMANHAP550_DataSheet.pdf. Accessed 29 february 2008
19. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74(1):106-20.
20. The International HapMap Project. *Nature* 2003;426(6968):789-96.

Chapter 3.5.

Common variation in the *NOS1AP* gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea

ABSTRACT

Background: The single nucleotide polymorphism rs10494366 in the *nitric oxide synthase 1 adaptor protein* (*NOS1AP)* gene is associated with QTc prolongation, through an effect on the intracellular Ca^{2+} levels. As sulfonylurea stimulate insulin secretion by an increased influx of Ca^{2+}, we hypothesized that this polymorphism is associated with the glucose lowering effect and mortality risk in sulfonylurea users.

Methods: Associations between the *NOS1AP* polymorphism, prescribed doses and mortality rates in sulfonylurea, metformin and insulin users were assessed in the Rotterdam Study, a population based cohort study of 7,983 elderly people.

Results: We identified 619 participants who were prescribed oral antidiabetic drugs during follow-up. In glibenclamide users carrying the TG genotype, the prescribed doses were higher compared with the glibenclamide users carrying the TT genotype (0.38 DDD units; 95% CI 0.14, 0.63). Glibenclamide users with the TG or GG genotype had an increased mortality risk compared with glibenclamide users with the TT genotype (HR 2.80; 95% CI 1.09, 7.22). Tolbutamide users with the TG or GG genotype (HR 0.30; 95% CI 0.14, 0.63) and glimepiride users with the TG or GG genotype (HR 0.18; 95% CI 0.04, 0.74) had a decreased mortality risk compared with tolbutamide and glimepiride users with the TT genotype.

Conclusion: In participants with the TG or GG genotype at rs10494366 in the *NOS1AP* gene, glibenclamide is less effective in reducing glucose levels and mortality rates were higher compared with glibenclamide users with the TT genotype. In tolbutamide and glimepiride users the TG and GG genotype were associated with a reduced mortality rate.

INTRODUCTION

Sulfonylurea have been used extensively for decades in the treatment of type 2 diabetes mellitus. Since the publication of the University Group Diabetes Program trial in 1970, in which tolbutamide treatment was compared with other treatments and placebo, sulfonylurea have been associated with an increased risk of cardiovascular mortality.[1] However, there was criticism on this study in subsequent publications.[2-4] In 1998 the results of another trial with sulfonylurea were published. In this UK Prospective Diabetes Study trial, in which treatment with sulfonylurea (chlorpropamide, glibenclamide or glipizide) was compared with insulin treatment and conventional policy with diet, no detrimental effects of sulfonylurea were seen.[5] Ever since, controversy remains as to whether sulfonylurea may increase the risk of cardiovascular death.

Sulfonylurea stimulate insulin secretion by the pancreatic β cells.[6-8] The sulfonylurea receptor (SUR) is part of the ATP-sensitive K^+ (K_{ATP}) channel. Binding of the sulfonylurea to SUR causes inhibition of the K_{ATP}-channel, decreasing the K^+ efflux and depolarization of the cell membrane. This triggers the opening of voltage dependent Ca^{2+} channels, eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+}. In the pancreatic β cell, this rise stimulates the exocytosis of insulin-containing secretory granules.

Nitric oxide synthases (NOS) are the enzymes responsible for nitric oxide generation. Nitric oxide regulates cardiovascular homeostasis.[9] Recently, two nearby single nucleotide polymorphisms (SNP) rs10494366 and rs10918594 in the gene encoding nitric oxide synthase 1 adaptor protein (NOS1AP) have been found to be associated with QTc-interval prolongation in electrocardiograms.[10-12] NOS1AP is a regulator of neuronal NOS (nNOS encoded by NOS1), one of the isoforms of NOS. The nNOS enzyme is believed to regulate intracellular calcium levels.[9,13] It is thought that nNOS inhibits the inward Ca^{2+} current through voltage dependent calcium channels, reducing the intracellular calcium concentrations. Thereby it suppresses β-adrenoreceptor stimulation of the heart. nNOS has also been associated with insulin release.[14,15]

Similarities exist between the effects of nNOS and sulfonylurea. Both nNOS and sulfonylurea influence the calcium influx through voltage dependent calcium channels. Moreover nNOS and sulfonylurea modulate the release of insulin by pancreatic β cells. Both might be associated with cardiovascular mortality. In view of these similarities we hypothesized that genetic variation in the *NOS1AP* gene influences the glucose-lowering effect of sulfonylurea and mortality risk in patients using sulfonylurea.

METHODS

Setting

The data were obtained from the Rotterdam Study, a prospective population-based, closed cohort study in the suburb Ommoord in Rotterdam. All inhabitants who were 55 years of age or older and had lived in the district for at least one year were invited between 1990 and 1993 to participate in the study. Of the 10,275 eligible persons, 7,983 participated and were followed since then. At baseline, trained interviewers administered a questionnaire during a home interview covering socioeconomic background and medical history, among other topics. During subsequent visits to the study center, laboratory assessments and clinical examinations were performed, including recording of electrocardiograms. Follow-up examinations were carried out periodically (every four to five years). All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases. The design of the Rotterdam Study has been described before.[16,17] All mortality cases were identified by obtaining the vital status of the participants from the municipal population registry at regular intervals. After notification of death, cause and circumstances were established by information from the general practitioner, letters, 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-tenth edition.[18] In case of disagreement, consensus was sought. The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2005 was available and included the product name of the drug, the anatomical therapeutical chemical code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[19]

Cohort Definition

All participants of the Rotterdam Study, who received a prescription for an oral antidiabetic drug in the period between January 1st 1991 and January 1st 2005, were included in the study population at the time of the first prescription. These subjects were followed until death or end of the study period whichever came first.

Outcomes

Associations between the SNPs rs10494366 and rs10918594 in the *NOS1AP* gene, and prescribed doses of sulfonylurea, all-cause and cardiovascular mortality and first myocardial infarction were assessed. We used two types of study outcome, the change in prescribed dose of oral antidiabetic drugs compared with the first prescription and mortality while using antidiabetic drugs.

First, we analyzed differences between genotypes in prescribed doses in incident oral antidiabetic drug users. All subjects who received a first prescription for a sulfonylureum after July 1st 1991 were included in this analysis and followed until the last prescription of that particular antidiabetic drug. July 1st 1991 was chosen to ensure that we would have complete medication histories for at least half a year from January 1st 1991. For every prescription of the oral antidiabetic drug the participant started on, the change in prescribed daily dose compared with the first prescription was calculated. As doses are titrated to avoid hypoglycemia and diabetes mellitus is a progressive disease, the prescribed doses of oral antidiabetic drugs usually increase over time. The prescribed daily dose is given as the number of defined daily dose (DDD) units, established by the World Health Organization, to make prescribed doses comparable between different drugs.[19] If participants received more than one oral antidiabetic drug, the number of DDD units of the other drugs was added to the drug that was prescribed first.

Second, we analyzed differences in all-cause mortality between genotypes within users of the same antidiabetic drug. We also analyzed differences in mortality in patients using metformin and insulin. Subsequent analysis was restricted to events that were coded as cardiovascular mortality. Similarly, we analyzed differences in the risk for a first (fatal and nonfatal) myocardial infarction.

Cofactors

The following characteristics were considered as potential determinants for affecting the change in prescribed daily dose of sulfonylurea after start: age, sex and calendar time. Determinants potentially affecting the mortality rates were age, sex, QTc interval at baseline, the cumulative prescribed dose of all oral antidiabetic drugs at the index date, the number of days the sulfonylureum of interest was prescribed until the index date, and whether the participant used insulin at the index date. We also adjusted for current dihydropyridine calcium channel blocker use, because we recently found an association between genetic variation in *NOS1AP* and mortality in dihydropyridine calcium channel blocker users. The time of entrance in the Rotterdam Study was regarded as baseline and the results of physical examinations at the first visit were used in the analysis.

Genotyping

All participants were genotyped for the *NOS1AP* SNP rs10494366 T>G previously shown to be associated with QTc interval in five independent samples.[10-12] The correlated SNP rs10918594 C>G, was also genotyped. These two SNPs are in linkage disequilibrium (r^2=0.63, D'=0.89). Both were genotyped using Taqman assays C_1777074_10 and C1777009_10 (Applied Biosystems, Foster City, Ca., USA) in 1 ng of genomic DNA extracted from leukocytes, as previously reported.[10,20]

Statistical analysis

A χ^2 test was used to test for deviation from Hardy-Weinberg equilibrium. We used unbalanced repeated measurements analysis to analyze the difference per genotype in the change in prescribed daily dose (in DDD units) in series of all consecutive prescriptions of oral antidiabetic drugs for the same participant compared with the prescribed daily dose of the first prescription. For these analyses, we used the PROC Mixed module of SAS (version 8.2; SAS, Cary, NC). Cox proportional hazards analysis was used to analyze the difference in mortality between genotypes in users of the same antidiabetic drug. For each antidiabetic drug, all subjects in the study population who died between July 1st 1991 and January 1st 2005, while using that antidiabetic drug were identified as cases. The mortality date was taken as the index date. To each case we matched all persons in the cohort using that antidiabetic drug on the index date of the corresponding case. Participants with missing values were excluded from the analyses. Cox proportional hazards analysis was also used for analyzing differences in first myocardial infarction between genotypes. These analyses were performed using SPSS software (version 11.0.1; SPSS, Chicago IL).

RESULTS

In the Rotterdam Study, we identified 784 subjects who were prescribed oral antidiabetic drugs. One hundred thirty-four participants were excluded because a blood sample was not available and 31 participants were excluded because of failure to genotype successfully. Consequently, 619 participants were available for the analysis (table 1). We analyzed the associations between both SNPs rs10494366 and rs10918594 and the study outcomes. As the associations with the SNP rs10494366 were stronger, only these results are presented. The minor allele frequency was 0.38 (G allele) and genotype distribution was in the Hardy-Weinberg equilibrium (χ^2=1.94; p=0.16).

Four hundred fifty-two participants received a first prescription for sulfonylurea between July 1st 1991 and January 1st 2005, and these patients were considered as incident users. No significant differences were observed in starting dose among the genotypes. The average increase in prescribed daily dose for all consecutive prescriptions compared with the first prescriptions is given in table 2. Among 74 patients using glibenclamide, patients with the TG genotype received on average a prescribed daily dose that was 0.38 DDD higher (95% CI 0.14, 0.63) than patients with the TT genotype. The difference between patients with the GG genotype and the TT genotype was not significantly different (0.11 DDD; 95% CI –0.32, 0.55). The change in prescribed daily dose for consecutive prescriptions of glibenclamide is given in figure 1. Patients with the GG genotype starting on glibenclamide were on average given fewer prescriptions for glibenclamide than patients with the TG genotype (20.4 versus 27.4; p=0.04). For the other sulfonylurea, no differences in prescribed doses were found.

Table 1 Characteristics of the study population by *NOS1AP* rs10494366 genotype

rs10494366 genotype	TT	TG	GG
Number	247	275	97
Gender, male	103 (41.7%)	118 (42.9%)	44 (45.4%)
Age (SD)	69.7 (8.3) years	69.1 (7.9) years	69.8 (8.5) years
Follow-up time (SD)	11.1 (3.3) years	11.0 (3.7) years	10.5 (4.0) years
Body mass index (SD)	28.0 (3.6) kg/m^2	28.2 (3.8) kg/m^2	28.6 (4.5) kg/m^2
Serum creatinine (SD)	85.0 (16.1) µmol/l (n=198)	84.9 (17.0) µmol/l (n=213)	84.3 (17.9) µmol/l (n=70)
Drug use during follow up			
Glibenclamide	87 (35.2%)	109 (39.6%)	37 (38.1%)
Tolbutamide	137 (55.5%)	155 (56.4%)	55 (56.7%)
Gliclazide	43 (17.4%)	41 (14.9%)	10 (10.3%)
Glimepiride	56 (22.7%)	77 (28.0%)	23 (23.7%)
Metformin	141 (57.1%)	165 (60.0%)	55 (56.7%)
Insulin	49 (19.8%)	62 (22.5%)	19 (19.6%)

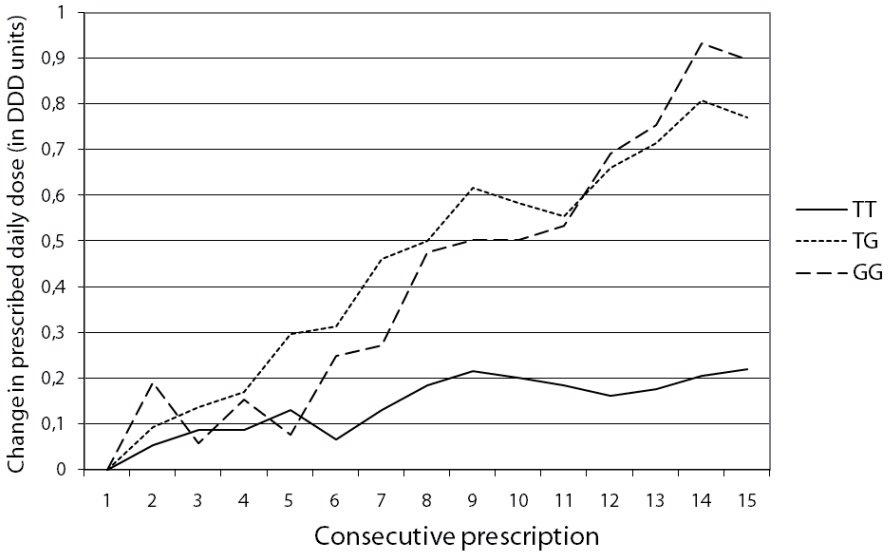


Figure 1 Change in prescribed daily dose (in DDD units) of glibenclamide plus co-prescribed oral antidiabetic drugs in patients starting with glibenclamide compared to the first prescription

Table 2 Average change in prescribed dose of oral antidiabetic drugs (in DDD units) in incident sulfonylurea users by rs10494366 genotype

Drug	TT		TG				GG			
	N		N	Change [a]	95% CI	p-value	N	Change [a]	95% CI	p-value
Glibenclamide	32	ref.	28	**0.38**	**(0.14, 0.63)**	**0.003**	14	0.11	(-0.32, 0.55)	0.60
Tolbutamide	115	ref.	116	-0.02	(-0.12, 0.08)	0.66	50	-0.05	(-0.19, 0.08)	0.43
Gliclazide	12	ref.	11	0.05	(-0.45, 0.55)	0.84	2	0.37	(-0.65, 1.39)	0.44
Glimepiride	24	ref.	41	0.16	(-0.22, 0.53)	0.40	7	0.26	(-0.43, 0.95)	0.44

[a] Adjusted for: age, sex and calendar time.

In the cohort of 619 subjects, 156 subjects died during follow-up while using antidiabetic drugs. In 142 of the 156 cases we had complete follow-up information, including QTc interval, and these subjects were used for the mortality analyses. In the group of glibenclamide users, both users with the TG genotype (Hazard Ratio (HR) 2.95; 95% CI 1.02, 8.52) and users with the GG genotype (HR 4.42; 95% CI 1.23, 15.9) had a higher mortality rate than users with the TT genotype (table 3). For tolbutamide (TG genotype HR 0.26; 95% CI 0.11, 0.59 and GG genotype HR 0.27; 95% CI 0.09, 0.27) and glimepiride (TG genotype HR 0.15; 95% CI 0.05, 0.75) the ef-

Table 3 Association between the polymorphism rs10494366 in the *NOS1AP* gene and all-cause mortality in sulfonylurea users

		Cases [a]	Unadjusted	Adjusted [b]		
			HR	HR	95% CI	p-value
Glibenclamide						
	TT	6	ref.	ref.		
	TG	15	2.30	**2.95**	**(1.02, 8.52)**	**0.046**
	GG	8	2.97	**4.42**	**(1.23, 15.9)**	**0.022**
Tolbutamide						
	TT	21	ref.	ref.		
	TG	13	0.34	**0.26**	**(0.11, 0.59)**	**0.001**
	GG	6	0.48	**0.27**	**(0.09, 0.87)**	**0.028**
Gliclazide						
	TT	9	ref.	ref.		
	TG	3	0.41	0.32	(0.03, 3.27)	0.33
	GG	0	- [c]	- [c]		
Glimepiride						
	TT	9	ref.	ref.		
	TG	7	0.50	**0.15**	**(0.05, 0.75)**	**0.021**
	GG	2	- [c]	- [c]		
Metformin						
	TT	10	ref.	ref.		
	TG	12	0.86	0.82	(0.33, 2.06)	0.68
	GG	7	1.45	1.65	(0.59, 4.55)	0.34
Insulin						
	TT	16	ref.	ref.		
	TG	22	1.03	1.00	(0.48, 2.06)	1.00
	GG	8	0.90	1.23	(0.41, 3.68)	0.71

[a] As patients can use more than one antidiabetic drug, these numbers do not add up to 142. [b] Adjusted for: age, sex, QTc time, prescribed doses oral antidiabetic drugs, duration of use, insulin use and dihydropyridine calcium channel blocker use. [c] Too few cases were available to calculate HR's.

fects were the opposite. Participants on these drugs with the TG and GG genotype had lower mortality rates, although the number of participants with the GG genotype using glimepiride was too small to calculate hazard rates. In gliclazide users, a nonsignificant protective effect for the TG genotype was found (TG genotype HR 0.32; 95% CI 0.03, 3.27). No associations were found between all-cause mortality and *NOS1AP* genotype in metformin or insulin users.

In 63 of the 142 participants who died during follow-up, the cause of death was categorized as cardiovascular. In table 4 the associations between *NOS1AP* genotype and cardiovascular mortality are given. Since the hazard estimates for the all-cause mortality are suggestive of a dominant effect of the G allele and the power is too low for separate analyses, we grouped patients with the TG and GG genotypes. In glibenclamide users, no differences were found in cardiovascular mortality between genotypes (HR 1.45; 95% CI 0.34, 6.19). With tolbutamide, the decreased mortality in users with the TG or GG genotype seemed to be caused by a decrease in cardiovascular mortality (HR 0.09; 95% CI 0.02, 0.40). For metformin and insulin, no differences in cardiovascular mortality were found.

Table 4 Association between the polymorphism rs10494366 in the *NOS1AP* gene and cardiovascular mortality in sulfonylurea users

	All-cause mortality				Cardiovascular mortality			
	N	HR [a]	95% CI	p-value	N [b]	HR [a]	95% CI	p-value
Glibenclamide								
TT	6	ref.			4	ref.		
TG or GG	23	**2.80**	**(1.09, 7.22)**	**0.033**	8	1.45	(0.34, 6.19)	0.61
Tolbutamide								
TT	21	ref.			11	ref.		
TG or GG	19	**0.30**	**(0.14, 0.63)**	**0.001**	**6**	**0.09**	**(0.02, 0.40)**	**0.002**
Gliclazide								
TT	9	ref.			5	ref.		
TG or GG	3	0.23	(0.02, 2.34)	0.21	0	- [c]		
Glimepiride								
TT	9	ref.			3	ref.		
TG or GG	9	**0.18**	**(0.04, 0.74)**	**0.018**	3	- [c]		
Metformin								
TT	10	ref.			5	ref.		
TG or GG	19	1.12	(0.50, 2.51)	0.79	7	1.10	(0.29, 4.23)	0.89
Insulin								
TT	16	ref.			7	ref.		
TG or GG	30	1.03	(0.52, 2.01)	0.94	14	1.23	(0.43, 3.50)	0.70

[a] Adjusted for: age, sex, QTc time, prescribed doses oral antidiabetic drugs, duration of use, insulin use and dihydropyridine calcium channel blocker use. [b] As patients can use more than one antidiabetic drug, these numbers do not add up to 63. [c] Too few cases were available to calculate HR's.

Forty-nine cases of myocardial infarction were identified in participants using antidiabetic drugs. The number of first myocardial infarctions in participants using glibenclamide was too low to examine. In the group of participants using tolbutamide, gliclazide or glimepiride, the

hazard ratio for a myocardial infarction was 0.89 (95% CI 0.27, 2.97) for users with the TG or GG genotype compared with users with the TT genotype, albeit only 12 cases were identified. For participants using insulin, the hazard ratio was 1.34 (95% CI 0.48, 3.73).

DISCUSSION

In this population-based cohort study, the glucose-lowering response of glibenclamide seems to be less effective in users with the TG or GG genotype, because over time their daily dose is significantly higher than in users with the TT genotype. Moreover, for all sulfonylurea differences were found in mortality between patients with the TG or GG genotype and patients with the TT genotype. The effects of glibenclamide on mortality were opposite to the other sulfonylurea. In participants using glibenclamide, the TG and GG genotype were associated with an increased risk of mortality, whereas in participants using tolbutamide and glimepiride, these genotypes were associated with a reduced risk of mortality. No differences were found in subjects using metformin or insulin.

Participants with the TG genotype using glibenclamide were prescribed higher doses than subjects with the TT genotype. As prescribed doses are titrated according to glucose levels, it is likely that this is caused by a difference in glucose-lowering effect. Participants with the GG genotype starting on glibenclamide stopped using glibenclamide sooner than subjects with the TG genotype. This may explain why no difference in the average prescribed dose was found for users with the GG genotype, although the changes in prescribed dose for users with the TG and GG genotype are similar in figure 1.

As the hazard rates for the TG and GG genotypes are similar, we suggested a dominant effect of the G allele. If the underlying genetic effect operated through a recessive or additive effect, larger differences between the TG and GG would be expected. In the analyses of cardiovascular mortality we analyzed participants with the TG or GG genotype as one group, because numbers were too low to analyze them separately. Only in participants with the TG or GG genotype using tolbutamide, a decreased hazard rate for cardiovascular mortality was found.

The reduced all-cause mortality observed in subjects with the TG or GG genotype using tolbutamide and glimepiride may be caused by *NOS1AP* influencing the pharmacologic pathway of sulfonylurea. In metformin and insulin users no differences were found, indicating that the differences are related to sulfonylurea use and not to the underlying disease. Also in gliclazide users a reduced mortality was observed, although not significant. Although the wide confidence intervals preclude a precise estimation, a two to three times larger sample size would be needed for the HR in this study to become significantly different. Both NOS1AP and sulfonylurea regulate the Ca^{2+} influx by voltage dependent calcium channels. Sulfonylurea stimulate Ca^{2+} influx by blocking the K_{ATP}-channels, whereas the exact mechanism of

nNOS is not known. In subjects with the TG or GG genotype using tolbutamide a reduced risk of cardiovascular mortality was seen. In participants with the TG or GG genotype using gliclazide and glimepiride, a reduction in cardiovascular mortality was the most likely explanation for the reduced all-cause mortality, although the differences were not significant. These effects on cardiovascular mortality in participants using tolbutamide, gliclazide and glimepiride may be caused by the effect sulfonylurea have on the heart. More than one isoform of the SUR exist.[8,21-23] The SUR1 isoform is found in the pancreas, the SUR2A isoform in the heart and skeletal muscle and the SUR2B isoform in vascular smooth muscles. The glucose-lowering effect of sulfonylurea is accomplished by binding to the SUR1 receptor on the β cell. Sulfonylurea also bind to other SUR isoforms. It is suggested that the affinity to the SUR2A isoform could be responsible for the effects on cardiovascular mortality.[21,24-26] Under normal conditions the K_{ATP} channels in the heart are closed. They open in response to metabolic stress such as ischemia, and the increasing total outward K^+ current shortens the action potential duration, decreases Ca^{2+} influx and contraction, and conserves ATP. These channels are involved in a phenomenon called ischemic preconditioning. This refers to the observation that a brief period of ischemia may render a less severe, subsequent, and more prolonged episode.[26,27] Binding to the SUR2A isoform by sulfonylurea may block this ATP conserving pathway and possibly influences survival of ischemic events. Mutations in the gene encoding the SUR2A gene have been associated with heart failure and rhythm disturbances, confirming the importance of K_{ATP} channels and SUR2A.[28] In a study of 185 patients undergoing direct coronary angioplasty for acute myocardial infarction, sulfonylurea use was associated with an increased mortality.[29]

The effects observed in participants using glibenclamide were different from that observed in participants using other sulfonylurea. Glibenclamide has a higher affinity for the SUR2A receptor than the other sulfonylurea.[8,22,24,30-32] This difference in affinity by glibenclamide for the SUR2A receptor cannot explain all the results. Since SUR2A is only found on cardiac tissue, no differences would be expected in prescribed doses.

Glibenclamide is also an inhibitor of other channels than the K_{ATP} channel.[33,34] Studies have shown that beside the K_{ATP} channel, other potassium channels are present in the β cell, such as the Ca^{2+}-dependent K^+ channel.[35,36] Blocking one or more of these channels by glibenclamide may be an alternative explanation for the results found in this study. A possible explanation for our results may be that there is a difference in effect on the Ca^{2+}-dependent K^+ channel between glibenclamide and other sulfonylurea. This explanation is supported by two observations. First, Ca^{2+}-dependent K^+ channel are also found in the pancreatic β cell, influencing the firing of action potentials and possibly insulin release. Second, nitric oxide directly activates these Ca^{2+}-dependent K^+ channels, which could explain the role of NOS1AP.[37] As we are not aware of studies assessing the influence of other sulfonylurea than glibenclamide on Ca^{2+}-dependent K^+ channels, we do not know whether differences in blocking

these channels do attribute to the differences between glibenclamide and other sulfonylurea found in this study.

Although nNOS has previously been associated with insulin release, we do not think that this association can explain the differences in prescribed doses and mortality risk in sulfonylurea users. The association with insulin release was too weak to explain the results and the associations were not found for metformin and insulin, suggesting that the association is related to sulfonylurea. As we adjusted for the QTc interval, also the QTc prolongating effect of NOS1AP is less likely to explain the observed results.

In population-based studies, bias may affect the obtained results. We believe that bias in our study is minimal. As diabetes mellitus is a progressive disease, co-prescription of other antidiabetic drugs and switching is common. Confounding by indication may have occurred if the risks at the start of a drug were different between genotypes, owing to differences in the effect of previously prescribed drugs. This is, for example, the case if the genotype influences the rate of switching or co-prescription during previously prescribed drugs. However, if we adjusted for previously prescribed sulfonylurea, the results did not change. Therefore we do not think that confounding by indication did influence our results. Information bias is unlikely, since information was collected prospectively without prior knowledge of the study hypothesis. It is also unlikely that selection bias has occurred since we identified all patients with diabetes mellitus in a population based cohort study, and the absence of a blood sample and difficulties with genotyping were probably independent of the genotype.

Although there is always the possibility that the results are a chance finding, we think that this is probably not the case in our study. First, the analyses were not part of a genome wide association study. The SNP rs10494366 was associated with QTc prolongation in five independent populations before and we were testing whether this SNP affected prescribed doses and all-cause mortality in sulfonylurea users. Therefore, multiple testing did not bias our results. Second, significant associations with all-cause mortality were found for tolbutamide and glimepiride, whereas no significant associations were found for metformin and insulin. The point estimate for gliclazide was similar to the point estimate for tolbutamide and glimepiride, although not significant. Probably, this was because of lack of power in this group. For glibenclamide, we also found an association with all-cause mortality, although opposite to the effects of the other sulfonylurea. Differences in effect between glibenclamide and other sulfonylurea were observed before, although the differences were ascribed to differences in the affinity to the SUR2A receptor.

To conclude, the glucose-lowering effect of glibenclamide in patients with the TG or GG genotype seems to be less effective. Moreover, genetic variation in the *NOS1AP* gene seems to predict the risk of mortality in patients using sulfonylurea. Although the exact mechanism has not been revealed, our results give a new insight into the pharmacologic association between sulfonylurea use and cardiovascular mortality.

REFERENCES

1. Klimt CR, Knatterud GL, Meinert CL, Prout TE. A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. *Diabetes* 1970;19:Suppl:747-830.
2. Schor S. The University Group Diabetes Program. A statistician looks at the mortality results. *JAMA* 1971;217(12):1671-5.
3. Seltzer HS. A summary of criticisms of the findings and conclusions of the University Group Diabetes Program (UGDP). *Diabetes* 1972;21(9):976-9.
4. Cornfield J. The University Group Diabetes Program. A further statistical analysis of the mortality findings. *JAMA* 1971;217(12):1676-87.
5. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998;352(9131):837-53.
6. Ashcroft FM, Rorsman P. Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 1989;54(2):87-143.
7. Boyd AE, Aguilar-Bryan L, Nelson DA. Molecular mechanisms of action of glyburide on the beta cell. *Am J Med* 1990;89(2A):3S-10S
8. Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. *Diabetes* 2002;51 Suppl 3:S368-76.
9. Schulz R, Rassaf T, Massion PB, Kelm M, Balligand JL. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005;108(3):225-56.
10. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006;38(6):644-51.
11. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, et al. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. *Circulation* 2007;116(1):10-6.
12. Post W, Shen H, Damcott C, et al. Associations between genetic variants in the NOS1AP (CAPON) gene and cardiac repolarization in the old order Amish. *Hum Hered* 2007;64(4):214-9.
13. Massion PB, Pelat M, Belge C, Balligand JL. Regulation of the mammalian heart function by nitric oxide. *Comp Biochem Physiol A Mol Integr Physiol* 2005;142(2):144-50.
14. Lajoix AD, Reggio H, Chardes T, et al. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. *Diabetes* 2001;50(6):1311-23.
15. Gunawardana SC, Rocheleau JV, Head WS, Piston DW. Mechanisms of time-dependent potentiation of insulin release: involvement of nitric oxide synthase. *Diabetes* 2006;55(4):1029-33.
16. Hofman A, Breteler MMB, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):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(4):403-22.
18. World Health Organization. International statistical classification of diseases and related health problems. Tenth revision. Geneva: World Health Organization; 1992.
19. Complete ATC index 2007. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 30 October 2007.
20. Fang Y, van Meurs JB, d'Alesio A, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet* 2005;77(5):807-23.
21. Yokoshiki H, Sunagawa M, Seki T, Sperelakis N. ATP-sensitive K+ channels in pancreatic, cardiac, and vascular smooth muscle cells. *Am J Physiol* 1998;274(1 Pt 1):C25-37.

22. Song DK, Ashcroft FM. Glimepiride block of cloned beta-cell, cardiac and smooth muscle K(ATP) channels. *Br J Pharmacol* 2001;133(1):193-9.
23. Dorschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M. Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. *Mol Pharmacol* 1999;55(6):1060-6.
24. Ashcroft FM, Gribble FM. Tissue-specific effects of sulfonylureas: lessons from studies of cloned K(ATP) channels. *J Diabetes Complications* 2000;14(4):192-6.
25. Venkatesh N, Lamp ST, Weiss JN. Sulfonylureas, ATP-sensitive K+ channels, and cellular K+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ Res* 1991;69(3):623-37.
26. Engler RL, Yellon DM. Sulfonylurea KATP blockade in type II diabetes and preconditioning in cardiovascular disease. Time for reconsideration. *Circulation* 1996;94(9):2297-301.
27. Brady PA, Terzic A. The sulfonylurea controversy: more questions from the heart. *J Am Coll Cardiol* 1998;31(5):950-6.
28. Sattiraju S, Reyes S, Kane GC, Terzic A. K(ATP) channel pharmacogenomics: from bench to bedside. *Clin Pharmacol Ther* 2008;83(2):354-7.
29. Garratt KN, Brady PA, Hassinger NL, Grill DE, Terzic A, Holmes DR, Jr. Sulfonylurea drugs increase early mortality in patients with diabetes mellitus after direct angioplasty for acute myocardial infarction. *J Am Coll Cardiol* 1999;33(1):119-24.
30. Lee TM, Chou TF. Impairment of myocardial protection in type 2 diabetic patients. *J Clin Endocrinol Metab* 2003;88(2):531-7.
31. Legtenberg RJ, Houston RJ, Oeseburg B, Smits P. Effects of sulfonylurea derivatives on ischemia-induced loss of function in the isolated rat heart. *Eur J Pharmacol* 2001;419(1):85-92.
32. Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. *Circulation* 2001;103(25):3111-6.
33. Rosati B, Rocchetti M, Zaza A, Wanke E. Sulfonylureas blockade of neural and cardiac HERG channels. *FEBS Lett* 1998;440(1-2):125-30.
34. Schaffer P, Pelzmann B, Bernhart E, et al. The sulphonylurea glibenclamide inhibits voltage dependent potassium currents in human atrial and ventricular myocytes. *Br J Pharmacol* 1999;128(6):1175-80.
35. Kanno T, Rorsman P, Gopel SO. Glucose-dependent regulation of rhythmic action potential firing in pancreatic beta-cells by K(ATP)-channel modulation. *J Physiol 2002*;545(Pt 2):501-7.
36. Gopel SO, Kanno T, Barg S, et al. Activation of Ca(2+)-dependent K(+) channels contributes to rhythmic firing of action potentials in mouse pancreatic beta cells. *J Gen Physiol* 1999;114(6):759-70.
37. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* 1994;368(6474):850-3.

Chapter 4.

Genetic factors affecting cardiovascular pharmacotherapy

Chapter 4.1.

Common genetic variation in the *ABCB1* gene is associated with the cholesterol lowering effect of simvastatin in males

ABSTRACT

Background: The cholesterol lowering drug simvastatin is a substrate for P-glycoprotein (P-gp). P-gp, encoded by *ABCB1*, is an efflux transporter and genetic variation in *ABCB1* is associated with drug levels and response. We studied in the Rotterdam Study, a population-based cohort study, whether the C1236T, G2677TA and C3435T polymorphisms and haplotypes in the *ABCB1* gene are associated with the total cholesterol and LDL cholesterol lowering effect of simvastatin.

Methods: We identified 85 incident simvastatin users, for whom a cholesterol measurement both before and after start of simvastatin therapy was available. Associations between *ABCB1* gene variants and reductions in cholesterol levels were analyzed. We stratified in our analysis for gender, because the level of P-gp expression in the liver is higher in men than in women.

Results: The three *ABCB1* polymorphisms were associated with total cholesterol reduction in the whole population. In men, both the 1236/2677/3435 TTT haplotype and the CGT haplotype were associated with larger reductions in total cholesterol (TTT -0.40 mmol/l 95% CI -0.63, -0.17; CGT -0.44 mmol/l 95% CI -0.77, -0.11) and LDL cholesterol levels (TTT -0.51 mmol/l 95% CI -0.81, -0.22; CGT -0.53 mmol/l 95% CI -0.92, -0.15) than the reference CGC haplotype. In women, genetic variation in the *ABCB1* gene was not associated with total and LDL cholesterol levels.

Conclusion: Male simvastatin users with the *ABCB1* 1236/2677/3435 TTT and CGT haplotype have larger reductions in total cholesterol and LDL cholesterol compared to the wildtype CGC haplotype. For women, no associations were found.

INTRODUCTION

Statins are widely used in the treatment of hypercholesterolemia. They inhibit the enzyme HMG-CoA reductase, which is involved in the synthesis of cholesterol. Inhibition results in a decrease in total cholesterol and LDL cholesterol levels and a reduction in morbidity and mortality.[1] The reduction in triglyceride levels is small. In general, statins are safe and effective in lowering total and LDL cholesterol levels, although they have the potential to cause myopathy and rhabdomyolysis.[2]

Transporters are involved in the carriage of drugs and other substances over membranes. One of these transporters is P-glycoprotein (P-gp), which is involved in the efflux of drugs such as digoxin and ciclosporin.[3,4] P-gp is mainly found in the liver, small intestine and blood-brain barrier.[5,6] The hepatic expression of P-gp is stronger in males than in females.[7] Simvastatin, a commonly used statin, is a substrate for P-gp.[8-10] Reduced P-gp activity may result in increased plasma levels due to a decrease in simvastatin efflux out of the body in the small intestine and liver.

The P-gp transporter is encoded by the *ATP-binding cassette B1* (*ABCB1*) gene with gene location 7q21.12, previously known as *multidrug resistance 1 (MDR1)* gene. Many single nucleotide polymorphisms (SNP) have been identified in the *ABCB1* gene.[11] It is established that three SNPs (C1236T, G2677TA and C3435T) affect the drug transporter function of P-gp, although less is known about the effect on the cholesterol lowering effect of simvastatin.[5,12-14]

In this population-based cohort study, we analyzed the association between these three SNPs and haplotypes in the *ABCB1* gene and reduction in total cholesterol and LDL cholesterol levels after start of simvastatin therapy.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[15,16] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included

the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[17]

For this study, we used the total cholesterol and LDL cholesterol assessments from the stichting trombosedienst en artsenlaboratorium rijnmond – medisch diagnostisch centrum (STAR-MDC), which performs all outpatient laboratory assessments for general practitioners in the Rijnmond area of Rotterdam with a potential source population of more than 1 million inhabitants. Hereby, we obtained all outpatient total cholesterol and LDL cholesterol assessments from all participants between April 1st 1997, the time at which a new computer system was introduced at STAR-MDC, and June 1st 2008.

Study Sample

All participants in the Rotterdam Study, who were incident simvastatin users in the period between April 1st 1997 and June 1st 2008, were included in this analysis. Incident simvastatin use was defined as a first dispensed prescription for simvastatin in the database, without prior prescriptions for other statins. The study sample consisted of all incident simvastatin users who had a measurement of total cholesterol and/or LDL cholesterol level in the period of 180 days before the first prescription of simvastatin and in the period between 7 and 180 days following the first prescription of simvastatin. Patients who discontinued simvastatin therapy before the first measurement after start were excluded. We also excluded patients who were co-prescribed fibrates, bile acid sequestrants, nicotinic acids or ezetimibe at the time of one of the measurements.

Outcomes

Simvastatin reduces the total and LDL cholesterol levels. The goal of simvastatin therapy is a LDL cholesterol level below 2.6 mmol/l in patients with coronary heart disease and below 3.4 mmol/l in high risk patients.[18,19] We analyzed the association between the SNPs C1236T, G2677TA and C3435T in the *ABCB1* gene and derived haplotypes, and reductions in total cholesterol and LDL cholesterol level between the last measurement before start of simvastatin therapy and the first measurement after start.

Cofactors

Characteristics considered as potential determinants affecting the change in total cholesterol and LDL cholesterol level were age, gender, the level at the last measurement before start of simvastatin therapy and the daily prescribed dose of simvastatin at the time of the first measurement after start.

Genotyping

All participants of the Rotterdam Study for whom a blood sample was available were genotyped for the SNPs C1236T, G2677TA and C3435T in the *ABCB1* gene. Genotyping was done

using Taqman allelic discrimination assays on the ABI Prism 7900 HT Sequence detection system (Applied Biosystems, Foster City, Ca.,USA) on 1 ng of genomic DNA extracted from leukocytes, as previously reported.[13,20] For the tri-allelic variant G2677TA, two separate assays were designed, one detecting G2677T and one detecting G2677A. Haplotypes were estimated using the estimation maximization algorithm and software as described in the statistical analysis section.

Statistical analysis

Potential deviations from Hardy-Weinberg equilibrium and differences in genotypes between patients who continued and discontinued simvastatin therapy were analyzed using χ^2-tests. Expectation maximization for the haplotypes were performed with HaploStats 1.3. package for R, using haplo.em and haplo.glm respectively.[21,22] Inferred haplotypes with a frequency below 5% were pooled into one 'other haplotype' group, since estimates become unreliable for rare haplotypes in HaploStats.

Multivariate linear regression was used to analyze differences between *ABCB1* genotypes in time from start of simvastatin therapy and the first total cholesterol measurement after start and differences in baseline total cholesterol and LDL cholesterol level. Multivariate linear regression was also used to analyze differences between *ABCB1* genotypes in total cholesterol and LDL cholesterol level change after start of simvastatin therapy. For each polymorphism we calculated the association between the number of variant alleles and the difference in change in total cholesterol or LDL cholesterol levels. Also the association between the number of haplotypes and the difference in change was calculated. In the haplotype analyses, the haplotype with CGC at positions 1236-2677-3435, respectively, was considered the reference, to which the other haplotypes were compared. These analyses were performed with SPSS software (version 15.0; SPSS, Chicago, IL).

RESULTS

In 108 incident simvastatin users, cholesterol measurements were done both in the period of 180 days before and in the period between 7 and 180 days after start. Three participants were excluded because no blood sample was available for genotyping, and genotyping failed for C1226T in zero participants, for G2677TA in three participants and for C3435T in three participants. Nineteen participants discontinued simvastatin therapy before the first measurement after start, and one patient was co-prescribed ezetimibe at the time of the first cholesterol measurement after start. Eventually, we included 85 incident simvastatin users in our study (table 1). In all these patients, total cholesterol levels were measured both before and after start of simvastatin therapy. In 76 patients, LDL-cholesterol levels were measured both before and after start of simvastatin therapy.

Table 1 Baseline Characteristics of the study population

Characteristic		Study population	Males	Females
Number		85	38	47
Age (SD)		71.5 (5.1) yr	71.1 (5.5) yr	71.8 (4.8) yr
BMI (SD)		26.5 (3.6) kg/m^2	26.1 (3.4) kg/m^2	26.9 (3.7) kg/m^2
Baseline level	Total cholesterol	6.86 (1.10) mmol/l	6.61 (1.12) mmol/l	7.06 (1.05) mmol/l
	LDL cholesterol	4.64 (1.01) mmol/l	4.37 (1.05) mmol/l	4.85 (0.94) mmol/l
Simvastatin dose	10 mg	46 (54%)	25 (66%)	21 (45%)
	20 mg	37 (44%)	13 (34%)	24 (51%)
	40 mg	2 (2%)	0 (0%)	2 (4%)
ABCB1 genotype				
C1236T	CC	28 (33%)	12 (32%)	16 (34%)
	CT	40 (47%)	15 (39%)	25 (53%)
	TT	17 (20%)	11 (29%)	6 (13%)
G2677TA	GG	24 (29%)	9 (26%)	15 (32%)
	GT	39 (48%)	14 (40%)	25 (53%)
	TT	16 (20%)	10 (29%)	6 (13%)
	GA	3 (4%)	2 (6%)	1 (2%)
C3435T	CC	17 (21%)	6 (17%)	11 (23%)
	CT	42 (51%)	15 (43%)	27 (57%)
	TT	23 (28%)	14 (40%)	9 (19%)
Haplotype	CGC	42%	30%	50%
(allele frequency)	TTT	42%	48%	38%
	CGT	11%	14%	10%
	other	4%	8%	2%

The average time from the last cholesterol measurement and start of simvastatin therapy was 26 (SD 38) days and the average time from start of simvastatin therapy until the first measurement after start was 51 (SD 35) days. No differences between genotypes were found in baseline total cholesterol or LDL cholesterol levels, in genotype distributions between participants who continued simvastatin therapy and participants who discontinued, or in time from start of simvastatin therapy and the first cholesterol measurement after start. The average decrease in total cholesterol and LDL cholesterol level after start of simvastatin therapy was 2.1 (SD 0.7) mmol/l and 2.0 (SD 0.7) mmol/l, respectively. The average prescribed simvastatin dose was 15.1 mg.

The variant allele frequency for C1236T, G2677T, G2677A and C3435T were 0.44, 0.45, 0.06 and 0.54, respectively. The genotype distribution for all genotypes was in Hardy-Weinberg equilibrium and no differences were seen in genotype frequency between patients who discontinued simvastatin therapy before the first measurement and patients who continued

simvastatin therapy. The SNP C1236T was in strong linkage disequilibrium with G2677T (r^2=0.95, D'=0.97), the linkage disequilibria between C1236T and C3435T (r^2=0.49, D'=0.85), and between G2677T and C3435T (r^2=0.49, D'=0.89) were smaller.

The reduction in total cholesterol level after start of simvastatin therapy was associated with the three SNPs in the *ABCB1* gene (table 2). For each minor 1236T allele, the reduction in total cholesterol was 0.19 mmol/l (95% CI 0.04, 0.33) larger. The reduction in total cholesterol was 0.17 mmol/l (95% CI 0.01, 0.32) larger for each minor 2677T allele and 0.18 mmol/l (95% CI 0.02, 0.33) for each variant 3435T allele. No associations were found for the reduction in LDL cholesterol levels.

Table 2 The total cholesterol and LDL cholesterol lowering effect of simvastatin by *ABCB1* genotype [a]

Allele	Total cholesterol			LDL cholesterol		
	Difference	95% CI	p-value	Difference	95% CI	p-value
T^{1236}	**-0.19**	**(-0.33, -0.041)**	**0.012**	-0.14	(-0.31, 0.031)	0.11
T^{2677} [b]	**-0.17**	**(-0.32, -0.011)**	**0.036**	-0.14	(-0.31, 0.042)	0.13
T^{3435}	**-0.18**	**(-0.33, -0.021)**	**0.027**	-0.17	(-0.35, 0.002)	0.053

[a] Difference in change in cholesterol level between the last measurement before start and the first measurement after start per copy of the minor allele in mmol/l. Adjusted for age, gender, cholesterol level at the last measurement before start and the daily prescribed dose of simvastatin. [b] 3 participants with GA genotype were excluded.

Haplotype analyses revealed that the TTT haplotype was associated with a statistically significant reduction in total cholesterol (table 3). For each TTT haplotype the reduction in total cholesterol level was 0.26 mmol/l (95% CI 0.08, 0.43) larger. Both the TTT and the CGT haplotype were associated with a reduction in LDL cholesterol level. For each TTT and CGT haplotype the reduction was 0.25 mmol/l (95% CI 0.06, 0.45) and 0.30 mmol/l (95% CI 0.04, 0.57) larger, respectively.

Table 3 The total cholesterol and LDL cholesterol lowering effect of simvastatin by *ABCB1* haplotype [a]

Haplotype allele	Total cholesterol			LDL cholesterol		
	Difference	95% CI	p-value	Difference	95% CI	p-value
CGC	ref.			ref.		
TTT	**-0.26**	**(-0.43, -0.083)**	**0.004**	**-0.25**	**(-0.45, -0.061)**	**0.011**
CGT	-0.22	(-0.47, 0.029)	0.082	**-0.30**	**(-0.57, -0.038)**	**0.026**
other	-0.27	(-0.67, 0.13)	0.19	-0.34	(-0.75, 0.065)	0.098

[a] Difference in change in cholesterol level between the last measurement before start and the first measurement after start per copy of the minor allele in mmol/l. Adjusted for age, gender, cholesterol level at the last measurement before start and the daily prescribed dose of simvastatin.

The effect of the SNPs in the *ABCB1* gene was stronger in males than in females (table 4). Both the TTT and the CGT haplotype were associated with more total cholesterol reduction (TTT 0.40 mmol/l 95% CI 0.17, 0.63; CGT 0.44 mmol/l 95% CI 0.11, 0.77) and LDL cholesterol reduction (TTT 0.51 mmol/l 95% CI 0.22, 0.81; CGT 0.53 mmol/l 95% CI 0.15, 0.92) in males.

The amount of variability in total cholesterol and LDL cholesterol reduction in men explained by the haplotypes (r^2) was 27.9 and 35.2, respectively. No significant associations with total cholesterol or LDL cholesterol reduction were found in females.

Table 4 Gender differences in the effect of *ABCB1* haplotypes on the total cholesterol and LDL cholesterol lowering effect of simvastatin [a]

Gender	Haplotype allele	Total cholesterol			LDL cholesterol		
		Difference	95% CI	p-value	Difference	95% CI	p-value
Males							
	CGC	ref.			ref.		
	TTT	**-0.40**	**(-0.63, -0.17)**	**0.001**	**-0.51**	**(-0.81, -0.22)**	**0.002**
	CGT	**-0.44**	**(-0.77, -0.11)**	**0.010**	**-0.53**	**(-0.92, -0.15)**	**0.009**
	other	-0.12	(-0.54, 0.31)	0.58	-0.27	(-0.78, 0.23)	0.27
Females							
	CGC	ref.			ref.		
	TTT	-0.20	(-0.45, 0.054)	0.12	-0.13	(-0.40, 0.14)	0.34
	CGT	-0.072	(-0.43, 0.29)	0.69	-0.14	(-0.52, 0.23)	0.44
	other	-0.57	(-1.37, 0.22)	0.15	-0.52	(-1.32, 0.28)	0.19

[a] Difference in change in cholesterol level between the last measurement before start and the first measurement after start per copy of the minor allele in mmol/l. Adjusted for age, gender, cholesterol level at the last measurement before start and the daily prescribed dose of simvastatin.

DISCUSSION

In this population-based cohort study, SNPs in the *ABCB1* gene were associated with the total cholesterol and LDL cholesterol lowering effect in incident male simvastatin users. In males, each TTT haplotype was associated with a 0.40 mmol/l larger reduction in total cholesterol and a 0.51 mmol/l larger reduction in LDL cholesterol. Each CGT haplotype was associated with a 0.44 mmol/l larger reduction in total cholesterol and a 0.53 mmol/l larger reduction in LDL cholesterol. For females no associations were found with the total cholesterol or LDL cholesterol reduction. In this study we could include only 85 incident simvastatin users, of whom 38 were men. In spite of the small sample size, significant associations between the *ABCB1* haplotypes and total cholesterol and LDL cholesterol reduction in men were found, with p-values below 0.01. The *ABCB1* haplotypes explain around one-third of the total variation in cholesterol reduction in men, which indicates that the association between the *ABCB1* haplotypes and cholesterol reduction is a strong one.

Most likely, the TTT and CGT haplotypes are associated with a reduced efflux functioning of the P-gp transporter, resulting in increased simvastatin levels and a stronger reduction in total cholesterol and LDL cholesterol levels. The hepatic expression of P-gp is approximately 2.4 times higher in men than in women.[7] Consequently, the pharmacokinetics of simvastatin is much more dependent on P-gp functioning in men than in women and the polymorphisms in the *ABCB1* gene will have more effect in men than in women.[6] However, *ABCB1* is also

expressed in the small intestine and less is known about gender differences in *ABCB1* expression in the small intestine.

In hepatocytes, *ABCB1* is co-expressed with *CYP3A4* [6] and simvastatin is a substrate for both P-gp and CYP3A4.[8-10] A reduction in efflux out of the hepatocyte by P-gp will result in higher intracellular simvastatin levels and an increase in simvastatin available for CYP3A4 metabolism. This increase in availability may result in increased metabolism, reduced simvastatin plasma levels and less LDL and total cholesterol reduction. Apparently, the effect of the *ABCB1* polymorphisms on efflux out of the body is stronger than on CYP3A4 metabolism due to reduced efflux out of the hepatocyte.

In the study by Fiegenbaum et al., the TTT haplotype was associated with a larger reduction in total cholesterol and LDL cholesterol levels with simvastatin therapy.[23] No stratification on gender was presented in this study. Kajinami et al. studied the effect of the G2677TA and C3435T polymorphism in atorvastatin users.[24] The C3435T SNP was associated with a larger reduction in total cholesterol and LDL cholesterol levels and a larger increase in HDL cholesterol level, whereas the G2677TA SNP was not. Strikingly, for atorvastatin the effects were stronger in women than in men.

The Rotterdam Study is a population-based cohort study, not primarily designed to assess the effect of simvastatin therapy. Although many participants were incident simvastatin users, only for 85 participants cholesterol levels were available both in the period of 180 days before and 180 days after start of simvastatin therapy. In this study, we used the cholesterol measurements done by general practicioners. Cholesterol levels measured, for example, in hospitals could not be included. In spite of the limited number of users, we found significant associations between *ABCB1* haplotypes and total cholesterol and LDL cholesterol reductions in men. The absence of significant associations in women does not preclude that genetic variation in *ABCB1* does affect the cholesterol lowering effect of simvastatin in women, but most likely the effect is weaker in women than in men.

In population-based studies bias may occur. We identified all incident simvastatin users in the Rotterdam Study. The *ABCB1* polymorphisms apparently affect simvastatin therapy, but it is unlikely that differences in cholesterol levels are present before start of therapy. *ABCB1* polymorphisms were in Hardy-Weinberg equilibrium, suggesting that Mendelian randomization was present. Also permission of patients to take blood and isolate DNA for scientific research was most likely independent from the genetic variation. Therefore, selection bias was unlikely. The information in the Rotterdam Study was collected prospectively without prior knowledge of the study hypothesis. Cholesterol measurements were done in daily practice and both the prescribing physician and the patient were not aware of genetic variation in the *ABCB1* gene. No differences between genotypes were seen in discontinuation of simvastatin therapy or in time from start of simvastatin therapy to the first cholesterol measurement after start, making information bias unlikely.

To conclude, both the TTT and CGT haplotype are associated with a stronger reduction in total cholesterol and LDL cholesterol levels in men compared to the CGC haplotype. In women, no significant associations between genetic variation in the *ABCB1* gene and total cholesterol or LDL cholesterol was found. These results suggest that polymorphisms in the *ABCB1* gene do affect simvastatin pharmacokinetics in men, but to a lesser extent in women. These differences may be attributable to a higher *ABCB1* expression in men than in women.

REFERENCES

1. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344(8934):1383-9.
2. Armitage J. The safety of statins in clinical practice. *Lancet* 2007;370(9601):1781-90.
3. Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics* 2008;9(1):105-27.
4. Chinn LW, Kroetz DL. ABCB1 pharmacogenetics: progress, pitfalls, and promise. *Clin Pharmacol Ther* 2007;81(2):265-9.
5. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004;75(1):13-33.
6. Cummins CL, Wu CY, Benet LZ. Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein. *Clin Pharmacol Ther* 2002;72(5):474-89.
7. Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* 1995;275(2):1011-8.
8. Chen C, Lin J, Smolarek T, Tremaine L. P-glycoprotein has differential effects on the disposition of statin acid and lactone forms in mdr1a/b knockout and wild-type mice. *Drug Metab Dispos* 2007;35(10):1725-9.
9. Hochman JH, Pudvah N, Qiu J, et al. Interactions of human P-glycoprotein with simvastatin, simvastatin acid, and atorvastatin. *Pharm Res* 2004;21(9):1686-91.
10. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* 2006;80(6):565-81.
11. Single Nucleotide Polymorphism. 2008. National Center for Biotechnology Information. Available from http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=5243. Accessed 5 September 2008)
12. Crettol S, Venetz JP, Fontana M, et al. Influence of ABCB1 genetic polymorphisms on cyclosporine intracellular concentration in transplant recipients. *Pharmacogenet Genomics* 2008;18(4):307-15.
13. Aarnoudse AJ, Dieleman JP, Visser LE, et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. *Pharmacogenet Genomics* 2008;18(4):299-305.
14. Aarnoudse AL, van Schaik RH, Dieleman J, et al. MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine. *Clin Pharmacol Ther* 2006;80(4):367-74.
15. 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.
16. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
17. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 28 April 2008.
18. Safeer RS, Ugalat PS. Cholesterol treatment guidelines update. *Am Fam Physician* 2002;65(5):871-80.
19. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285(19):2486-97.
20. Fang Y, van Meurs JB, d'Alesio A, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet* 2005;77(5):807-23.
21. Schaid DJ. Evaluating associations of haplotypes with traits. *Genet Epidemiol* 2004;27(4):348-64.

22. Stram DO, Leigh Pearce C, Bretsky P, et al. Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case-control study of unrelated individuals. *Hum Hered* 2003;55(4):179-90.
23. Fiegenbaum M, da Silveira FR, Van der Sand CR, et al. The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. *Clin Pharmacol Ther* 2005;78(5):551-8.
24. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. Polymorphisms in the multidrug resistance-1 (MDR1) gene influence the response to atorvastatin treatment in a gender-specific manner. *Am J Cardiol* 2004;93(8):1046-50.

Chapter 4.2.

Influence of genetic variation in *CYP3A4* and *ABCB1* on dose decrease or switching during simvastatin and atorvastatin therapy

ABSTRACT

Background: Simvastatin and atorvastatin are metabolized by the CYP3A4 enzyme and transported by the ABCB1 transporter. We studied whether the polymorphism *CYP3A4*1B* and the polymorphisms C1236T, G2677A/T and C3435T in the *ABCB1* gene were associated with a decrease of the prescribed dose or a switch to another cholesterol lowering drug during simvastatin and atorvastatin therapy. These events may indicate that statin plasma levels were too high and resulted in an adverse drug reaction or a too strong reduction in cholesterol level.

Methods: We identified 1,239 incident simvastatin and atorvastatin users in the Rotterdam Study, a population-based cohort study. Associations between the polymorphisms in the *CYP3A4* and *ABCB1* gene and the time to a decrease in dose or a switch to another cholesterol lowering drug were studied using Cox proportional hazards.

Results: Simvastatin and atorvastatin users with the *CYP3A4*1B* variant G allele had a lower risk (HR 0.46; 95% CI 0.24, 0.90) for these events than users with the wild-type AA genotype. No significant associations were found for the *ABCB1* polymorphisms. The association with the *CYP3A4*1B* polymorphism was found in women (HR 0.33; 95% CI 0.12, 0.89) and was absent in men (HR 0.69; 95% CI 0.28, 1.70). This association was stronger in patients with the *ABCB1* 3435T variant allele versus the C allele.

Conclusion: In simvastatin and atorvastatin users, the low expression *CYP3A4*1B* G allele is associated with a lower risk of elevated statin plasma levels, particularly in women and in users with the *ABCB1* 3435T variant allele.

INTRODUCTION

HMG-CoA reductase inhibitors or statins are widely prescribed for the treatment of hypercholesterolemia. Statins reduce morbidity and mortality by lowering LDL cholesterol levels.[1] In general, statins are well tolerated and safe, although myopathy and rhabdomyolysis are well-known serious adverse reactions associated with statin therapy.[2]

Two regularly used statins, simvastatin and atorvastatin, are mainly metabolized by the Cytochrome P450 (CYP) 3A4 enzyme.[3-6] The area under curve (AUC) of simvastatin increases five- to twenty fold if itraconazol, a potent CYP3A4 inhibitor, is co-prescribed, and the AUC of atorvastatin increases two to fourfold.[7] The risk of myopathy with these statins is markedly increased if combined with drugs inhibiting CYP3A4 enzymes.[7,8] Genetic variation in the *CYP3A4* gene affects the metabolism of simvastatin and atorvastatin.[9] The polymorphism *CYP3A4*1B* (-392A>G) is located in the promotor region of the *CYP3A4* gene, and the G allele is associated with enhanced CYP3A4 expression due to reduced binding of a transcriptional repressor.[10] This will lead to a decrease in simvastatin and atorvastatin levels and eventually their cholesterol lowering effect.

The CYP3A4 enzymes are mainly located in the cells in the intestinal wall and hepatocytes. The ATP-binding cassette B1 (ABCB1) protein, also known as P-glycoprotein, is an efflux transporter that is co-located in the cells expressing CYP3A4.[11,12] This transporter does pump statins out of the cells in the intestinal wall back into the lumen and out of the hepatocytes into the bile.[7]

Three polymorphisms in the *ABCB1* gene, previously identified as multidrug resistance 1 (MDR1), (C1236T, G2677A/T and C3435T) are associated with an impaired efflux pump of the ABCB1 transporter, resulting in increased drug levels.[13] Since CYP3A4 and ABCB1 are co-located in the same cells, a reduced efflux by the ABCB1 transporter results in increased intracellular plasma levels and increased substrate availability for the CYP3A4 enzymes and vice versa. In women, the expression of *ABCB1* is lower than in men, and therefore a change in CYP3A4 activity will have more impact in women than in men. In our study, we assessed whether the *CYP3A4*1B* polymorphism and the polymorphisms in the *ABCB1* gene are associated with the occurrence of a dose decrease or a switch to another cholesterol lowering drug. These events may be the consequence of an elevated statin plasma level, resulting in either an adverse drug reaction or a too strong reduction in cholesterol level. We also assessed whether these effects are different for men and women, and whether there is interaction between the *CYP3A4* and *ABCB1* polymorphisms.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[14,15] The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[16] General practitioners introduced electronic patient records from 1997 onwards, although complete patient files including, for example, the reason for the visit and laboratory assessments, were only available for the most recent years. These electronic patient records were available for most of the participants in the Rotterdam Study.

Study Sample

All participants with at least one filled prescription for simvastatin or atorvastatin were included in the study sample. Follow-up started at the date of the first prescription for simvastatin or atorvastatin. Participants were followed until January 1st 2008, the end of the last prescription for simvastatin or atorvastatin, an increase in prescribed dose or the occurrence of an event, whichever came first. In an additional analysis we excluded all simvastatin and atorvastatin users with a first prescription before July 1st 1991, to ensure that all participants were incident users and did not use these drugs before January 1st 1991 for which we did not have the prescription data. In another additional analysis we excluded all participants with a prescription for any of the other statins, not metabolised by CYP3A4 before start of simvastatin or atorvastatin therapy to exclude a possible effect of the CYP3A4 enzyme on the other statins. We stratified the analysis on gender, because women have a lower *ABCB1* expression resulting in higher statin levels available for the CYP3A4 enzyme.

Outcome

As adverse reactions due to statin use were not registered as such in our database, we analyzed the occurrence of either a dose decrease or a switch to another statin as an indicator of an adverse drug reaction or a too strong reduction in cholesterol level. The first time after start of simvastatin or atorvastatin use that a patient had a dose decrease or switched to

another cholesterol lowering drug (statins, fibrates, bile acid sequestrants, nicotinic acid, acipimox or ezetimibe) was regarded as an event. After identification of these events in the dispensing data, we searched in the patient records that were available for the reason of the dose decrease or switch to another cholesterol lowering drug. These outcomes were chosen, because a physician facing an adverse drug reaction or a too strong reduction in cholesterol level has two possible options. First, the physician can lower the dose if he or she suspects a dose-effect relationship. Second, switching to another cholesterol lowering drug is an option. In case of ineffective therapy the most likely decision is to increase the dose before switching to another drug and therefore follow-up ended at the time of the first dose increase.

Cofactors

Age, gender and the prescribed dose of the first prescription for simvastatin or atorvastatin were considered as potential confounders of the association between the *CYP3A4* and *ABCB1* polymorphisms and the occurrence of the events.

Genotyping

Genotyping *CYP3A4*1B*, *ABCB1* C1236T, *ABCB1* G2677T/A and *ABCB1* C3435T was done using Taqman allelic discrimination assays on the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA) on 1 ng of genomic DNA extracted from leukocytes, as previously reported.[17] The primer and probe sequences were designed by Applied Biosystems. For the triallelic variant *ABCB1* G2677T/A, two separate assays were designed, one detecting G2677T and one detecting G2677A. Haplotypes were estimated using the estimation maximization algorithm with Haplostats 1.3.0 package for R (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm).

Statistical analysis

A χ^2-test was used to test for deviations from Hardy-Weinberg equilibrium. Cox proportional hazard models were constructed to test for differences in the incidence of medication change events between genotypes. Additive models were used, in which we analyzed the association between the number of minor (variant) alleles and the occurrence of events. We tested for multiplicative interaction between the *CYP3A4* and *ABCB1* polymorphisms in the Cox models. Kaplan-Meier curves were constructed to present the results visually. Analyses were performed with SPSS software (version 11.0.1; SPSS Chicago IL) and SAS software (version 8.02; SAS Institute Cary NC).

RESULTS

In the Rotterdam Study, we identified 1,380 participants who were prescribed simvastatin or atorvastatin during follow-up. 1,058 participants had a first prescription for simvastatin in the database and 322 participants for atorvastatin. For the *CYP3A4* genotyping 1,239 of the 1,380 blood samples were available and genotyping failed in 41 participants (table 1). For the *ABCB1* genotyping 1,255 blood samples were available and genotyping failed in 23 (C1236T), 46 (G2677TA) and 50 (C3435T) participants. The allele frequencies were 0.04 (*CYP3A4*1B* A>G), 0.44 (C1236T), 0.42 (G2677T), 0.03 (G2677A) and 0.53 (C3435T). Only one patient had the *CYP3A4*1B* GG genotype. All genotype frequencies were in Hardy-Weinberg equilibrium (p>0.01). The *ABCB1* polymorphisms were in strong linkage disequilibrium with each other (r^2>0.8). Simvastatin and atorvastatin users were followed on average 5.3 year (SD 4.8 year). In 250 patients, the prescribed dose of simvastatin or atorvastatin was increased. Events occurred in 271 patients, in 163 patients the prescribed dose was decreased and 108 patients switched to another cholesterol lowering drug.

Table 1 Baseline characteristics of 1,198 incident atorvastatin and simvastatin users by *CYP3A4*1B* genotype

***CYP3A4 *1b* genotype**	**AA**	**AG or GG [a]**
Number	1,102	96
Gender, male	474 (43 %)	40 (42%)
Age (SD)	71.3 (7.0) year	71.9 (6.9) year
Body mass index (SD) [b]	26.6 (3.4) kg/m^2	26.8 (3.8) kg/m^2
Simvastatin start	851 (77 %)	74 (77 %)
Atorvastatin start	251 (23 %)	22 (23 %)
ACBC1 genotypes		
1236CC	343 (31 %)	22 (23 %)
1236CT	548 (50 %)	57 (60 %)
1236TT	201 (18 %)	16 (17 %)
2677GG	324 (30%)	21 (23 %)
2677GT	515 (48 %)	50 (54 %)
2677GA	27 (3 %)	3 (3 %)
2677TT	185 (17 %)	16 (17 %)
2677TA	21 (2 %)	2 (2 %)
2677AA	3 (0 %)	0
3435CC	242 (23 %)	12 (13 %)
3435CT	547 (51 %)	55 (58 %)
3435TT	286 (27 %)	28 (29 %)

[a] Number of participants with the AG genotype is 95 and with the GG genotype is 1. [b] At the time of entrance in the Rotterdam Study.

One hundred and fifty-two events occurred after January 1st 1997, the date that electronic patient records were introduced (table 2). Forty-two patient records were not available. For 78 cases, the reason of change in the cholesterol lowering medication could not be recovered, for example because a medical specialist had changed the medication or because the general practitioner used paper files at the time of the event to record this. The reason was given in the electronic patient records in 32 cases. In 17 cases (53%), an adverse drug reaction was the reason for the decrease in dose or switch to another cholesterol lowering drugs, and in 13 cases (41%) the reason was a too strong reduction in cholesterol level. Two patients (6%) switched to another cholesterol lowering drug after a cholesterol measurement and ineffective drug therapy was the most likely reason for these switches.

Table 2 Reason for the dose decrease or switch to another cholesterol lowering drug for all events after January 1st 1997

Data retrieval	**N**	
Patient file not available	42	(28 %)
By specialist, reason unknown	14	(9 %)
Unknown	64	(42 %)
Reason given (see below)	32	(21 %)
	152	
Reason	**N**	
Adverse drug reaction	17	(53 %)
Muscle pain	4	
Malaise	2	
Allergy or itching	2	
Pain or neuropathy	2	
Other [a]	3	
Not specified	4	
Too strong cholesterol reduction (certain)	8	(25 %)
Too strong cholesterol reduction (possible)	5	(16 %)
Statin not effective (possible)	2	(6 %)
	32	

[a] Other: hair loss (n=1), diarrhea (n=1), hepatic failure (n=1).

The risk of a dose decrease or switch to another drug was smaller in patients with the G allele at *CYP3A4*1B* than in patients with the wild-type AA genotype (HR 0.46; 95% CI 0.24, 0.90) (table 3, figure 1). The hazard ratios for participants using simvastatin (HR 0.47; 95% CI 0.23, 0.96) and atorvastatin (HR 0.44; 95% CI 0.06, 3.22) were similar, although for atorvastatin it was not statistically significant. Excluding patients with a first prescription before July 1st 1991 (HR 0.45; 95% CI 0.21, 0.95) or excluding patients with prescriptions for other statins before start of simvastatin or atorvastatin therapy (HR 0.48; 95% CI 0.25, 0.93) did not change the results substantially. No differences in hazard ratio were found for the C3435T polymorphism in the *ABCB1* gene (HR 1.14; 95% CI 0.94, 1.38). The results for the other polymorphisms in the *ABCB1* gene were comparable to the C3435T polymorphism. The hazard rates for the

haplotype analysis gave similar results with wider confidence intervals due to lower numbers of patients in each of the subgroups.

Table 3 The association between the *CYP3A4*1B* A>G and *ACBC1* C3435T polymorphisms [a], and dose decreases or switching to another cholesterol lowering drug in simvastatin and atorvastatin users

	Unadjusted	Adjusted [b]		
	HR	HR	95% CI	p-value
***CYP3A4*1B* A>G**				
Simvastatin	0.47	**0.47**	**(0.23, 0.96)**	**0.039**
Atorvastatin	0.30	0.44	(0.06, 3.23)	0.42
Simvastatin and atorvastatin	0.44	**0.46**	**(0.24, 0.90)**	**0.023**
***ABCB1* C3435T**				
Simvastatin	1.16	1.15	(0.93, 1.42)	0.20
Atorvastatin	1.12	1.07	(0.69, 1.67)	0.76
Simvastatin and atorvastatin	1.14	1.14	(0.94, 1.38)	0.18

[a] Additive model with the AA (for *CYP3A4*1B*) and CC (for *ABCB1* C3435T) genotype as reference. [b] Adjusted for age, gender and starting dose.

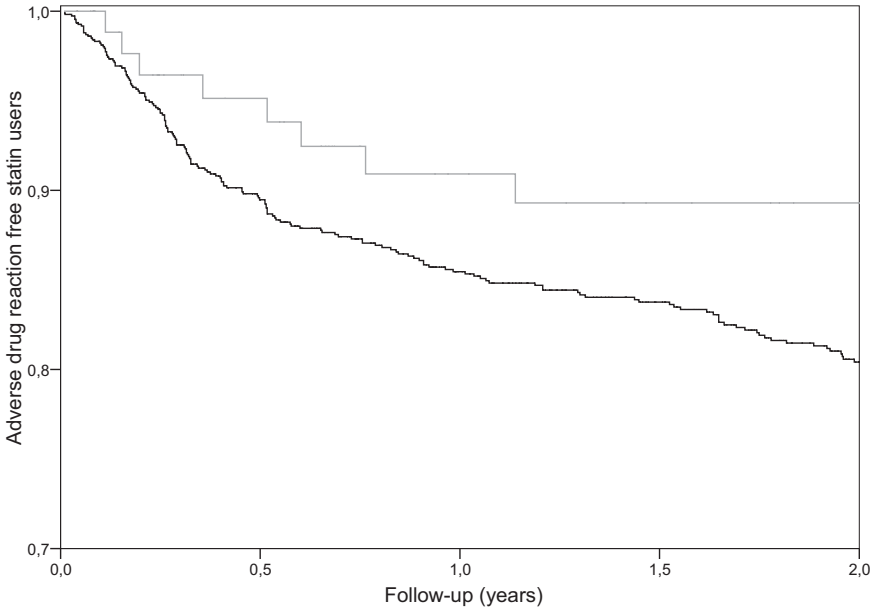


Figure 1 Kaplan-Meier curve for the association between the CYP3A4*1B A>G polymorphism and dose decreases or switching to another cholesterol lowering drug during the first two years of simvastatin and atorvastatin therapy
Black line: *CYP3A4*1B* AA genotype
Grey line: *CYP3A4*1B* AG or GG genotype

The differences in hazard ratio for the *CYP3A4*1B* polymorphism were caused by the women in the group. The hazard ratio was 0.33 (95% CI 0.12, 0.89) for women, while the hazard ratio was not significant for men (HR 0.69; 95% CI 0.28, 1.70). In table 4 the associa-

tion between *CYP3A4*1B* genotype and the occurrence of events is stratified for the *ABCB1* C3435T genotype to analyze possible interaction. In patients with the 3435CC genotype, the differences in hazard ratios between the *CYP3A4*1B* genotypes were smaller (HR 0.89; 95% CI 0.21, 3.69) than in patients with the 3435CT genotype (HR 0.55; 95% CI 0.24, 1.24) and patients with the 3435TT genotype (HR 0.15; 95% CI 0.021, 1.09). Combining the 3435CT and TT genotypes, gave a significant difference between the *CYP3A4*1B* genotypes (HR 0.39; 95% CI 0.19, 0.84), that was smaller than the overall hazard ratio. Testing for multiplicative interaction between the *CYP3A4*1B* polymorphism and the *ABCB1* C3435T polymorphism revealed no significance for patients with the 3435CT genotype (HR 0.53; 95% CI 0.10, 2.76) and a trend towards an effect for the 3435TT genotype (HR 0.15; 95% CI 0.013, 1.75), although not significant (p=0.13). The results were similar for the C1236T and G2677T/A polymorphisms in the *ABCB1* gene or for testing additive interaction.

Table 4 Association between the *CYP3A4*1B* A>G polymorphism [a] and dose decreases or switching to another cholesterol lowering drug in simvastatin and atorvastatin users, stratified for the *ABCB1* C3435T genotype

***CYP3A4*1B* A>G**	**Unadjusted**	**Adjusted [b]**		
	HR	**HR**	**95% CI**	**p-value**
ABCB1 3435CC	1.15	0.89	(0.21, 3.69)	0.87
ABCB1 3435CT	0.53	0.55	(0.24, 1.24)	0.15
ABCB1 3435TT	0.13	0.15	(0.021, 1.09)	0.061
ABCB1 3435CT or TT	0.37	**0.39**	**(0.19, 0.84)**	**0.016**

[a] Additive model with the AA genotype as reference. [b] Adjusted for age, gender and starting dose.

DISCUSSION

In this population based closed cohort study, we found a two times lower risk for a dose decrease or a switch to another cholesterol lowering drug, in simvastatin and atorvastatin users with the *CYP3A4*1B* variant allele. In the majority of cases, the reason for these medication changes was an adverse drug reaction or a too strong reduction in cholesterol level. Both are most likely caused by elevated statin plasma levels, due to reduced metabolism of simvastatin and atorvastatin. The association was only significant in women, and not in men. No associations were found with polymorphisms in the *ABCB1* gene. Although not statistically significant, the results suggest that in patients with the *ABCB1* C3435T wild-type genotype the occurrence of these events is not associated with the *CYP3A4*1B* polymorphism, while in patients with one or two variant alleles at *ABCB1* C3435T the risk is more than two times lower in simvastatin and atorvastatin users with the *CYP3A4*1B* variant allele.

The results of our study are in line with previous publications. Patients with the *CYP3A4*1B* G variant allele have an enhanced CYP3A4 expression[10] and less reduction in LDL cholesterol.[18] This indicates most likely lower simvastatin and atorvastatin levels in patients with the

*CYP3A4*1B* AG or GG genotype. Fiegenbaum et al., however, found no association between the *CYP3A4*1B* polymorphism and adverse drug reactions in simvastatin users.[19] For the *ABCB1* C3435T polymorphism we found a non-significant increase in risk for the variant T allele, suggesting a decreased functioning of the efflux pump. Previous studies on simvastatin[19,20] and other substrates[21,22] indicate higher plasma levels and reduced functioning of the C3435T variant alleles, although Fiegenbaum et al.[19] found, contradictorily, a decreased risk of adverse drug reactions with the C3435T variant allele.

Beside simvastatin and atorvastatin, the CYP3A4 enzyme metabolizes also lovastatin and cerivastatin, but these statins were not analyzed in this study. Lovastatin was not marketed in the Netherlands and therefore not dispensed to the study participants. Cerivastatin is also metabolised by other cytochrome P450 enzymes, such as CYP2C8, and therefore the effect of CYP3A4 inhibition on cerivastatin levels is limited because other routes of metabolism compensate this inhibition.[7] Moreover, the number of participants who were prescribed cerivastatin was small, due to its withdrawal from the market in 2001.

The *CYP3A4* gene is expressed both in the liver and the wall of the intestine. Simvastatin and atorvastatin are lipophilic drugs and oxidized to inactive or modestly active metabolites by CYP3A4. Before entering the circulation, the majority of simvastatin and atorvastatin is metabolized, resulting in a bioavailability of five and twelve percent, respectively. This low bioavailability is an explanation why inhibition of CYP3A4 and genetic variation in the *CYP3A4* gene has a substantial impact on the pharmacokinetics of these drugs.

ABCB1 is expressed in the wall of the intestine, kidney, liver and brain and protects against xenobiotics by transporting these out of the body. For simvastatin and atorvastatin, the effects of ABCB1 in the wall of the intestine and in the liver are most relevant. ABCB1 transports simvastatin and atorvastatin out of the intestinal wall into the lumen, and out of the hepatocytes into the bile. After transportation into the lumen or into the bile, the simvastatin and atorvastatin is still available for reabsorption and uptake in the circulation. This may explain why the effect of polymorphisms in the *ABCB1* gene was smaller than the effect of the polymorphism in the *CYP3A4* gene. In patients with the *ABCB1* 3435TT genotype, the intracellular simvastatin and atorvastatin concentrations in the intestinal wall and hepatocyte are probably higher due to the impaired efflux functioning of the ABCB1 transporter. Therefore, more simvastatin and atorvastatin is available for metabolism by CYP3A4 and, consequently, the effects of the *CYP3A4*1B* polymorphism is stronger in patients with the *ABCB1* 3435TT genotype than in patients with the CC genotype.

In our study we found an association between the *CYP3A4*1B* polymorphism and a dose decrease or switch to another cholesterol lowering drug in women using simvastatin or atorvastatin. It has been reported that CYP3A4 activity is higher in women compared to men,[23,24] and therefore polymorphisms in the *CYP3A4* gene may have a larger effect in women. However, differences may also be attributable to differences in *ABCB1* expression between men and women.[12] Indeed, *ABCB1* expression was reported to be lower in women than in men,

leading to increased intracellular drug concentrations and thereby potentially have a higher susceptibility for changes in CYP3A4 activity.

In population-based studies, bias may affect the obtained results. In the patient records, we could not retrieve the reasons for all dose decreases or switches to other cholesterol lowering drugs. We assumed that these events were associated with elevated statin plasma levels, resulting in adverse drug reactions or too strong reductions in cholesterol levels. However, part of the events will be caused by other reasons, such as ineffective cholesterol lowering due to too low statin plasma levels. For those events that we could retrieve the reason of the change in medication in the patient files, only a minority of the events was due to ineffectiveness. This may have given an underestimation of the true effect. On the other hand, we probably will have missed events of adverse drug reactions or too strong reduction in cholesterol levels, because, for example, people stopped using statins at all. Both the patient and the prescriber were blinded to the genotype and misclassifications were therefore random. Random misclassification gives a dilution of the true effect, and the effect sizes in our study were most likely an underestimation. Our results are consistent with what would be expected on theoretical grounds and previous reports. We identified all simvastatin and atorvastatin users in the Rotterdam Study and information was collected prospectively without knowledge of the study hypothesis. The permission of patients to take blood and isolate DNA for scientific research was probably random. Therefore, selection bias and information bias were minimal. Although we included a large number of simvastatin and atorvastatin users, our study was complicated by the low minor allele frequency of the *CYP3A4*1B* polymorphism, making further stratification of the results not possible. It is likely that this was also the cause for not finding a statistically significant interaction between the *CYP3A4*1B* and *ABCB1* C3435T polymorphism, although an interaction is suggested based on the stratified data.

To conclude, simvastatin and atorvastatin users with the *CYP3A4*1B* variant G allele have a two times lower risk for a dose decrease or switch to another cholesterol lowering drug. The *ABCB1* C1236T, G2677A/T and C3435T polymorphisms did not affect the risk. In women and in patients with the *ABCB1* 3435CT or 3435TT genotype the effects of the *CYP3A4*1B* polymorphism on the risk of these events were stronger, although the interaction term did not reach statistical significance.

REFERENCES

1. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344(8934):1383-9.
2. Armitage J. The safety of statins in clinical practice. *Lancet* 2007;370(9601):1781-90.
3. Jacobson TA. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *Am J Cardiol* 2004;94(9):1140-6.
4. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine. Available from http://medicine.iupui.edu/flockhart/table.htm. Accessed 18 April 2008.
5. Prueksaritanont T, Gorham LM, Ma B, et al. In vitro metabolism of simvastatin in humans identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos* 1997;25(10):1191-9.
6. Jacobsen W, Kuhn B, Soldner A, et al. Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. *Drug Metab Dispos* 2000;28(11):1369-78.
7. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* 2006;80(6):565-81.
8. Bottorff M, Hansten P. Long-term safety of hepatic hydroxymethyl glutaryl coenzyme A reductase inhibitors: the role of metabolism-monograph for physicians. *Arch Intern Med* 2000;160(15):2273-80.
9. Zineh I. HMG-CoA reductase inhibitor pharmacogenomics: overview and implications for practice. *Future Medicine* 2005;1(2):191-206.
10. 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(4):299-305.
11. Benet LZ, Cummins CL, Wu CY. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm* 2004;277(1-2):3-9.
12. Cummins CL, Wu CY, Benet LZ. Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein. *Clin Pharmacol Ther* 2002;72(5):474-89.
13. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004;75(1):13-33.
14. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
15. 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.
16. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 18 April 2008.
17. Fang Y, van Meurs JB, d'Alesio A, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet* 2005;77(5):807-23.
18. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. CYP3A4 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin in primary hypercholesterolemia. *Am J Cardiol* 2004;93(1):104-7.
19. Fiegenbaum M, da Silveira FR, Van der Sand CR, et al. The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. *Clin Pharmacol Ther* 2005;78(5):551-8.

20. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. Polymorphisms in the multidrug resistance-1 (MDR1) gene influence the response to atorvastatin treatment in a gender-specific manner. *Am J Cardiol* 2004;93(8):1046-50.
21. Aarnoudse AL, van Schaik RH, Dieleman J, et al. MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine. *Clin Pharmacol Ther* 2006;80(4):367-74.
22. Aarnoudse AJ, Dieleman JP, Visser LE, et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. *Pharmacogenet Genomics* 2008;18(4):299-305.
23. Tanaka E. Gender-related differences in pharmacokinetics and their clinical significance. *J Clin Pharm Ther* 1999;24(5):339-46.
24. Parkinson A, Mudra DR, Johnson C, Dwyer A, Carroll KM. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* 2004;199(3):193-209.

Chapter 4.3.

Genetic variation in the *NOS1AP* gene is associated with the incidence of diabetes mellitus in users of calcium channel blockers

ABSTRACT

Background: Insulin release from the pancreatic beta cell is triggered by an influx of calcium through the voltage dependent calcium channel. It is suggested that nitric oxide, produced by neuronal nitric oxide synthase (nNOS) is essential for normal beta cell functioning. Both nitric oxide and calcium channel blockers (CCBs) have an effect on voltage dependent calcium channels. Recently, the single nucleotide polymorphism rs10494366 T>G in the *NOS1AP* gene, a regulator of nNOS was associated with QTc prolongation. Because both NOS1AP and CCBs have an effect on calcium channels, we hypothesized that variant alleles in the *NOS1AP* gene are associated with the incidence of diabetes mellitus in CCB users.

Methods: We identified all incident CCB users between 1991 and 2008 in the Rotterdam Study, a population based cohort study of 7,983 participants of 55 years and older. Differences in incidence of diabetes mellitus between *NOS1AP* genotypes in CCB users were assessed using Cox proportional hazard models. We adjusted for age, gender and body-mass index.

Results: We identified 816 incident CCB users, of whom 55 developed diabetes mellitus during CCB therapy. The risk of incident diabetes mellitus was lowest in CCB users with the TG or GG genotype (HR 0.56; 95% CI 0.33, 0.97). Differences in risk were small at start of CCB therapy and increased over time.

Conclusion: The polymorphism rs10494366 T>G in the *NOS1AP* gene is associated with the development of diabetes mellitus in CCB users.

INTRODUCTION

Nitric oxide (NO) is an important regulator of a number of intracellular processes, including the secretion of insulin by the pancreatic beta cell. NO, produced by neuronal nitric oxide synthases (nNOS) is essential for normal beta cell functioning, and it has also been suggested that nNOS might have a role in the pathogenesis of diabetes mellitus.[1-3]

Recently, the single nucleotide polymorphism (SNP) rs10494366 in the nitric oxide synthase 1 adaptor protein gene (*NOS1AP*), a regulator of nNOS, has been associated with QTc prolongation.[4,5] nNOS regulates calcium handling in the heart through the voltage-dependent calcium channels.[6] In the beta cell, exocytosis of insulin granules is triggered by an influx of calcium through these calcium channels.[7] Calcium channel blockers (CCBs) bind to a receptor on the voltage-dependent calcium channels and they regulate calcium handling by reducing the influx of calcium into the cell. As calcium channels are also situated in the pancreatic beta cell, CCBs could affect insulin secretion.

In view of these similarities in calcium handling between the myocyte and the pancreatic beta cell, we hypothesized that the SNP rs10494366 in the *NOS1AP* gene is associated with the incidence of diabetes mellitus in patients using CCBs. We studied this in the Rotterdam Study, a prospective population-based closed cohort study in the suburb Ommoord in Rotterdam, in which 7,983 inhabitants participated.[8] Patients were followed from 1991 onwards. Clinical examinations were carried out every 4 to 5 years and participants were monitored through linkage with files from general practitioners and pharmacies. Diabetes mellitus was diagnosed according to the World Health Organization criteria. All participants in the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC.

METHODS

All participants of the Rotterdam Study who received a first prescription for a CCB between July 1st 1991 and January 1st 2008 were included in the study cohort at the time of the first prescription. We excluded participants with a prescription for a CCB between January 1st 1991 and July 1st 1991 in order to have a complete medication history at the time of the first prescription and to ascertain that use of CCB after July 1st 1991 was really incident. We also excluded all participants who stopped using CCBs within one year, because diabetes mellitus is a disease that often has a long latent period and we assumed that a duration of exposure of less than one year would be too short to show actual effect modification. Participants with diabetes mellitus at the first prescription for a CCB were excluded from the study cohort. Participants were followed until 90 days after the end of the last prescription for a CCB, death or end of the study period, whichever came first.

Associations between the SNP rs10494366 in the *NOS1AP* gene, associated with QTc prolongation, and incidence of diabetes mellitus in current users of CCB were assessed. We adjusted for age, sex and body mass index. We developed Cox-proportional hazard models and Kaplan–Meier curves with SPSS software (version 11.0.1; SPSS Chicago, IL, USA).

RESULTS

In the Rotterdam Study, we identified 816 incident CCB users who were genotyped for rs10494366. The average age was 68.3 years and 342 participants were men (41.9%). Five-hundred and forty-six participants (67%) started with dihydropyiridine CCBs, 77 participants (9%) with verapamil and 193 participants (24%) with diltiazem. The minor allele frequency for rs10494366 was 0.35 (G allele).

In the study cohort, 55 participants developed diabetes mellitus while using CCBs. CCB users with the TG or GG genotype had a lower risk of incident diabetes mellitus than CCB users with the TT genotype (HR 0.56; 95% CI 0.33, 0.97). The HR in CCB users with the TG genotype (HR 0.59; 95% CI 0.33, 1.04) was similar to the HR of CCB users with the GG genotype (HR 0.49; 95% CI 0.19, 1.26), suggesting a dominant effect of the G allele.

In the whole Rotterdam Study of 6,292 genotyped participants, irrespective of CCB use, no associations were found between the SNP rs10494366 and the incidence of diabetes mellitus (TG or GG genotype versus TT HR 0.97; 95% CI 0.83, 1.14), nor was there an association in participants who were not prescribed CCBs during follow-up (HR 1.07; 95% CI 0.86, 1.32).

The difference in prevalence of diabetes mellitus over time between CCB users with the TT genotype and CCB users with the TG or GG genotype is presented in figure 1. During the first years of CCB therapy, the HRs of incident diabetes mellitus were similar between the genotypes. Over time the curves diverged and differences between the genotypes became larger. After 6 years of CCB therapy, the HR for CCB users with the TG or GG genotype compared with CCB users with the TT genotype was 0.42 (95% CI 0.15, 1.21). Diabetes mellitus is a progressive disease with deteriorating beta cell functioning. CCB seems to affect the progression of this deterioration differently between the rs10494366 genotype.

DISCUSSION

In the Rotterdam Study, the total follow-up time of the participants genotyped for rs10494366 during the study period was 79,000 person-years and the total time of CCB use was 5,000 person-years. In spite of these large numbers, we only identified 55 incident diabetes mellitus patients while they were using CCBs. Our results could be a false positive result given the small number of incident cases. Therefore, to confirm this association, a large cohort in which

both genotype data and drug dispensing data are available is necessary. Nevertheless, it has been demonstrated that the SNP rs10494366 is probably functional and affects calcium handling in the myocyte. Calcium handling plays a major role in insulin secretion. If replicated, our results may give a new perspective on the pathogenesis of diabetes mellitus and on the relationship between CCB use and the risk of developing diabetes mellitus.

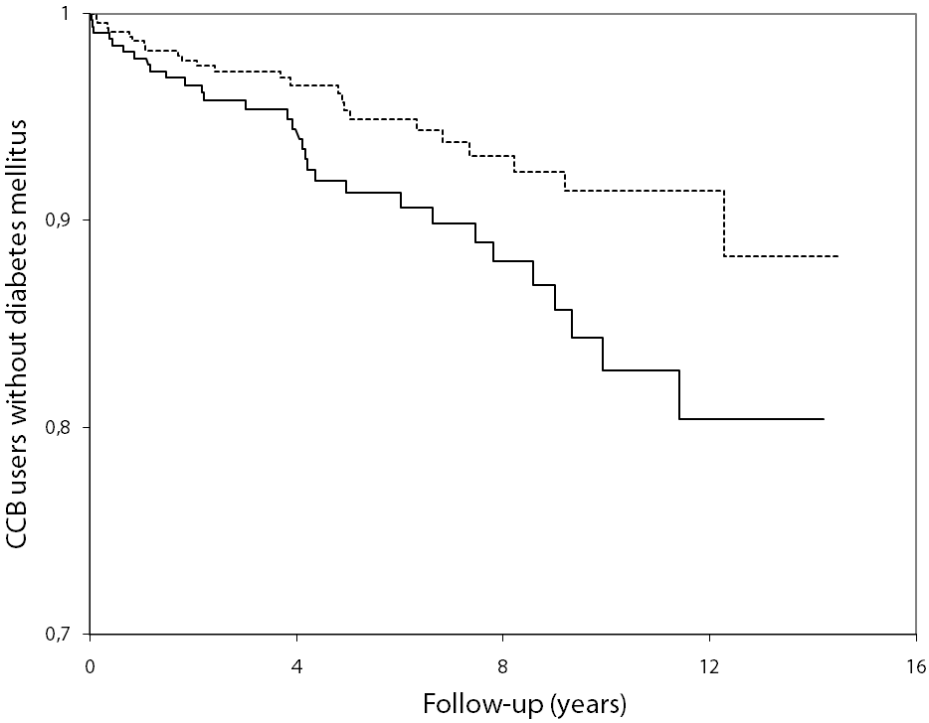


Fig. 1 Kaplan–Meier curve of differences in diabetes mellitus-free CCB users between NOS1AP genotypes.
Continuous line, TT genotype; dashed line, TG or GG genotype. Logrank test, p=0.024. Number of participants at risk (n): TT 337, 194, 92 and 26 at 0, 4, 8 and 12 years of follow-up, respectively; TG/GG 479, 269, 130 and 32 at 0, 4, 8 and 12 years of follow-up, respectively.

REFERENCES

1. Lajoix AD, Reggio H, Chardes T, et al. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. *Diabetes* 2001;50(6):1311-23.
2. Lajoix AD, Pugniere M, Roquet F, et al. Changes in the dimeric state of neuronal nitric oxide synthase affect the kinetics of secretagogue-induced insulin response. *Diabetes* 2004;53(6):1467-74.
3. Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD. Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes* 2000;49(5):684-7.
4. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006;38(6):644-51.
5. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, et al. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. *Circulation* 2007;116(1):10-6.
6. Schulz R, Rassaf T, Massion PB, Kelm M, Balligand JL. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005;108(3):225-56.
7. Ashcroft FM, Rorsman P. Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 1989;54(2):87-143.
8. Hofman A, Breteler MMB, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.

Chapter 4.4.

A common *NOS1AP* genetic polymorphism is associated with increased cardiovascular mortality in users of dihydropyridine calcium channel blockers

ABSTRACT

Background: Recently, a polymorphism in the *NOS1AP* gene (rs10494366), a regulator of neuronal Nitric Oxide Synthase (nNOS), was associated with QTc prolongation. Both nNOS and Calcium Channel Blockers (CCBs) regulate intracellular calcium levels and have an important role in cardiovascular homeostasis. The aim was to investigate whether this polymorphism is associated with cardiovascular mortality in users of CCBs.

Methods: The data from the Rotterdam study, a population-based closed cohort study of Caucasian individuals of 55 years of age or over, were used. We identified 1,113 participants in the Rotterdam Study who were prescribed CCBs for the first time between 1991 and 2005. All-cause and cardiovascular mortality were assessed in participants who were prescribed CCBs with different *NOS1AP* rs10494366 genotypes using Cox proportional hazard models.

Results: In participants starting on dihydropyridine CCBs (amlodipine, nifedipine and others) all-cause mortality (n=79) risks were higher in participants with the TG (hazard ratio (HR) 2.57; 95% CI 1.24, 5.34) or the GG genotype (hazard ratio 3.18; 95% CI 1.18, 8.58) than in participants with the referent TT genotype. Cardiovascular mortality (n=54) risks were 3.51 (95% CI 1.41, 8.78) for the TG genotype and 6.00 (95% CI 1.80, 20.0) for the GG genotype. No differences in all-cause mortality or cardiovascular mortality were seen in participants starting with the non-dihydropyridine CCBs verapamil or diltiazem.

Conclusion: The minor G-allele of rs10494366 in the *NOS1AP* gene is associated with increased all-cause and cardiovascular mortality in Caucasian users of dihydropyridine CCBs. The mechanism underlying the observed association is unknown.

INTRODUCTION

Nitric Oxide (NO) is an important regulator of intracellular calcium handling, and controls many processes in cardiovascular homeostasis, such as myocardial contraction.[1,2] Nitric Oxide Synthase (NOS) produces endogenous NO from the amino acid L-arginine. Recently, the single nucleotide polymorphism (SNP) rs10494366 in the *NOS1 Adaptor Protein* (*NOS1AP*) gene was associated with a prolonged QTc interval in five independent populations.[3-5] NOS1AP is a regulator of neuronal NOS (nNOS, encoded by NOS1), one of the isoforms of NOS. Contraction of the cardiomyocyte is triggered by a short calcium influx through the voltage gated L-type calcium channels on the cell membrane.[6] Intracellular calcium is stored in the sarcoplasmic reticulum (SR) and two calcium channels control the release of calcium to and reuptake from the cytosol.[1] First, the ryanodine receptor releases calcium from the SR into the cytosol, which causes contraction of the cardiomyocyte. Second, the sarcoplasmic reticulum Ca^{2+}-ATPase regulates reuptake of calcium in the SR and stops the contraction of the cardiomyocyte. In the cardiomyocyte nNOS is localized on the SR, and it is hypothesized that nNOS has an effect on one or more of these calcium channels and transporter.[7-11]

Calcium channel blockers (CCB) bind to a receptor on the voltage gated L-type calcium channel, promoting the closed position of the calcium channel and reducing calcium influx into the cell. Dihydropyridine CCBs, such as amlodipine and nifedipine preferentially affect the blood vessels, causing vasodilatation, whereas the non-dihydropyridine CCBs verapamil and diltiazem have a higher affinity for the heart and have a negative chronotropic and inotropic effect.[12]

Since nNOS affects the intracellular calcium levels and either directly or indirectly the calcium currents through the L-type calcium channel, the target of the CCBs, we hypothesized that the *NOS1AP* polymorphism rs10494366 might be associated with mortality in users of CCBs.

METHODS

Setting

Data were obtained from the Rotterdam Study, a population-based prospective cohort study of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases. All inhabitants of the suburb Ommoord in Rotterdam, who were over 55 years of age and had lived in the suburb for more than one year, were invited to participate in the study between 1990 and 1993. Of the 10,275 eligible persons, 7,983 participated (78%) and have been followed since then.[13,14] All participants were of Caucasian origin. The study was approved by the medical ethical committee of the Erasmus MC and all participants gave written informed consent.

At baseline, trained interviewers administered a questionnaire during a home interview, covering socioeconomic background and medical history, among other topics. During subsequent visits to the study center, additional interviewing, laboratory assessments and clinical examinations were performed, including recording of electrocardiograms (ECGs). Follow-up examinations were carried out periodically (every 4-5 years). The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2005 was available and included the product name of the drug, the amount dispensed, the prescribed dosage regimen and the date of dispensing. The duration of each prescription was calculated by dividing the total number of tablets or capsules dispensed by the daily prescribed number of tablets or capsules.

Cohort Definition

We identified all participants in the Rotterdam Study, who received a first prescription for a CCB between the baseline interview and January 1st 2005. Participants who were prescribed CCBs between January 1st 1991 and July 1st 1991 were excluded from the analysis, because they might have been using CCBs before January 1st 1991 in a period for which we had no pharmacy data. Participants who did not receive CCBs in the period of at least six months before the first prescription in the database were regarded as incident users. The time of the first prescription for a CCB was regarded as the date of entry into the study cohort. Participants were followed until one of the following events led to censoring: the end of the last prescription for a CCB, a period of no CCB use of more than 90 days calculated from the prescription data, switch to another CCB than the one on which the participant started, the occurrence of one of the study outcomes, or the end of the study period whichever came first.

Outcomes

All mortality cases in the study cohort were identified, by obtaining at regular intervals the vital status of the participants from the municipal population registry. After notification of death, cause and circumstances were established by information from the general practitioner, letters, and in case of hospitalization, discharge reports from medical specialists. Two research physicians coded all mortality independently according to the international classification of diseases (ICD), tenth edition.[15] In case of disagreement, consensus was sought. Participants who died within fourteen days after the end of the last prescription for a CCB were included in the analysis as current users.

In a subsequent analysis, cases of mortality that were coded as cardiovascular (ICD codes I00 through I99) were selected and cardiovascular mortality risks analyzed. In these analyses, we also included the ICD codes R96 (other sudden death, cause unknown), R98 (unattended death) and R99 (other ill-defined and unspecified causes of mortality). We also analyzed differences in the risk of a first myocardial infarction and fatal myocardial infarction as secondary outcomes.

Cofactors

Information was gathered at baseline on several potential covariates such as age and gender. All Cox proportional hazard models were adjusted for age and gender. To test whether the association between *NOS1AP* genotype and mortality or cardiovascular mortality was caused via an effect on the QTc interval, diabetes mellitus or hypertension, we also adjusted for these covariates. Diabetes mellitus was defined as any participant who had been diagnosed with diabetes mellitus at baseline. Diastolic and systolic blood pressures from the right upper arm were measured twice with a random-zero sphygmomanometer with the participant in sitting condition. The mean of the two readings was used to determine blood pressure levels. Hypertension was defined as use of antihypertensive drugs for the indication of high blood pressure, or a diastolic blood pressure of ≥90 mm Hg, or a systolic blood pressure of ≥140 mm Hg. The heart rate corrected QT interval (QTc) was calculated from the ECG readings, using the Bazett's formula ($QTc=QT/\sqrt{RR}$). Since this CCB subcohort was nested in the Rotterdam Study, baseline characteristics were assessed before the time of the first prescription for a CCB. Because there was little reason to assume that this biased our results, these baseline characteristics are used in the analyses. In additional analyses we adjusted for the time-varying determinants heart failure, diabetes mellitus, sulfonylurea co-medication (glibenclamide, tolbutamide, gliclazide and glimepiride) and cardiovascular co-medication (loop diuretics, other diuretics, β-blockers and angiotensin converting enzyme-inhibitors / angiotensin II antagonists) at the time of event.

Genotyping

All participants were genotyped for the *NOS1AP* SNP rs10494366 T>G which has previously been shown to be associated with a prolonged QTc interval.[3-5] This SNP was genotyped using Taqman assay C_1777074_10 (Applied Biosystems, Foster City, Ca., USA) in 1 ng of genomic DNA extracted from leukocytes, as previously reported.[16]

Statistical analysis

Deviation from Hardy-Weinberg equilibrium was tested using a χ^2-test. To test whether differences between genotypes were present at start of CCB therapy, we analyzed differences in time from baseline to start of CCB therapy with Cox proportional hazard models and differences in starting dose with one-way Anova.

Multivariate Cox proportional hazard models were constructed for the outcomes occurring during follow-up. First, all-cause and cardiovascular mortality in the whole Rotterdam Study were analyzed. Participants were followed from entrance in the Rotterdam Study, until death or end of the study period. Second, all-cause mortality, cardiovascular mortality, incident myocardial infarction and fatal myocardial infarction were analyzed in participants who were prescribed CCBs. The date of the first prescription was regarded as start of follow-up. We analyzed participants starting on dihydropyridine CCBs, non-dihydropyridine CCBs, and the individual drugs amlodipine, nifedipine, verapamil and diltiazem separately.

RESULTS

In the whole Rotterdam Study, 6,571 blood samples from participants were available for analysis; 6,292 people were successfully genotyped for the SNP rs10494366 and in 279 participants genotyping failed. The minor allele frequency was 0.36 (G allele). The genotype distribution of rs10494366 was in Hardy-Weinberg equilibrium in the Rotterdam Study (χ^2=1.04; p=0.59). No associations were found between *NOS1AP* genotype and all-cause mortality or cardiovascular mortality risks in the total group of participants, independent of whether they were prescribed CCBs or not. People with the TG or GG genotype had similar all-cause mortality risks (TG genotype hazard ratio (HR) 1.05; 95% CI 0.96, 1.14; GG genotype HR 1.08; 95% CI 0.96, 1.22) and cardiovascular mortality risks (TG genotype HR 1.01; 95% CI 0.90, 1.14; GG genotype HR 1.04; 95% CI 0.88, 1.23) as people in the reference group with the TT genotype.

1,113 of the 6,292 genotyped people (17.7%) were prescribed a first CCB during the study period and were included in the study cohort (table 1). The genotype distribution of rs10494366 was in Hardy-Weinberg equilibrium in the study cohort (χ^2=0.45; p=0.80). No differences among genotypes were seen in time from enrolment in the Rotterdam Study to a first prescription for a CCB, or in prescribed daily dose of the first prescription for a CCB.

During follow-up, 79 of the 1,113 participants (7.1%) who were prescribed CCBs for the first time during follow-up died while they were prescribed the CCB they started on. In participants with a first prescription for a dihydropyridine CCB, all-cause mortality risk was significantly higher in participants with the TG (HR 2.57; 95% CI 1.24, 5.34) or GG (HR 3.18; 95% CI 1.18, 8.58) genotype than in participants with the TT genotype (table 2). No associations were found between *NOS1AP* and all-cause mortality for participants with a first prescription for the non-dihydropyridine CCBs as a class or on verapamil or diltiazem individually.

In 54 of the 79 mortality cases, the cause of death was categorized as cardiovascular. In table 3 the associations between *NOS1AP* genotypes and cardiovascular mortality are given. Here as well, the cardiovascular mortality risk was significantly higher in participants with the TG (HR 3.51; 95% CI 1.41, 8.78) or GG (HR 6.00; 95% CI 1.80, 20.0) genotype with a first prescription for a dihydropyridine CCB, than in participants with the TT genotype. No differences were found in participants starting on verapamil or diltiazem.

The HRs for both all-cause mortality and cardiovascular mortality after adjustment for QTc interval, hypertension or diabetes mellitus are given in table 2 and 3. Adjusting for these covariates or for heart failure, diabetes mellitus, sulfonylurea co-medication and cardiovascular co-medication at the time of the event (data not shown) did not change the results essentially.

We also analyzed 34 cases of nonfatal and fatal myocardial infarction in the study population. Since numbers were too small to analyze the TG and GG genotype separately, these genotypes were grouped. In the participants who were prescribed dihydropyridine CCB the HR of any myocardial infarction (n=23) for participants with the TG or GG genotype was 1.31

Table 1 Characteristics of the study population of incident CCB users (n=1,113)

rs10494366 genotype	TT	TG	GG
Number	467	500	146
Gender, male (%)	44.8 %	40.2 %	43.8 %
Age (SD)	68.9 (7.9) years	68.0 (7.3) years	69.1 (7.5) years
Follow-up time (SD) [a]	11.4 (2.9) years	11.5 (2.9) years	10.7 (3.8) years
QTc (SD)	430.2 (27.8) msec	431.3 (28.8) msec	434.9 (25.1) msec
Hypertension (%)	69.1 %	68.6 %	74.4 %
Diabetes mellitus (%)	7.6 %	6.9 %	5.6 %
Body mass index (SD)	26.6 (3.6) kg/m^2	26.8 (3.5) kg/m^2	26.9 (4.1) kg/m^2
Serum creatinine (SD)	85.0 (25.1) µmol/l (n=367)	83.4 (16.5) µmol/l (n=392)	82.6 (15.5) µmol/l (n=99)
Start drug			
Dihydropyridine calcium channel blockers	283 (60.6%)	332 (66.4%)	86 (58.9%)
Non-dihydropyridine calcium channel blockers	184 (39.4%)	168 (33.6%)	60 (41.1%)
Dihydropyridine calcium channel blockers			
Amlodipine	22.9%	28.2%	29.5%
Nifedipine	26.6%	27.4%	21.2%
Felodipine	1.3%	0.4%	1.4%
Isradipine	2.6%	5.0%	2.7%
Nicardipine	2.4%	3.0%	1.4%
Nisoldipine	0.9%	0.4%	0%
Nitrendipine	0.2%	0%	0%
Lacidipine	0.6%	0.2%	0.7%
Barnidipine	2.6%	1.4%	2.1%
Lercanidipine	0.4%	0.4%	0%
Mibefradil	0.2%	0%	0%
Non-dihydropyridine calcium channel blockers			
Verapamil	11.6%	11.2%	13.0%
Diltiazem	27.8.%	22.4%	28.1%

[a] Follow-up time in the Rotterdam Study.

(95% CI 0.52, 3.31) compared with participants with the TT genotype. The risk of dying from a myocardial infarction (n=11) was higher in patients with the TG or GG genotype (HR 6.69; 95% CI 0.83, 53.8), although not statistically significant.

Table 2 Association between *NOS1AP* genotype and all-cause mortality (n=79) in 1,113 incident CCB users

			Model 1 [a]		Model 2 [b]		Model 3 [c]	
		Cases	HR	95% CI	HR	95% CI	HR	95% CI
Dihydropyridine calcium channel blockers (n=52)								
	TT	12	ref.		ref.		ref.	
	TG	30	**2.57**	**(1.24, 5.34)**	**2.50**	**(1.20, 5.19)**	**2.50**	**(1.19, 5.22)**
	GG	10	**3.18**	**(1.18, 8.58)**	**3.18**	**(1.18, 8.61)**	**3.25**	**(1.19, 8.85)**
Amlodipine								
	TT	4	ref.		ref.		ref.	
	TG	9	1.47	(0.45, 4.84)	1.48	(0.45, 4.86)	1.25	(0.38, 4.18)
	GG	4	2.65	(0.63, 11.1)	2.39	(0.58, 9.89)	2.56	(0.61, 10.8)
Nifedipine								
	TT	7	ref.		ref.		ref.	
	TG	16	**3.48**	**(1.19, 10.2)**	**3.68**	**(1.22, 11.1)**	**3.95**	**(1.28, 12.2)**
	GG	5	2.65	(0.53, 13.3)	2.54	(0.50, 12.8)	2.27	(0.42, 12.1)
Non-dihydropyridine calcium channel blockers (n=27)								
	TT	13	ref.		ref.		ref.	
	TG	9	0.83	(0.35, 2.01)	0.81	(0.34, 1.98)	0.80	(0.33, 1.96)
	GG	5	0.94	(0.30, 2.97)	0.95	(0.30, 3.01)	1.02	(0.29, 3.54)
Verapamil								
	TT	5	ref.		ref.		ref.	
	TG	4	0.65	(0.15, 2.86)	0.56	(0.12, 2.57)	0.46	(0.09, 2.35)
	GG	3	0.33	(0.03, 3.15)	0.50	(0.04, 6.22)	0.38	(0.02, 5.67)
Diltiazem								
	TT	8	ref.		ref.		ref.	
	TG	5	0.76	(0.24, 2.41)	0.76	(0.24, 2.41)	0.73	(0.22, 2.37)
	GG	2	0.73	(0.15, 3.53)	0.75	(0.15, 3.63)	0.73	(0.12, 4.22)

[a] Model 1: adjusted for age and gender. [b] Model 2: adjusted for age, gender and QTc interval. [c] Model 3: adjusted for age, gender, QTc interval, hypertension and diabetes mellitus.

DISCUSSION

In our study of 1,113 participants, we found a statistically significant three- to six fold increased cardiovascular mortality risk for participants with a G-allele at SNP rs10494366 while they were prescribed dihydropyridine CCBs. In the whole Rotterdam Study no differences were seen in cardiovascular mortality, indicating that the association between *NOS1AP* genetic variation and cardiovascular mortality is present only in participants who were prescribed dihydropyridine CCBs.

The precise mechanisms by which the common variation in the *NOS1AP* gene causes differences in mortality in participants who were prescribed dihydropyridine CCB is not known. Both nNOS, regulated by *NOS1AP*, and CCBs have an effect on intracellular calcium homeostasis. nNOS has negative feedback regulation of calcium release in the cytosol, because increases in calcium levels stimulate nNOS synthesis of NO, which in turn inhibits calcium

Table 3 Association between *NOS1AP* genotype and cardiovascular mortality (n=54) in 1,113 incident CCB users

			Model 1 [a]		Model 2 [b]		Model 3 [c]	
		Cases	HR	95% CI	HR	95% CI	HR	95% CI
Dihydropyridine calcium channel blockers (n=38)								
	TT	7	ref.		ref.		ref.	
	TG	22	**3.51**	**(1.41, 8.78)**	**3.40**	**(1.36, 8.51)**	**3.33**	**(1.32, 8.39)**
	GG	9	**6.00**	**(1.80, 20.0)**	**5.91**	**(1.77, 19.7)**	**6.38**	**(1.38, 22.2)**
Amlodipine								
	TT	2	ref.		ref.		ref.	
	TG	6	2.39	(0.47, 12.1)	2.41	(0.47, 12.3)	2.23	(0.44, 11.3)
	GG	3	4.49	(0.73, 27.8)	2.99	(0.47, 19.3)	3.23	(0.48, 21.7)
Nifedipine								
	TT	4	ref.		ref.		ref.	
	TG	12	**4.98**	**(1.32, 18.9)**	**5.22**	**(1.34, 20.3)**	**5.90**	**(1.49, 23.4)**
	GG	5	**11.0**	**(1.13, 107)**	8.80	(0.84, 92.0)	**14.7**	**(1.17, 184)**
Non-dihydropyridine calcium channel blockers (n=16)								
	TT	8	ref.		ref.		ref.	
	TG	5	0.85	(0.26, 2.80)	0.83	(0.25, 2.75)	0.77	(0.23, 2.63)
	GG	3	1.10	(0.21, 5.77)	1.10	(0.21, 5.83)	1.12	(0.18, 6.85)
Verapamil								
	TT	3	ref.		ref.		ref.	
	TG	1	0.49	(0.04, 6.09)	0.43	(0.03, 5.29)	0.56	(0.03, 8.92)
	GG	1	- [d]		- [d]		- [d]	
Diltiazem								
	TT	5	ref.		ref.		ref.	
	TG	4	1.01	(0.25, 4.08)	1.02	(0.25, 4.10)	0.96	(0.21, 4.26)
	GG	2	1.57	(0.27, 9.18)	1.48	(0.26, 8.45)	1.41	(0.19, 10.7)

[a] Model 1: adjusted for age and gender. [b] Model 2: adjusted for age, gender and QTc interval. [c] Model 3: adjusted for age, gender, QTc interval, hypertension and diabetes mellitus. [d] Numbers were too low to calculate hazard ratios.

release[7-11,17,18] Although the effects of nNOS have been mostly assessed in the cardiomyocyte, calcium plays a vital role in many other cells.

Differences in all-cause and cardiovascular mortality were only found for the dihydropyridine CCBs and not for verapamil and diltiazem, although modest sample sizes preclude definitive conclusions. The clinical relevance of our findings could be high because 16.5% of our population used a dihydropyridine CCB at any time during follow-up. Dihydropyridine CCBs have a higher affinity for vascular calcium channels, while verapamil and diltiazem have a higher affinity for the cardiac calcium channels. Verapamil and diltiazem are also used for the treatment of heart rhythm disturbances, such as atrial fibrillation, and angina pectoris, but adjusting for cardiovascular drugs co-prescribed with these indications did not change the results. It is suggested that dihydropyridine CCB relax coronary arteries by a NO mediated mechanism.[19,20] Although this has been attributed to the role of endothelial NOS, it is also possible that nNOS is involved. This may explain why differences were found for the dihydropyridine CCBs and not for verapamil and diltiazem.

Participants carrying a TG or GG genotype have a prolonged QTc interval, and therefore they might have an increased risk of arrhythmias and sudden cardiac death.[21] However, we do not think that this can explain our results. First, no associations between rs10494366 genotypes and all-cause mortality were seen in the whole Rotterdam Study. Second, adjusting for the QTc-interval at baseline did not change the results materially. It is suggested that the CCBs isradipine, nicardipine, verapamil and diltiazem can cause QTc prolongation, although the evidence is weak.[22, 23] The number of participants in the study cohort starting on isradipine or nicardipine therapy was small, so any QTc prolonging effect of these drugs could not have changed the results much. Recently, we identified an association between genetic variation in the *NOS1AP* gene and mortality in users of sulfonylurea.[24] Adjusting for diabetes mellitus, both at baseline and at the time of the event, and sulfonylurea use at the time of the event did not change the results either. Therefore, the effect of dihydropyridine CCBs on all-cause or cardiovascular mortality is not mediated by an effect on diabetes mellitus or prescribed sulfonylurea.

The risk of acute myocardial infarction was not increased but the risk of dying from a myocardial infarction was increased, albeit non-significantly. Increased mortality in users of nifedipine with myocardial infarction has also been observed in two double-blind randomized clinical trials, but no genetic determinants were assessed.[25,26] Although the number of cases was low and the results non significant, this may be an interesting issue for further research.

In population-based studies, bias might affect the obtained results. We believe that bias in our study is minimal. Information in the Rotterdam Study is collected prospectively, without prior knowledge of the study hypothesis. Therefore information bias is unlikely. We identified all participants who started on CCB therapy during follow-up. Selection bias may have occurred if there were differences in severity of disease or in allocation to CCB therapy among genotypes at entry in the study cohort caused by the *NOS1AP* polymorphism. However, the genotypes in this population were in Hardy-Weinberg equilibrium and no differences were found in time to start of CCB therapy or starting dose. The absence of blood samples and difficulties with genotyping were most likely independent of the genotype. It is also unlikely that confounding has influenced the results of our study, because all participants were incident users, and because physicians were unaware of the participant's genotype and could not base their drug choice on this information. In this study, drug use was calculated from filled prescriptions. In an earlier study published in this journal, we demonstrated that there was a high agreement in the Rotterdam Study for filled cardiovascular chronic medication and actual drug use as stated by the patient during interview.[27] There is always the possibility that the results are a chance finding. However, we think that this is probably not the case in our study. These analyses are not part of a genome wide association study, but we were testing an a priori hypothesis. Given the small number of cases in our study, it is necessary that the results will be replicated in further studies.

In the Caucasian population around 40 percent of the population has the TT genotype, while in Yoruba in Ibadan (Nigeria), Japanese in Tokyo and Han Chinese in Beijing only 10-15% of the population have the TT genotype.[28] As a consequence of this, the results of trials with dihydropyridine CCBs performed in a Caucasian population cannot be extrapolated unconditionally to other populations and vice versa. Regarding the polymorphism in the *NOS1AP* gene, it could be hypothesized that the risk of cardiovascular mortality in users of dihydropyridine CCB in Yoruba, Japanese and Chinese populations in general will be higher than in Caucasian populations.

To conclude, our results show that the genetic variation in the *NOS1AP* gene is associated with mortality risk in participants using dihydropyridine CCB. Participants with a TG or GG genotype at SNP rs10494366 have a higher all-cause and cardiovascular mortality risk than participants with the TT genotype. Because both the use of dihydropyridine CCBs and the allele frequencies of both alleles of the *NOS1AP* SNP rs10494366 are high, our results seem to be of substantial clinical impact, if replicated in further studies.

REFERENCES

1. Massion PB, Feron O, Dessy C, Balligand JL. Nitric oxide and cardiac function: ten years after, and continuing. *Circ Res* 2003;93(5):388-98.
2. Schulz R, Rassaf T, Massion PB, Kelm M, Balligand JL. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005;108(3):225-56.
3. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006;38(6):644-51.
4. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, et al. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. *Circulation* 2007;116(1):10-6.
5. Post W, Shen H, Damcott C, et al. Associations between genetic variants in the NOS1AP (CAPON) gene and cardiac repolarization in the old order Amish. *Hum Hered* 2007;64(4):214-9.
6. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol* 1983;245(1):C1-14.
7. Xu KY, Huso DL, Dawson TM, Bredt DS, Becker LC. Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci U.S.A.* 1999;96(2):657-62.
8. Ashley EA, Sears CE, Bryant SM, Watkins HC, Casadei B. Cardiac nitric oxide synthase 1 regulates basal and beta-adrenergic contractility in murine ventricular myocytes. *Circulation* 2002;105(25):3011-6.
9. Burkard N, Rokita AG, Kaufmann SG, et al. Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. *Circ Res* 2007;100(3):e32-44.
10. Sears CE, Bryant SM, Ashley EA, et al. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. *Circ Res* 2003;92(5):e52-9.
11. Xu L, Eu JP, Meissner G, Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 1998;279(5348):234-7.
12. Richard S. Vascular effects of calcium channel antagonists: new evidence. *Drugs* 2005;65 Suppl 2:1-10.
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, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
15. World Health Organization. International statistical classification of diseases and related health problems. Tenth revision. Geneva: World Health Organization; 1992.
16. Fang Y, van Meurs JB, d'Alesio A, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet* 2005;77(5):807-23.
17. Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J Biol Chem* 1993;268(35):26286-95.
18. Dittrich M, Jurevicius J, Georget M, et al. Local response of L-type Ca(2+) current to nitric oxide in frog ventricular myocytes. *J Physiol* 2001;534(Pt 1):109-21.
19. Ding Y, Vaziri ND. Nifedipine and diltiazem but not verapamil up-regulate endothelial nitric-oxide synthase expression. *J Pharmacol Exp Ther* 2000;292(2):606-9.
20. Lenasi H, Kohlstedt K, Fichtlscherer B, Mulsch A, Busse R, Fleming I. Amlodipine activates the endothelial nitric oxide synthase by altering phosphorylation on Ser1177 and Thr495. *Cardiovasc Res* 2003;59(4):844-53.

21. Straus SM, Kors JA, De Bruin ML, et al. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol* 2006;47(2):362-7.
22. QT drug lists. Arizona CERT. Available from http://www.torsades.org/medical-pros/drug-lists/drug-lists.htm Accessed 3 June 2007
23. De Ponti F, Poluzzi E, Cavalli A, Recanatini M, Montanaro N. Safety of non-antiarrhythmic drugs that prolong the QT interval or induce torsade de pointes: an overview. *Drug Saf* 2002;25(4):263-86.
24. Becker ML, Aarnoudse AJ, Newton-Cheh C, et al. Common variation in the NOS1AP gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea. *Pharmacogenet Genomics* 2008;18(7):591-7.
25. Goldbourt U, Behar S, Reicher-Reiss H, Zion M, Mandelzweig L, Kaplinsky E. Early administration of nifedipine in suspected acute myocardial infarction. The Secondary Prevention Reinfarction Israel Nifedipine Trial 2 Study. *Arch Intern Med* 1993;153(3):345-53.
26. Muller JE, Morrison J, Stone PH, et al. Nifedipine therapy for patients with threatened and acute myocardial infarction: a randomized, double-blind, placebo-controlled comparison. *Circulation* 1984;69(4):740-7.
27. 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(6):591-5.
28. The International HapMap Project. *Nature* 2003;426(6968):789-96.

Chapter 5.

Genetic factors affecting pharmacotherapy for Parkinson's disease

Chapter 5.1.

The OCT1 polymorphism rs622342 A>C is associated with decreased drug response and shorter survival time in Parkinson's disease

ABSTRACT

Background: The transporter OCT1, encoded by the *SLC22A1* gene, is responsible for the transportation of a wide variety of compounds including the anti-Parkinson drugs amantadine, pramipexole and, possibly, levodopa. Recently, we identified that the rs622342 A>C polymorphism in the *SLC22A1* gene is associated with the glucose lowering effect of metformin. We tested whether this polymorphism is also associated with response to anti-Parkinson drugs.
Methods: We identified all incident levodopa users in the Rotterdam Study, a population based cohort study. First, associations between the rs622342 polymorphism and the change in prescribed doses of levodopa and co-prescribed anti-Parkinson drugs in incident levodopa users was analyzed. Second, the association between the polymorphism and time from start of levodopa therapy to death was analyzed.
Results: We identified 99 incident levodopa users. Between the first and fifth prescription for levodopa, for each minor rs622342 C allele the prescribed doses of anti-Parkinson drugs increased with 0.35 defined daily dose (95% CI 0.073, 0.64; p=0.014). The differences were mainly caused by higher prescribed doses for amantadine and selegiline. With each minor C allele the mortality ratio after start of levodopa therapy was 1.47 times higher (95% CI 1.01, 2.13; p=0.045).
Conclusion: The rs622342 minor C allele is associated with higher prescribed doses of anti-Parkinson drugs and a shorter survival time after start of levodopa therapy. Most likely, transporters encoded by this variant allele transport anti-Parkinson drugs less efficient to the brain, resulting in more severe symptoms.

INTRODUCTION

Transporter proteins have a major role in the absorption, distribution and elimination of a wide variety of drugs and endogenous compounds. The family of organic cation transporters (OCT) is involved in the carriage of organic cations with at least one positively charged amine moiety at physiological pH. Substrates for OCT are the endogenous compounds epinephrine, histamine, serotonin and dopamine.[1,2] The three most important members of the OCT family are OCT1, OCT2 and OCT3, encoded by the *SLC22A1*, *SLC22A2* and *SLC22A3* gene respectively. These transporters differ in their substrate specificity and location in the body.[3] Besides endogenous compounds, several drugs are substrates for OCT. The hepatic uptake of the antidiabetic drug metformin by OCT1 is essential for its glucose lowering effect.[4,5] Several drugs, used in the treatment of Parkinson's disease are also substrates for OCT. Pramipexole, a selective dopamine receptor agonist, and amantadine, which has dopaminergic and anticholinergic properties, are substrates for the OCT1 and OCT2 subtypes.[2,6,7] Levodopa, a precursor of dopamine which crosses the blood-brain barrier, is also a substrate for OCT, although the subtype has not yet been identified.[8,9] Levodopa and dopamine agonists are indicated for the initial treatment of Parkinson's disease, and levodopa seems to be more effective than the dopamine receptor agonists.[10] Other drugs that can be used for the initial treatment are anticholinergic drugs, especially in cases where tremor is predominant, amantadine and selegiline.

The uptake of levodopa in the brain through transporters has an important role in suppressing symptoms of Parkinson's disease. The amino acids phenylalanine, leucine and isoleucine, structurally related to levodopa, competitively inhibit the transportation of levodopa to the brain and combining levodopa with these amino acids results in a reduced efficacy of levodopa.[11]

Single nucleotide polymorphisms (SNP) in the genes encoding transport proteins may result in transporters with reduced efficacy. Recently, we identified that the rs622342 A>C SNP in the *SLC22A1* gene, coding for OCT1, was associated with the glucose lowering effect in incident metformin users.[12] As this SNP might also be involved in the transportation efficacy of drugs used in the treatment of Parkinson's disease, it may affect the response to these drugs. In this population based cohort study, we studied whether prescribed doses for levodopa and co-prescribed anti-Parkinson drugs differed between rs622342 genotypes in incident levodopa users. We also studied the difference in survival time after start of levodopa therapy.

METHODS

Setting

Data for these analyses were obtained from the Rotterdam Study, a prospective population-based cohort study of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam. Participants were invited between 1990 and 1993 and have been continuously followed since then. All participants of the Rotterdam Study gave written informed consent. Ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. The rationale, ethical approval and design of this study have been described before.[13,14] All cases of mortality were identified, by obtaining at regular intervals the vital status of the participants from the municipal population registry. The seven pharmacies in Ommoord dispense the prescriptions of more than 99% of all participants. Information on all filled prescriptions from January 1st 1991 until January 1st 2008 was available and included the product name of the drug, the anatomical therapeutical chemical (ATC) code, the amount dispensed, the prescribed dosage regimen and the date of dispensing.[15]

Study Sample

The study sample consisted of all subjects in the Rotterdam Study who had a first prescription for levodopa between July 1st 1991 and January 1st 2008. Participants who had prescriptions for levodopa between January 1st 1991 and July 1st 1991 were excluded to ensure that only incident levodopa users were included. We also excluded all participants who were prescribed less than three prescriptions for levodopa, because, most likely, levodopa treatment was used as a diagnostic tool instead of treating Parkinson's disease. Participants were followed until death or end of the study period whichever came first.

Outcomes

We used two types of study outcome, the change in prescribed daily dose of levodopa and co-prescribed drugs to treat Parkinson's disease, and the difference in all-cause mortality.

First, for every prescription of levodopa, the change in prescribed daily dose for levodopa plus the dose of co-prescribed other anti-Parkinson drugs compared to the first prescription of levodopa was calculated. The influence of the rs622342 genotype on the change in prescribed daily doses was analyzed. To make the prescribed doses of different anti-Parkinson drugs comparable to each other, we divided the prescribed daily dose by the defined daily dose (DDD).[15] The DDD is a standardized dosing measure representing the recommended daily dose for the main indication in an adult. Second, we analyzed the difference between rs622342 genotypes in time from the first prescription for levodopa until death due to any cause.

Cofactors

Age at the time of the first levodopa prescription and gender were considered as potential determinants affecting the change in prescribed dose of anti-Parkinson drugs and the difference in all-cause mortality.

Genotyping

In this study we used the tagging SNPs on the Illumina 550k SNP array for genotyping according to the manufacturer's instruction. The tagging SNPs on the array were selected using an algorithm with which in a Caucasian population ninety percent of all phase I and II Hapmap SNPs are covered by at least one SNP on the array.[16-18] This coverage arises because genetic variation is transmitted in blocks, in which haplotype alleles exist. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. For this study we selected the tagging SNP rs622342 in the *SLC22A1* gene that was previously associated with metformin response.[12]

Statistical Analysis

Deviation from Hardy-Weinberg equilibrium was tested using a χ^2-test. To ensure that the rs622342 SNP did not affect the occurrence of Parkinson's disease, we analyzed the difference in time from July 1st 1991 to the first levodopa prescription between rs622342 genotypes in the whole Rotterdam Study using Cox proportional hazard models, and in incident levodopa users we analyzed the difference in prescribed daily dose of anti-Parkinson drugs at the time of the first levodopa prescription using multivariate linear regression and differences in prior use of other anti-Parkinson medication using a χ^2-test. For each prescription of levodopa, we calculated the change in prescribed daily doses of levodopa and co-prescribed anti-Parkinson drugs compared with the prescribed daily doses at the first prescription of levodopa. We analyzed the association between the number of rs622342 variant C alleles and change in prescribed daily doses of levodopa and co-prescribed anti-Parkinson drugs. As we analyzed the sequence of levodopa prescriptions, we tested whether there was a difference between genotypes in the average duration of levodopa prescriptions using unbalanced repeated measurements. Cox proportional hazard models were used to analyze the association between the number of rs622342 C variant alleles and time from the first levodopa prescription until death. Analyses were performed with SPSS software (version 11.0.1; SPSS, Chicago, IL), except for the unbalanced repeated measurements, which were performed with SAS (version 8.2; SAS, Cary, NC).

RESULTS

In the Rotterdam Study 186 participants were identified who were incident levodopa users. Forty-six levodopa users were excluded because a blood sample for genotyping was not available and one user was excluded because genotyping failed. Twenty-three levodopa users were prescribed levodopa before July 1st 1991 and 17 users were prescribed only one or two prescriptions for levodopa, and these participants were excluded. Eventually, 99 incident levodopa users were included (table 1).

Table 1 Baseline characteristics

rs622342 genotype		AA	AC	CC
N		39	49	11
Gender	Male (%)	17 (43%)	22 (45%)	3 (27%)
Age (SD)		77.7 (7.1) yr	78.0 (7.4) yr	78.8 (7.5) yr
Follow-up (SD)	In Rotterdam Study	11.8 (4.5) yr	10.0 (4.1) yr	9.4 (5.0) yr
	After start levodopa	5.0 (3.8) yr	4.6 (3.6) yr	3.5 (2.6) yr
Body-mass index (SD) [a]		28.2 (6.3) kg/m^2	26.2 (3.9) kg/m^2	27.6 (4.8) kg/m^2
Creatinine level (SD) [a]		85.9 (16.5) µmol/l	79.9 (14.2) µmol/l	79.8 (9.7) µmol/l
Prior use before start levodopa therapy				
	Anticholinergic drugs	5 (13%)	7 (14%)	1 (9%)
	Dopamine agonists	3 (8%)	4 (8%)	1 (9%)
	Amantadine	12 (31%)	11 (22%)	5 (45%)
	Selegiline	13 (33%)	15 (31%)	4 (36%)
Average prescribed dose of non levodopa anti-Parkinson drugs at start levodopa therapy		0.84 DDD	0.80 DDD	0.68 DDD

[a] At time of entrance in the Rotterdam Study.

The minor allele frequency (C allele) was 0.36 and the genotype distribution was in Hardy-Weinberg equilibrium (χ^2=0.57; p=0.45). The SNP rs622342 did not affect the time from July 1st 1991 until the first prescription for levodopa in all participants in the Rotterdam Study, genotyped for rs622342 (HR 0.96; 95% CI 0.72, 1.28). The drugs used to treat Parkinson's disease during the total follow-up time and the DDD of these drugs are given in table 2. No associations were found between the genotype and prescribed doses of anti-Parkinson drugs at start of levodopa therapy (-0.049 DDD; 95% CI –0.36, 0.26). Fifty-seven participants (58%) were prescribed other anti-Parkinson drugs before start of levodopa therapy and this percentage did not differ between genotypes (χ^2=0.32; p=0.85). The average time that drugs were dispensed for per levodopa prescription was 52.5 days and the rs622342 genotype was not associated with this duration (-1.8 days; 95% CI –7.5, 3.8).

The average prescribed dose of levodopa and co-prescribed drugs increased after start of levodopa therapy. The increase in prescribed dose was higher in patients with the rs622342

Table 2 Drugs used for the treatment of Parkinson's disease during total follow-up time

Drug class	Drug	Number of users	Number of prescriptions	Daily defined dose
Anticholinergics	Trihexyphenidyl	11	51	10 mg
	Biperiden	6	59	10 mg
	Metixene	1	2	40 mg
	Dexetimide	2	5	0.5 mg
	Orphenadrine	7	63	0.2 g
Levodopa	Levodopa	99	2,735	0.6 g
Amantadine	Amantadine	37	634	0.2 g
Dopamine agonists	Bromocriptine	3	37	40 mg
	Pergolide	13	593	3 mg
	Ropinirole	8	116	6 mg
	Pramipexole	3	11	2.5 mg
Selegiline	Selegiline	47	1,172	5 mg
Entacapone	Entacapone	11	273	1 g

CC genotype than in patients with the AA genotype, while patients with the AC genotype were in between (figure 1). The changes in prescribed daily doses of all anti-Parkinson drugs were significantly different from the third until the eighth prescription. With each minor C allele, the change in prescribed daily dose of all anti-Parkinson drugs between the first and fifth prescription for levodopa was 0.35 DDD higher (95% CI 0.073, 0.64). The prescribed daily dose of levodopa was 0.02 DDD higher (95% CI -0.037, 0.084) and the prescribed daily dose of other anti-Parkinson drugs was 0.33 DDD higher (95% CI 0.049, 0.61). Five patients (6%) were

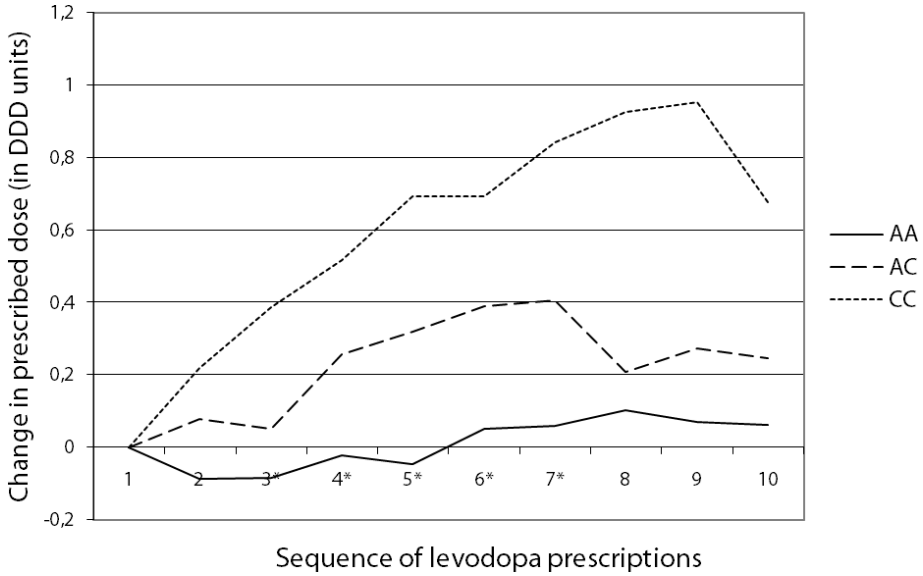


Figure 1 rs622342 genotype and change in prescribed daily doses of anti-Parkinson drugs (levodopa, dopamine agonists, selegiline, amantadine, anticholinergics, entacapone)

co-prescribed anticholinergic drugs, nineteen patients (22%) amantadine, twenty-nine patients (33%) selegiline and none entacapone. The change in prescribed daily dose was 0.038 DDD (95% CI -0.007, 0.083) for the anticholinergic drugs, 0.11 DDD (95% CI 0.01, 0.20) for amantadine and 0.19 DDD (95% CI -0.07, 0.45) for selegiline. Only six patients were prescribed dopamine agonists at the fifth prescription and in none of these patients the prescribed daily dose had changed between the first and fifth levodopa prescription and therefore changes in dopamine agonist doses did not contribute to the change in total prescribed daily dose of anti-Parkinson drugs.

During follow-up, 77 of the 99 participants (78%) died. The average survival time after start of levodopa therapy differed between rs622342 genotypes. The average survival time was 6.9 year for patients with the AA genotype, 5.2 year for patients with the AC genotype and 4.4 year for patients with the CC genotype (table 3). The mortality ratio was significantly raised with the number of minor C alleles (HR 1.47; 95% CI 1.01, 2.13).

Table 3 rs622342 and survival after start of levodopa therapy

rs622342	N	Mortality cases	Mean survival time (year)	95% CI	Mortality ratio [a] HR	95% CI	p-value
AA	39	24	6.9	(5.2, 8.6)	ref.		
AC	49	41	5.2	(4.0, 6.3)	1.50	(0.70, 2.49)	0.12
CC	11	8	4.4	(2.6, 6.2)	2.04	(0.87, 4.77)	0.10
Additive [b]					**1.47**	**(1.01, 2.13)**	**0.045**

[a] Adjusted for age and gender. [b] Number of variant C alleles.

DISCUSSION

In this population-based cohort study, the minor C allele of rs622342 in the *SLC22A1* gene, encoding the transporter OCT1, was associated with higher prescribed doses of anti-Parkinson drugs and a shorter survival after start of levodopa therapy. This SNP was previously associated with metformin response and the rs622342 minor C allele encodes most likely a less functioning OCT1 transporter. In this study, we could not exactly identify which drug or drugs contributed to the difference in prescribed daily dose of anti-Parkinson drugs. The only individual drug for which the prescribed daily doses were significantly associated with the rs622342 genotype was amantadine, and amantadine has previously been identified as substrate for OCT1.[19] The difference in prescribed daily doses of selegiline was not statistically significant, although the difference was larger for selegiline than for amantadine. Forty-six of the 88 patients were co-prescribed either amantadine or selegiline at the fifth prescription for levodopa. We cannot exclude that the rs622342 C variant allele is associated with a lower response to levodopa therapy and that this effect is compensated with higher prescribed doses of amantadine, selegiline and possibly other anti-Parkinson drugs. The shorter survival

period associated with the C allele of rs622342 suggests that multiple anti-Parkinson drugs are involved and that the lower response to one or more anti-Parkinson drugs is not compensated by higher prescribed doses of other drugs.

More transporters than OCT1 are involved in the transportation of anti-Parkinson drugs. Most research has focused on the transporters involved in levodopa carriage. Other transporters are involved, such as the other members of the OCT family, OCT2 and OCT3 encoded by the *SLC22A2* and *SLC22A3* genes and the L-type amino acid transporter1 and 2, encoded by *SLC7A5* and *SLC7A8*. The three OCTs are all involved in dopamine transport, although with varying affinity.[20-25] They differ in their expression throughout the body, distinguishing different roles in absorption, distribution and elimination of levodopa. OCT2 is predominantly expressed in the small intestine, kidney and brain and OCT3 in the heart and placenta.[2,22,24] LAT1 is located in the brain and LAT2 in the kidney and intestine.[26] Most likely, each transporter has its own role in the absorption, distribution and elimination process of drugs and other compounds.

OCT1 is expressed mainly in the liver and small intestine.[1,24] With metformin, the C allele is associated with a decreased transporter functioning in the liver. In this study the C allele was associated with reduced anti-Parkinson drug response, suggesting that OCT1 is not involved in the excretion of anti-Parkinson drugs. It is possible that the rs622342 C allele results in a reduced anti-Parkinson drug uptake from the small intestine resulting in a decreased biological availability.[24] However, OCT1 is also located in small amounts in the brains and we cannot exclude that these transporters are for example the rate-limiting step for uptake of anti-Parkinson drugs in the brain and responsible for the difference in anti-Parkinson drug response between rs622342 genotypes.[1]

In this study, we analyzed the consecutive prescriptions of levodopa and the anti-Parkinson drugs co-prescribed with levodopa. Both levodopa and dopamine agonists are the main drugs for the initial treatment of Parkinson's disease. In the Rotterdam Study, levodopa was used more frequently for this indication than dopamine agonists. Ninety-one participants were incident levodopa users without prior prescriptions for dopamine agonists, of whom eight participants started dopamine agonist therapy and later started levodopa therapy. Only nine participants were prescribed dopamine agonists and received no prescriptions for levodopa during follow-up, and these participants were not included in this study. In this group, we cannot exclude that the dopamine agonists were prescribed for other indications, such as restless-legs.

In population-based studies, bias may affect the obtained results. We believe that bias in our study is minimal. We identified all incident levodopa users in the Rotterdam Study and information was collected prospectively, without prior knowledge of the study hypothesis, making selection and information bias unlikely. The permission of patients to take blood and isolate DNA for scientific research was most likely independent from the genotype we studied. We did not find any difference in time to levodopa therapy, prior use of other anti-Parkinson

medication or prescribed doses of anti-Parkinson drugs at start of levodopa therapy, making it unlikely that the rs622342 polymorphism affects the progression to Parkinson's disease. The number of Parkinson's disease patients who were prescribed levodopa was limited. Therefore we cannot exclude that our results were a false-positive finding and replication of these results in another cohort is indicated.

To conclude, in this population-based cohort study, the rs622342 minor C variant allele in the *SLC22A1* gene, encoding OCT1, was associated with higher prescribed doses of drugs used to treat Parkinson's disease and had a shorter survival time after start of levodopa therapy. Most likely, this variant allele reduces the efficacy of the transportation of anti-Parkinson drugs by OCT1 to the brain. The results suggest that patients with the AC or CC genotype have less response to these drugs and more severe symptoms, resulting in a shorter survival period.

REFERENCES

1. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 2007;24(7):1227-51.
2. Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* 2004;308(1):2-9.
3. Shitara Y, Horie T, Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* 2006;27(5):425-46.
4. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* 2002;302(2):510-5.
5. Shu Y, Brown C, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
6. Okura T, Ito R, Ishiguro N, Tamai I, Deguchi Y. Blood-brain barrier transport of pramipexole, a dopamine D2 agonist. *Life Sci* 2007;80(17):1564-71.
7. Ishiguro N, Saito A, Yokoyama K, Morikawa M, Igarashi T, Tamai I. Transport of the dopamine D2 agonist pramipexole by rat organic cation transporters OCT1 and OCT2 in kidney. *Drug Metab Dispos* 2005;33(4):495-9.
8. Gomes P, Serrao MP, Viera-Coelho MA, Soares-da-Silva P. Opossum kidney cells take up L-DOPA through an organic cation potential-dependent and proton-independent transporter. *Cell Biol Int* 1997;21(4):249-55.
9. Pinto-do OP, Soares-da-Silva P. Studies on the pharmacology of the inward transport of L-DOPA in rat renal tubules. *Br J Pharmacol* 1996;118(3):741-7.
10. Miyasaki JM, Martin W, Suchowersky O, Weiner WJ, Lang AE. Practice parameter: initiation of treatment for Parkinson's disease: an evidence-based review: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2002;58(1):11-7.
11. Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The "on-off" phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. *N Engl J Med* 1984;310(8):483-8.
12. Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the Organic Cation Transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J.* 2009 Accepted.
13. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
14. 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.
15. Complete ATC index 2008. WHO. Collaborating Centre for Drug Statistics Methodology Web site. Available from http://www.whocc.no/atcddd. Accessed 6 August 2008.
16. Sentrix® HumanHap550 Genotyping Beadchip. Illumina Inc. Available from http://www.illumina.com/downloads/HUMANHAP550_DataSheet.pdf. Accessed 29 february 2008
17. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74(1):106-20.
18. The International HapMap Project. *Nature* 2003;426(6968):789-96.

19. Goralski KB, Lou G, Prowse MT, et al. The cation transporters rOCT1 and rOCT2 interact with bicarbonate but play only a minor role for amantadine uptake into rat renal proximal tubules. *J Pharmacol Exp Ther* 2002;303(3):959-68.
20. Breidert T, Spitzenberger F, Grundemann D, Schomig E. Catecholamine transport by the organic cation transporter type 1 (OCT1). *Br J Pharmacol* 1998;125(1):218-24.
21. Chemuturi NV, Donovan MD. Role of organic cation transporters in dopamine uptake across olfactory and nasal respiratory tissues. *Mol Pharm* 2007;4(6):936-42.
22. Grundemann D, Koster S, Kiefer N, et al. Transport of monoamine transmitters by the organic cation transporter type 2, OCT2. *J Biol Chem* 1998;273(47):30915-20.
23. Vialou V, Balasse L, Callebert J, Launay JM, Giros B, Gautron S. Altered aminergic neurotransmission in the brain of organic cation transporter 3-deficient mice. *J Neurochem* 2008;106(3):1471-82.
24. Martel F, Grundemann D, Calhau C, Schomig E. Apical uptake of organic cations by human intestinal Caco-2 cells: putative involvement of ASF transporters. Naunyn Schmiedebergs *Arch Pharmacol* 2001;363(1):40-9.
25. Grundemann D, Liebich G, Kiefer N, Koster S, Schomig E. Selective substrates for non-neuronal monoamine transporters. *Mol Pharmacol* 1999;56(1):1-10.
26. Del Amo EM, Urtti A, Yliperttula M. Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. *Eur J Pharm Sci* 2008;35(3):161-74.

Chapter 6.

General discussion

INTRODUCTION

Drug response varies widely between individuals. Thirty to sixty percent of patients do not show an efficacious response to important classes of therapeutic drugs.[1] Drug therapy is, conversely, regularly the cause of adverse reactions. Two to four percent of all hospital admissions are due to adverse reactions to drugs with a quarter to a half of these admissions being preventable.[2,3] A better understanding of why people do not respond to drug therapy or have adverse drug reactions can avoid part of these events and lead to more safe and effective drug use.

In this thesis, we studied the effects of both co-prescribed drugs and genetic variation on the response to drug therapy. We studied both because the effects are interlinked. For example, both genetic variation in the genes encoding the CYP enzymes and drug use may result in decreased or increased activity in metabolizing enzymes and transporters.

MAIN RESULTS

Co-prescribed drugs

Drug-drug interactions (DDIs) are common in healthcare and contribute substantially to the total number of adverse drug reactions. We calculated that in the elderly (≥70 years), one in five elderly people has been exposed to a DDI, with one in thirty four exposed to a DDI that was categorized as potentially life-threatening. In a literature review, analyzing the number of hospital admissions caused by DDIs, we calculated that one in every two hundred hospital admissions was due to DDIs, but in the elderly this increased to one in every twenty hospital admissions.

The impact drug-drug interactions have on the total number of hospital admissions, suggest that co-prescribed drugs have the potential to alter pharmacokinetic and pharmacodynamic parameters substantially. Community pharmacists in the Netherlands are obliged to intervene if a prescription implies too high a risk of patient harm, for example, due to DDIs. In chapter 2.2 and 2.3 we studied the dispensing of high-risk DDIs by community pharmacies. The results suggest a high level of medication surveillance in the Netherlands.

Genetic variation

The prescribed dose of a drug is a balance between the anticipated effectiveness of a drug at a certain dose and the anticipated risk of adverse reactions. A too low initial dose implies that there is a long titration period before therapeutic goals are accomplished, while too high a dose has a higher risk of adverse reactions. Genetic variation alters pharmacokinetic and pharmacodynamic parameters and explains part of the variation in drug response. A difference in the effect of co-prescribed drugs and genetic variation is that the effect of co-

prescribed drugs varies over time, while the effect of genetic variation is stable over time. The effect of genetic variation will be noticeable at the start of therapy, during the titration phase.

In this thesis, we assessed the effect of genetic variation in the *CYP2C9* gene [4-8] and *nitric oxide synthase 1 adaptor protein* (*NOS1AP)* gene on sulfonylureum response.[9-12] Tolbutamide users with a CYP2C9*3 polymorphism, resulting in decreased CYP2C9 enzyme activity, were prescribed lower doses than users with the wildtype genotype or CYP2C9*2 polymorphisms (chapter 3.1). Recently, the rs10494366 SNP in the *NOS1AP* gene was associated with an increase in QTc interval time on the ECG.[13-15] *NOS1AP* is a regulator of neuronal NOS (nNOS) and regulates intracellular calcium levels.[16,17] In glibenclamide users, the rs10494366 TG and GG genotype are associated with less glucose reducing effect and higher mortality rates than in glibenclamide users with the TT genotype (chapter 3.5). In tolbutamide and glimepiride users these genotypes were associated with lower mortality rates.

Metformin is not metabolized, but mainly excreted unchanged by the kidneys.[18] The carriage of metformin over membranes depends on transporters. The organic cation transporter 1 (OCT1) transporter, encoded by the *SLC22A1* gene, pumps metformin into the hepatocytes. [19-21] The multidrug and toxin extrusion 1 (MATE1) transporter, encoded by the *SLC47A1* gene, is also situated in the hepatocyte and is an efflux pump, which opposes the effect of OCT1. [20,22,23] The role of the OCT1 and MATE1 transporter in the distribution and elimination of metformin is presented in figure 1.

The rs622342 A>C SNP in the *SLC22A1* gene resulted in a 0.28% smaller decrease in HbA1c levels after start with metformin therapy. Most likely, the C polymorphism results in a reduced influx function, lower intracellular metformin levels in the hepatocyte and higher plasma glucose levels (chapter 3.2). The rs2289669 G>A SNP in the *SLC47A1* gene was associated with a 0.30 % larger decrease in HbA1c level and the A polymorphism may code for a reduced MATE1 efflux functioning (chapter 3.3). In chapter 3.4 we describe an interaction between the SNPs rs622342 and rs2289669. In metformin users with a normal OCT1 influx pump (rs622342 AA genotype), the effect of polymorphisms in the MATE1 efflux pump was limited. In metformin users with a crippled OCT1 influx pump and a normal functioning MATE1 efflux pump (rs2289669 GG genotype), the response to metformin was limited. Most likely, the efflux pump outperforms the crippled influx pump, resulting in low intracellular levels of metformin in the hepatocyte.

Another class of drugs studied was that of the cardiovascular drugs (chapter four). Two cholesterol lowering drugs (statins), simvastatin and atorvastatin, are transported by P-glycoprotein (P-gp), encoded by the *ABCB1* gene, and metabolized by the CYP3A4 enzyme.[24-29] Three common SNPs in the *ABCB1* gene (C1236T, G2677TA and C3435T) have been associated with digoxin, ciclosporin, mefloquine and antiretroviral drug response in previous studies. [30-34] We studied whether these polymorphisms, and the haplotypes that were derived from it, were associated with the cholesterol lowering effect of simvastatin (chapter 4.1). In men, the

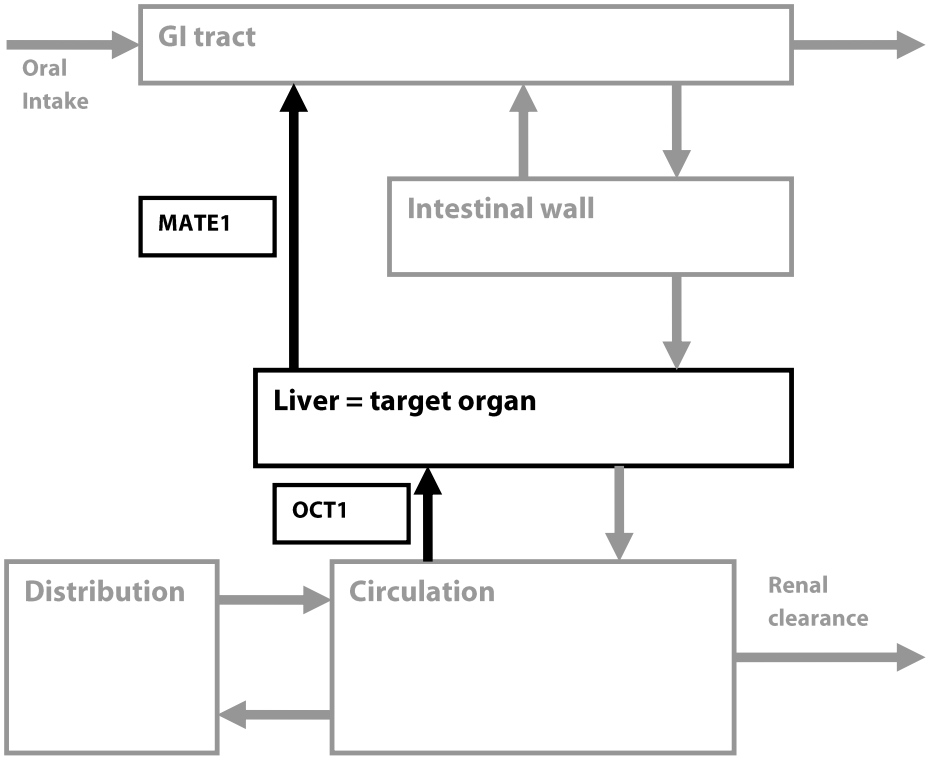


Figure 1 Diagram representing the distribution and elimination of metformin and the role of the transporters OCT1 and MATE1

haplotypes were associated with total and LDL cholesterol reduction. The reductions were larger in men with the TTT and CGT haplotype versus the CGC haplotype.

In chapter 4.2, we studied whether genetic variation in the *CYP3A4* or *ABCB1* genes is associated with a decrease of the prescribed dose or a switch to another cholesterol lowering drug during simvastatin and atorvastatin therapy. These events were used as a proxy and may indicate that statin plasma levels were too high and resulted in an adverse drug reaction or too large a reduction in cholesterol level. The CYP3A4*1B A>G SNP was associated with a decreased incidence of these events, while no associations were found for the *ABCB1* SNPs. The CYP3A4*1B A>G SNP results in higher CYP3A4 enzyme activity and decreased simvastatin and atorvastatin plasma levels.[35] The associations were stronger in women and in users with the *ABCB1* 3435 CT or TT genotype, although the interaction terms did not reach statistical significance.

The CYP3A4 enzymes and P-gp transporters are expressed both in the cells in the intestinal wall and hepatocytes (figure 2). In these cells, the *ABCB1* 3435 C>T SNP results in decreased P-gp efflux pump functioning, higher intracellular levels and more substrate availability for the CYP3A4 enzyme.[36-38] This may explain why the effect of the CYP3A4*1B polymorphism is stronger in users with the *ABCB1* 3435 CT or TT genotype. The gender differences in the effect

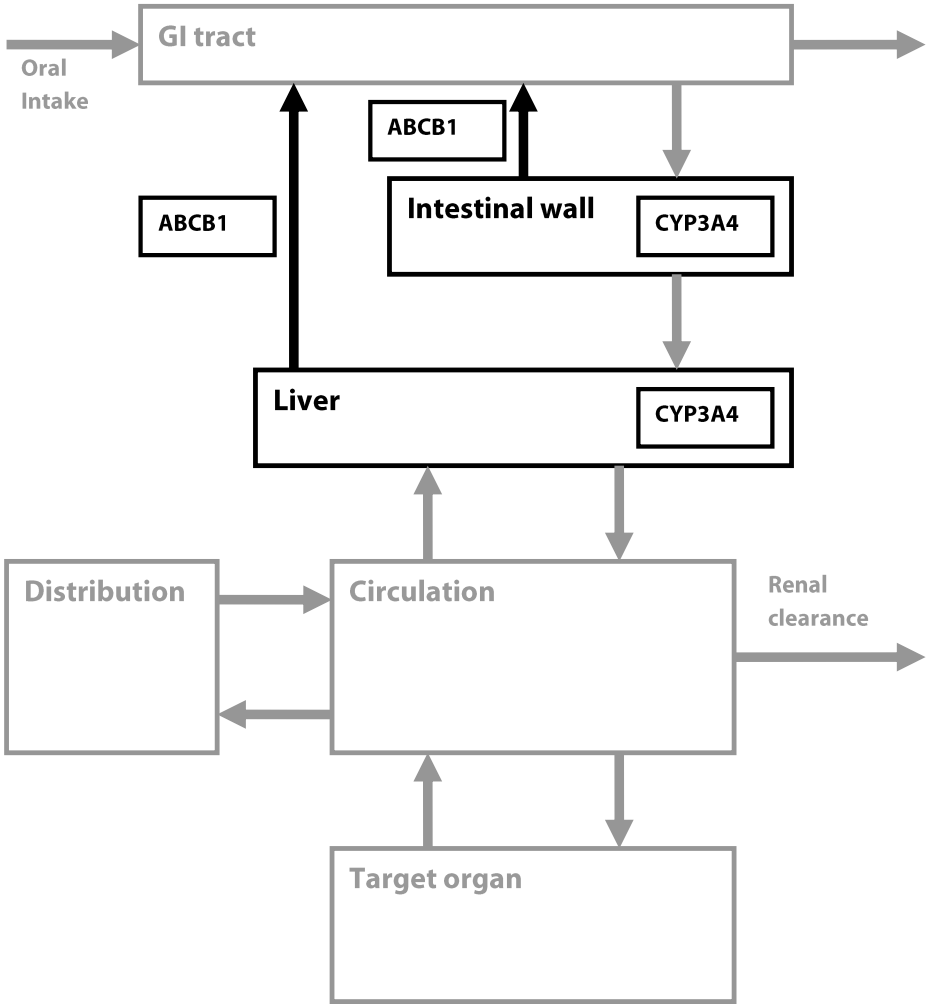


Figure 2 Diagram representing the distribution and elimination of simvastatin and atorvastatin and the role of the transporter ABCB1 and the enzyme CYP3A4

of polymorphisms in both the *ABCB1* and *CYP3A4* gene may be explained by differences in *ABCB1* expression. The expression of *ABCB1* is higher in men than in women.[39] Whether the CYP3A4 enzymes in the cells in the intestinal wall or the hepatocytes or both are involved cannot be concluded from the results in this thesis.

Calcium channel blockers (CCBs), a group of cardiovascular drugs, directly inhibit calcium currents through voltage dependent calcium channels. As both nNOS, regulated by *NOS1AP*, and CCBs regulate intracellular calcium levels, we studied the effect of the rs10494366 SNP in the *NOS1AP* gene on the incidence of diabetes mellitus (chapter 4.3) and on cardiovascular mortality (chapter 4.4) in CCB users. In CCB users with the TG or GG genotype at rs10494366,

the incidence of diabetes mellitus was two times lower than in users with the TT genotype. In the users of dihydropyridine CCB (e.g. amlodipine and nifedipine), the TG and GG genotype were associated with higher cardiovascular mortality rates than in users with the TT genotype. No associations were found in users of the non-dihydropyridine CCB (e.g. verapamil and diltiazem). The influx of calcium through voltage dependent calcium channels is a trigger in many physiological processes, such as contraction of the cardiomyocyte and insulin release by the pancreatic beta cells. This may be the explanation why this polymorphism in the *NOS1AP* gene is associated with the response to various classes of drugs.

Besides metformin, many drugs and other substances are substrates for the OCT1 transporter. OCT1 has a high affinity for substances with at least one positively charged amino moiety at physiological pH.[40] Drugs that are substrates for OCT1 are pramipexole, amantadine and, possibly, levodopa.[41-43] In chapter 5.1, we studied whether the SNP rs622342, which was associated with metformin response in chapter 3.2, was also associated with the response to anti-Parkinson drugs. In patients with one or more C alleles, the prescribed doses of anti-Parkinson drugs were 0.35 defined daily dose (DDD) higher per copy of the C allele. We also found that Parkinson patients with the CC genotype survived on average 4.4 years after the start of levodopa therapy versus 5.2 years for patients with the AC genotype and 6.9 years for patients with the AA genotype.

Genetic variation and co-prescribed drugs

The results on the effect of genetic variation on drug response give new insights into drug-drug interactions. For example, the OCT1 transporter is inhibited by several drugs, such as midazolam, quinidine, ritonavir and verapamil.[44] The results in chapter 3.2 suggest that combining these drugs with metformin therapy might result in less of a reduction in glucose levels, and the results of chapter 5.1 suggest a reduced effectiveness of anti-Parkinson drugs. However, these effects have not yet been proven in clinical practice. Inhibitors for the MATE1 transporter have not yet been described. Interestingly, the effect of selective MATE1 inhibitors, if they exist, may be stronger in metformin users with the rs622342 CC genotype on the OCT1 gene, as described in chapter 3.4.

The effect of CYP3A4*1B polymorphism and CYP3A4 inducers, such as carbamazepine and rifampicin, are similar. The effect of the CYP3A4*1B polymorphism suggest that the effects of CYP3A4 inducers on simvastatin and atorvastatin will be more pronounced in women and in patients with the *ABCB1* 3435 CT or TT genotype. Similarly, it may be hypothesized that the effects of CYP3A4 inhibitors, such as erythromycin, clarithromycin, itraconazol and voriconazol, on simvastatin and atorvastatin therapy will be stronger in women and in patients with the *ABCB1* 3435 CT or TT genotype. However, these drugs also inhibit the P-gp transporter, making it difficult to distinguish the individual effects.

CLINICAL PERSPECTIVE

Whether DDIs and genetic variation are clinically relevant to predict the response to a drug and are useful for individualizing pharmacotherapy, is not only dependent on the question of whether associations are statistically significant, but also depends on the strength of the association. Statistical significance depends on the effect size and the number of participants. For example, small differences may be clinically irrelevant but statistically significant in studies with large numbers of participants.

It is estimated that genetic variation has a major impact on drug response, although the majority have not yet been identified. Estimations vary from 12 to 98 percent.[45-48] These studies often assessed inter-individual and intra-individual differences in pharmacokinetic parameters such as metabolic ratios or clearance, and deduce from this information the estimated heritability.

In some analyses in this thesis, multivariate linear regression was used. In these analyses the coefficient of determination, r^2, is the proportion of variability in a data set that is accounted for by the statistical model (explained variance), or how well the predictors approximate the real values. These values give an indication of the practical usefulness of genetic testing. The r^2 values are given in table 1, for the non-genetic predictors, the genetic predictors and the total model. These values are adjusted for the number of predictors in the model.

The explained variance of the SNPs CYP2C9*3, rs622342 (OCT1), rs2289669 (MATE1) and *ABCB1* haplotypes on drug response in the total population of users varies from 0.0% to 7.0%. Although the associations are significant and may give new insights into the pharmacokinetics of these drugs, the effects of these genes on their own are too low to be clinically relevant. The explained variances of other predictors such as age, gender, drug doses and baseline values, which are easily available for the physician at the time of drug prescription, are similar to or larger than the explained variance of the SNPs.

Interestingly, the explained variance of genetic predictors increases considerably if the interaction between genes or the interaction with other predictors is taken into consideration. The glucose lowering effect of metformin is affected by the SNP rs622342 in the *SLC22A1* gene, coding for OCT1 and by the SNP rs2289669 in the *SLC47A1* gene, coding for MATE1. The explained variance of these SNPs individually is 5.3 percent and 7.0 percent, respectively. In a model in which both SNPs and the interaction term between these two SNPs are included, the explained variance increases to 25.1 percent. This is much higher than the explained variance of the two separate SNPs.

The effect of genetic variation in the *ABCB1* gene on the total and LDL cholesterol lowering effect of simvastatin is an example of drug-gender interaction. In the haplotype analyses for all simvastatin users, we found statistically significant differences between the TTT haplotype and the reference CGC haplotype in total cholesterol reduction, and differences between the TTT and CGT haplotype versus the reference CGC haplotype in LDL cholesterol reduction.

Table 1 Explained variance in drug response by genetic variation [a]

Drug	Genetic variation	Outcome	Covariates	Adjusted explained variance, r^2 [b]		
				Covariates, non-genetic	Genetic variation	Total model
Tolbutamide	CYP2C9*3 vs wildtype	Prescribed dose at tenth prescription	Age, sex	0.2 %	4.1 %	3.8 %
Metformin [c]	*SLC22A1* rs622342 (OCT1)	Delta HbA1c	Age, sex, HbA1c level before start, metformin dose, dose co-prescribed drugs	7.2 %	5.3 %	13.9 %
Metformin [c]	*SLC47A1* rs2289669 (MATE1)	Delta HbA1c	Age, sex, HbA1c level before start, metformin dose, dose co-prescribed drugs	8.5 %	7.0 %	16.4 %
Metformin [c,d]	rs622342 x rs2289669 (OCT1xMATE1)	Delta HbA1c	Age, sex, HbA1c level before start, metformin dose, dose co-prescribed drugs	8.0 %	25.1 %	46.5 %
Simvastatin	*ABCB1* haplotype	Delta total cholesterol	Age, sex, cholesterol level before start, simvastatin dose	47.6 %	0.0 %	52.8 %
Simvastatin	*ABCB1* haplotype	Delta LDL cholesterol	Age, sex, cholesterol level before start, simvastatin dose	48.8 %	0.0 %	57.5 %
Simvastatin	*ABCB1* haplotype	Delta total cholesterol in men	Age, sex, cholesterol level before start, simvastatin dose	23.3 %	27.9 %	43.1 %
Simvastatin	*ABCB1* haplotype	Delta LDL cholesterol in men	Age, sex, cholesterol level before start, simvastatin dose	33.4 %	35.2 %	62.3 %
Levodopa	*SLC22A1* rs622342 (OCT1)	Prescribed dose at fifth prescription for all anti-Parkinson drugs	Age, sex	3.7 %	6.1 %	8.1 %

[a] In this table, all studies are included in which linear regression was used. [b] As a percentage; zero (minimum) means model does not explain any variance at all, 100 (maximum) means model does explain all variance. [c] HbA1c level measurement in the period of 30 days before start and 15 to 100 days after start. [d] Genes plus interaction term.

In contradiction to these statistically significant associations is the minimal explained variance of these haplotypes. However, after stratification for gender, the amount of explained variance changes substantially. In men both the TTT and CGT haplotype are significantly associated with a stronger reduction in total cholesterol and LDL cholesterol than the CGC haplotype. The explained variance of the haplotypes in men is 27.9 percent for the reduction in total cholesterol and 35.2 percent for the reduction in LDL cholesterol.

We also found that the association between the CYP3A4*1B polymorphism and the incidence of adverse drug reactions in simvastatin and atorvastatin users was stronger in patients with the *ABCB1* 3435 CT or TT genotype than in patients with the CC genotype. This association was also stronger in men than in women. The interaction terms did not reach statistical significance. These associations were time-to-event associations and were analyzed with Cox proportional hazard analyses, and, therefore, the explained variance could not be calculated. These are other examples of gene-gene and gene-gender interactions.

OTHER GENETIC PREDICTORS

The genetic variation in genes coding for cytochrome P450 enzymes or transporters such as P-gp, OCT1 and MATE1 is responsible to an important extent for the phenotypic variation in pharmacokinetic parameters. Relatively little is known about genetic variation in pharmacodynamic parameters. For instance, several SNPs in the *ADRB1* and *ADRB2* gene, coding for the β_1 and β_2 receptor, have been identified. These SNPs have been associated with response to β blocker therapy and β agonist therapy in hypertension, heart failure and asthma.[49,50] The same applies for SNPs in genes coding for dopamine receptors and anti-Parkinson therapy.[51] Variation in drug response may also result from genetic variation in intracellular processes, such as intracellular messengers. In this thesis, we studied the effect of a common polymorphism in the *NOS1AP* gene. This polymorphism probably affects intracellular calcium levels, and with that the response to a wide variety of drugs. A previous study found associations with the SNP rs10494366 in the *NOS1AP* gene and response to digoxin.[52] In this thesis, we found associations with drug response to sulfonylurea users and CCBs.

As mentioned before, SNPs may affect the structure or amount of the protein they encode with consequences for biological function. A change in functioning in one protein may have consequences for the functioning or the expression of another protein. As presented in chapter 4.2, a reduced P-gp efflux functioning due to genetic variation in the *ABCB1* gene may result in an increase in substrate availability for the CYP3A4 enzyme and a larger effect of genetic variation in the *CYP3A4* gene.[36] It is also suggested that low *ABCB1* expression is compensated by overexpression of other transporters resulting, contradictorily, in low levels of HIV antiviral drugs.[34] High *ABCB1* expression has been associated with a reduction in HIV infectiveness.[34,53] These mechanisms compensate for the effects of genetic variation and result in a reduction of the phenotypic consequences of genetic variation, called phenotypic adaptation. Genetic variation in one gene may also directly affect the expression of another gene. For example, Hepatocyte Nuclear Factor-4α (HNF4A) is known to be involved in the expression of CYP enzymes.[54] The G60D SNP in this gene results in lower CYP2D6 metabolic activity, as measured by dextromethorphan metabolism.[55]

Copy number variations (CNV) are duplications of DNA fragments at least one kb in size and attribute to genetic variation in drug response.[56] In some people, the gene coding for the CYP2D6 enzyme is duplicated, resulting in higher expression of this gene (CYP2D6*xN).[57] Similarly, DNA fragments may be deleted, resulting in the absence of enzymes. For example the *CYP2D6* gene may be deleted (CYP2D6*5) resulting in the absence of CYP2D6 enzyme in homozygous persons. Drugs metabolized by CYP2D6 will reach high, probably toxic, plasma levels in patients without *CYP2D6* expression and low, probably ineffective, plasma levels in patients with duplicated *CYP2D6* expression.

Beside genetic variation, genes may also be switched on or off by the binding of methyl groups or other groups to the DNA, called epigenetics.[58,59] Methylation has traditionally been

associated with silenced genes. This information, although reversible, is contained during cell division and transferred from parents to children. These effects on gene expression may affect drug response, although little is yet known about the effects. The focus of research has partly shifted from genes to proteins. The latter area, proteomics, studies the structures and functions of proteins.[60] Proteomics may give us new insights into the function of proteins in drug response. In the treatment of leukemia, proteomics is used in an increasing extent to characterize the subtype, and individualize the pharmacotherapy.[61,62]

As genetic variation is much more than the effect of SNPs in metabolizing enzymes and transporters, the explained variance in drug response of up to 35 percent by the SNPs and interaction between them, as described in this thesis (table 1), is relatively high. However, for many drugs the percentage of explained variance that we can predict is much lower. As genetic variation is a major contributor to the variation in drug response [45-47], the remaining genetic variation may be explained by gene-gene interactions, SNPs in receptors and intracellular proteins, CNVs, deletions and epigenetics.

METHODOLOGICAL CONSIDERATIONS

The studies in chapter two, concerning the effect of co-prescribed drugs, differed in their study design. Two studies were literature reviews, one study was performed in a group of community pharmacies and one study was performed in the Rotterdam Study. The studies in chapter three, four and five were all performed in the Rotterdam Study and were similar in design.

Co-prescribed drugs

The main weakness in the studies on co-prescribed drugs or DDIs in chapter two, is the difference in definitions of a DDI. In chapter 2.1 we used the list of DDIs used by the Royal Dutch Association for the Advancement of Pharmacy (KNMP). The variation in prevalence of adverse drug reactions due to DDIs in chapter 2.4 may partly be explained by the variation in definitions used by the studies. Another drawback of chapter 2.4 is the difference in thoroughness of medication review, resulting in a varying number of missed cases. In chapter 2.3 we analyzed the dispensing of a limited number of DDIs, with a high risk of adverse patient outcomes. This definition may limit the generalisability of the study results, because the frequency of intermediate risk DDIs may be associated with other determinants. It is possible that more determinants would be identified if a broader range of DDIs was included.

Genetic variation

The studies on the effect of genetic variation in drug response were all performed in the Rotterdam Study.[63,64] The Rotterdam Study is a prospective population-based cohort study

of 7,983 Caucasians aged 55 years and older in the suburb Ommoord in Rotterdam, which was later extended with another cohort of approximately 3,000 people. All participants of the Rotterdam Study gave written informed consent and ethical approval was obtained from the medical ethical committee of the Erasmus MC. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, endocrine and ophthalmologic diseases. Observational studies may be hampered by selection bias, information bias and confounding. In the Rotterdam Study, only elderly Caucasian people were included, limiting the external validity. Moreover, observational studies and especially genetic studies are liable to false positive results.

In chapters three, four and five, we selected all (incident) users of sulfonylurea, metformin, simvastatin, atorvastatin, CCBs or levodopa. Selection bias may have occurred if the genetic variation under study was associated with the drug exposure itself. In the studies we did not find associations between the genetic variation and the baseline characteristics, making selection bias unlikely.

The study outcomes varied from prescribed doses, change in laboratory values, incident diabetes mellitus and mortality. The Rotterdam Study is a cohort study and data on incident diseases such as diabetes mellitus, and mortality was collected prospectively and independent of the study hypothesis, making information bias unlikely. In the studies evaluating prescribed doses or laboratory values, bias may have occurred if there were differences in duration of the prescriptions between genotypes or in the time from start of therapy until the laboratory measurement. No differences were found between genotypes, making information bias in these studies unlikely either.

The effect of genetic variation in genes coding for metabolizing enzymes or transporters will show itself only after the start of the drug, which is a substrate for these enzymes or transporters. Moreover, the patient and physician are both unaware of the patient's genotype. Therefore, genetic variation at baseline will be random, a phenomenon which is called Mendelian randomization. This random variation reduces the chance of confounding in our studies.

The Rotterdam Study only includes Caucasian people of at least 55 years of age. This age restriction limits the generalisability of our results because it has been suggested that the effect of genetic variation diminishes in older patients.[65] If true, this implies that the effect of genetic variation may be stronger in younger patients. Genetic variation differs between Caucasians and other races. As mentioned before, many other factors do affect drug response. These factors may vary between races, limiting the extrapolation of our results.

The primary aim of the Rotterdam Study was to study chronic diseases. The number of incident drug users represents drug use in the general population. Although almost 8,000 participants were included in the Rotterdam Study, the number of actual incident users was limited. For the change in glucose, HbA1c and cholesterol levels, we were dependent on laboratory measurements ordered by the general practitioners. These measurements were

not available for all incident users both before and after start. Therefore, the number of incident users for whom we had measurements both before and after start of drug therapy was further diminished. Limited sample sizes might have resulted in both false positive and false negative results. We identified associations not previously described in studies with limited sample size. This may result in false positive results and replication of these results is necessary. This is especially the case for the studies in which we used tagging SNPs in candidate genes to identify associations, although we adjusted for multiple testing using a Bonferroni correction.

FUTURE RESEARCH

Pharmacogenetic research used to focus on individual SNPs, which result in a change in amino acid sequence in the protein, and by that alter the function of the protein. Much effort was put into analyzing SNPs in the genes coding for CYP enzymes and more recently in analyzing SNPs in genes coding for transporters. Although many associations were found, the number of pharmacogenetic tests that have been incorporated into clinical practice is still limited. An exception is the test on genetic variation in the thiopurine methyltransferase (TPMT) enzyme which is now common practice before the start of therapy with azathioprine and mercaptopurine. This test being a predictor for the occurrence of bone marrow toxicity.[66] Testing on genetic variation in the CYP2D6 enzyme may also be useful in explaining why some people do not respond to antidepressant or antipsychotic therapy or develop adverse effects.

This genotype to phenotype approach, first determining the genetic variation and consequently determine the effect on drug response is now being increasingly replaced by a phenotype to genotype approach.[67] These phenotype to genotype studies may give us new insights into the question of how genetics affect drug response. Instead of studying SNPs that are well known for their effects on pharmacokinetics, other approaches like candidate-gene analyses and genome wide association (GWA) studies may increase the identification of new associations.

In candidate gene analyses, one gene or a limited number of genes that were previously associated with drug response, are selected. In each gene tagging SNPs are selected which represent variation in SNPs in the rest of the gene. This representation arises because genetic variation is transmitted in so-called haplotype blocks. Within these haplotypes, variant alleles are associated with each other. This more frequent occurrence of combinations of variant alleles than would be expected from a random formation is called linkage disequilibrium. The tagging SNP can be selected with the information on HapMap.[68] The major advantage of this method is that with a small number of genetic tests, a large degree of genetic variation can be analyzed. Until recently, the research focused on gene regions coding for amino acids,

called exons. The SNPs situated in the gene regions not coding for amino acids, called introns, were largely neglected. However, these SNPs in introns do affect transcription rates and have an impact on drug response. In this thesis we used candidate gene analysis to identify SNPs, not previously described, in the *SLC22A1* and *SLC47A1* gene coding for OCT1 and MATE1 and their association with metformin response. The rs622342 SNP in the *SLC22A1* gene and the rs2289669 SNP in the *SLC47A1* gene, associated in this thesis with metformin response, are situated in intron regions. These SNPs can be in linkage disequilibrium with other SNPs, making it difficult to identify the true SNP that affects drug response. With the use of tagging SNPs, new clinically relevant SNPs in introns may be identified in well studied genes such as the genes coding for CYP enzymes. A limitation of candidate gene analyses is that associations will not be found in genes which were not selected based on prior knowledge.

An example of the usefulness of this method is the study on hypersensitivity reactions with abacavir. Hypersensitivity reactions occurred relatively frequently in abacavir users, limiting the clinical applicability of this drug in the treatment of HIV infection. An association between hypersensitivity reaction in abacavir users and carriage of the major histocompatibility complex class I allele HLA-B*5701 was described.[69,70] A subsequent trial confirmed that genetic testing before the start of abacavir therapy could prevent this toxic adverse effect.[71] This test is now indicated before therapy.

A more advanced method is the genome wide association (GWA) study. In these studies the tagging SNPs are not limited to previously selected genes but cover the whole genome. The advantage being that because the whole genome is studied, genes are discovered which were not previously associated with drug response. The large number of tested SNPs, however, increases the risk of false positive results. To avoid this, only associations with very low p-values ($<10^{-6}$ - 10^{-8}) are regarded as significant. These low p-values can be attained either by very large study populations or by studying very strong associations. Including large numbers of participants in studies on drug use is difficult. However, some associations between SNPs and drug response are very strong. An example is a study on the risk of myopathy in statin users. A genome wide association (GWA) study tested whether genetic variation was associated with the incidence of myopathy in simvastatin users.[72] A SNP in the gene coding for the SLCO1B1 transporter revealed to be a strong predictor of myopathy in high-dose simvastatin users with a p-value of $4x10^{-9}$ and an attributable risk of more than 60 percent.

In this thesis GWA analyses were not performed, although we used the results of previous GWA studies. The SNP rs10494366 in the *NOS1AP* gene was identified to be associated with QTc prolongation in a GWA study and was later replicated in the Rotterdam Study.[13-15] These GWA studies gave us better insight into how intracellular calcium levels are handled. In this thesis, we assessed the effects of the SNP rs10494366 on drug response in sulfonylurea and calcium channel blocker users.

With these approaches, new SNPs can be identified that are associated with drug response. However, the prediction of drug response will be much better if interaction between SNPs

is taken into account. Testing of these interactions in studies without a priori knowledge requires very large populations, which is difficult to accomplish in pharmacogenetic research. The sample size which is required to test these interactions will increase by the square. A two-step approach is indicated. First, studies are needed that identify SNPs associated with drug response, irrespective of whether they are clinically relevant. Secondly, interaction with previously identified SNPs and interaction between other factors, such as gender and co-prescribed drugs, should be tested.

INDIVIDUALIZING PHARMACOTHERAPY

With the use of the genotype to phenotype approach, candidate gene analyses and GWA studies, new polymorphisms will be identified that are associated with drug response. One of the results of this thesis is that gene-gene interactions and gene-gender interactions can predict drug response much better than single SNPs. With the incorporation of gene-gene or gene-gender interactions the explained variance increases considerably, such as the interaction between OCT1 and MATE1 in metformin response and the effect of the CYP3A4*1B SNP in male statin users.

Two aspects of drug response are the (absence of) pharmacologic action, such as, in the case of antidiabetic drugs, the glucose lowering effect, and the occurrence of adverse reactions to a drug, such as myopathy in statin users. Studies on the genotyping may help in reaching targets sooner. In patients treated with the oral glucose lowering drug tolbutamide genotyping for the CYP2C9 genotype may help to shorten the time to reach the target dose. Similarly, genotyping for rs622342 and rs2289669 may help in the treatment with metformin. This may reduce costs due to fewer visits to the physician. In patients with the rs622342 CC genotype and rs2289669 AA genotype, with a low initial response to metformin, alternative treatments such as sulfonylurea and insulin could be used instead of metformin therapy.

In chapter five, the rs622342 SNP was associated with levodopa response and survival time. This may indicate that a reduced response to one or more anti-Parkinson drugs could not be compensated for by higher prescribed doses of other drugs. If true, genotyping will not be helpful in the identification of the right drug, although genotyping may be useful in predicting the progression of symptoms.

Chapter 4.2 focused primarily on the occurrence of adverse reactions. Adverse reactions during simvastatin and atorvastatin therapy were identified by using dose decreases or switches to other statins as a proxy. Around eight percent of the population is a carrier of the CYP3A4*1B variant G allele and this allele is associated with a lower incidence of adverse drug reactions. To be applicable in clinical practice, a polymorphism present in a small subgroup of the population and associated with a substantially increased risk would be more helpful.

In these people, the drug could be used more cautiously, for example, with lower doses, to avoid adverse drug reactions.

As described in chapter 3.5, 4.3 and 4.4, the rs10494366 SNP in the *NOS1AP* gene was associated with the response to sulfonylurea and calcium channel blockers (CCB). In dihydropyridine CCB users and in glibenclamide users, the G allele was associated with a higher mortality risk, while in tolbutamide and glimepiride users the G allele was associated with a lower mortality risk. The use of alternative drugs in patients with the genotype associated with a higher mortality risk might reduce mortality rates. However, replication of the results and clinical testing is necessary.

To give a definitive statement as to whether genetic testing is beneficial, a prospective trial with an alternative treatment in those with a lower response is mandatory. For example, in the treatment of metformin, all incident users should be randomized to two treatment arms. The first group receives conventional treatment with metformin, irrespective of their genotype. In the second group, patients with the genotype that corresponds to a poor response to metformin should be treated, for example, with sulfonylurea. Differences between these two groups in time to achieve treatment goals could be taken as an end-point.

The potential benefits of genetic testing must be weighted against the costs, and practical considerations must be taken into account. The costs of genetic testing are expressed both as the financial costs and the efforts for the patient, physician and others involved. Current knowledge on the effect of genetic variation on drug response is limited to a small number of SNPs per drug. For example, we identified two SNPs affecting the response to metformin treatment. In general, metformin treatment will be preceded by glucose or HbA1c measurements and blood will be available for genetic testing. With this information, a prediction can be made as to whether the starting dose should be lower or higher than normal and a small group of patients can be identified with a low response to metformin therapy. These patients could be treated preferentially with other oral glucose lowering drugs.

Pharmacists in the Netherlands are obliged to intervene if they suspect prescriptions with too high a risk of patient harm. Better knowledge on the effect of genetic variation on drug response will result in a better prediction of which patients have a higher risk of harm. If a patient is genotyped, this information should be made available for pharmacists. Pharmacists should incorporate this information into the medication surveillance program, and the pharmacist will be warned in case the patient is prescribed a high risk drug.

In the long term, the number of SNPs that are identified and associated with drug response will increase. With this information, a better prediction of drug response can be made. The costs of genotyping will decrease in the long term, making genotyping of a large number of SNPs or all SNPs on the whole genome available for daily practice. In the future, information on relevant SNPs will be available for all the patients before start of therapy. With a logarithm, the recommended dose and the chance of adverse drug reactions can be calculated, thereby individualizing pharmacotherapy.

The information on the effect of genetic variation should preferably be available at the time of prescribing. Nowadays medication surveillance is carried out at the time of dispensing. If the logarithm can calculate the dose and the risk of adverse drug reactions automatically and unambiguously, the prescribing physician can use this information instantaneously. The experience with medication surveillance software indicates that interpretation of the results is necessary and knowledge on the pharmacokinetic and pharmacodynamic properties of a drug essential. To make this information and knowledge available at the time of prescribing, a better cooperation between physician and pharmacist is required.

CONCLUSIONS

A better prediction of drug response will result in both the prevention of non-response and adverse reactions. In this thesis we studied the effect of both co-prescribed drugs and genetic variation on drug response.

DDIs are a major contributor to adverse reactions, which result in hospitalization. The exposure to DDIs has doubled between 1992 and 2005 in the elderly. There is a large similarity between DDIs and drug-gene interactions, for example in the case of inhibition or induction of the CYP enzymes, suggesting that drug-gene interactions are also a major contributor to adverse drug reactions.

In this thesis, the CYP2C9*3 SNP (tolbutamide), the rs622342 SNP in the *SLC22A1* gene (metformin, anti-Parkinson drugs), the rs2289669 SNP in the *SLC47A1* gene (metformin), the rs10494366 SNP in the *NOS1AP* gene (sulfonylurea, CCBs), *ABCB1* haplotypes (simvastatin) and the CYP3A4*1B SNP (simvastatin and atorvastatin) were associated with drug response. New approaches for the identification of SNPs were successfully used, such as the candidate gene analysis with tagging SNPs. These approaches may be useful in identifying new SNPs that are associated with drug response.

The prediction of drug response increased if the effect of interaction between two genes was analyzed or the effect of a single gene was analyzed in males or females separately. We identified a gene-gene interaction between OCT1 and MATE1. These two SNPs combined explain 25 percent of the variance in drug response versus five and seven percent for the individual SNPs, respectively. The prediction of *ABCB1* haplotypes on simvastatin response was stronger in men than in women, indicating a gene-gender interaction. Similar interactions were also suggested for *ABCB1* and *CYP3A4*, and *CYP3A4* and gender.

The results of our study may be helpful in identifying new DDIs or in identifying patients who are more susceptible to adverse reactions from DDIs. The results suggest that the combination of OCT1 inhibitors, such as midazolam and verapamil, with anti-Parkinson drugs may be detrimental in the suppression of symptoms. The effects of the DDI between CYP3A4

inducers and simvastatin or atorvastatin may be stronger in women and in users with the *ABCB1* 3435 CT or TT genotype.

Confirmation in other studies and randomized clinical trials is necessary before introducing these predictors in clinical practice. Further improvement beyond SNPs and interactions may be achieved by analyzing other genetic variation such as CNVs, deletions and epigenetics.

In the near future, medication surveillance will incorporate information on genetic variation, making it easier to identify prescriptions which involve too high a risk of patient harm and require intervention. In the long term, algorithms will be available which will calculate the recommended dose and risk of adverse drug reactions based on information on co-prescribed drugs and genetic variation among other variables. These algorithms will guide individualized pharmacotherapy.

REFERENCES

1. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends Mol Med* 2001;7(5):201-4.
2. Leendertse AJ, Egberts AC, Stoker LJ, van den Bemt PM. Frequency of and risk factors for preventable medication-related hospital admissions in the Netherlands. *Arch Intern Med* 2008;168(17):1890-6.
3. Beijer HJ, de Blaey CJ. Hospitalisations caused by adverse drug reactions (ADR): a meta-analysis of observational studies. *Pharm World Sci* 2002;24(2):46-54.
4. Kirchheiner J, Roots I, Goldammer M, Rosenkranz B, Brockmoller J. Effect of genetic polymorphisms in cytochrome P450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet* 2005;44(12):1209-25.
5. Kirchheiner J, Bauer S, Meineke I, et al. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002;12(2):101-9.
6. Kirchheiner J, Brockmoller J, Meineke I, et al. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002;71(4):286-96.
7. Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002;72(3):326-32.
8. Shon JH, Yoon YR, Kim KA, et al. Effects of CYP2C19 and CYP2C9 genetic polymorphisms on the disposition of and blood glucose lowering response to tolbutamide in humans. *Pharmacogenetics* 2002;12(2):111-9.
9. Gunawardana SC, Rocheleau JV, Head WS, Piston DW. Mechanisms of time-dependent potentiation of insulin release: involvement of nitric oxide synthase. *Diabetes* 2006;55(4):1029-33.
10. Lajoix AD, Reggio H, Chardes T, et al. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. *Diabetes* 2001;50(6):1311-23.
11. Ashcroft FM, Rorsman P. Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 1989;54(2):87-143.
12. Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. *Diabetes* 2002;51 Suppl 3:S368-76.
13. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006;38(6):644-51.
14. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, et al. Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. *Circulation* 2007;116(1):10-6.
15. Post W, Shen H, Damcott C, et al. Associations between genetic variants in the NOS1AP (CAPON) gene and cardiac repolarization in the old order Amish. *Hum Hered* 2007;64(4):214-9.
16. Schulz R, Rassaf T, Massion PB, Kelm M, Balligand JL. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. *Pharmacol Ther* 2005;108(3):225-56.
17. Massion PB, Pelat M, Belge C, Balligand JL. Regulation of the mammalian heart function by nitric oxide. *Comp Biochem Physiol A Mol Integr Physiol* 2005;142(2):144-50.
18. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med* 2002;137(1):25-33.
19. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;117(5):1422-31.

20. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics* 2008;9(4):415-22.
21. Shu Y, Brown C, Castro RA, et al. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *Clin Pharmacol Ther* 2008;83(2):273-80.
22. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci U.S.A.* 2005;102(50):17923-8.
23. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 2007;74(2):359-71.
24. Chen C, Lin J, Smolarek T, Tremaine L. P-glycoprotein has differential effects on the disposition of statin acid and lactone forms in mdr1a/b knockout and wild-type mice. *Drug Metab Dispos* 2007;35(10):1725-9.
25. Hochman JH, Pudvah N, Qiu J, et al. Interactions of human P-glycoprotein with simvastatin, simvastatin acid, and atorvastatin. *Pharm Res* 2004;21(9):1686-91.
26. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* 2006;80(6):565-81.
27. Jacobson TA. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *Am J Cardiol* 2004;94(9):1140-6.
28. Jacobsen W, Kuhn B, Soldner A, et al. Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. *Drug Metab Dispos* 2000;28(11):1369-78.
29. Prueksaritanont T, Gorham LM, Ma B, et al. In vitro metabolism of simvastatin in humans identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos* 1997;25(10):1191-9.
30. Chinn LW, Kroetz DL. ABCB1 pharmacogenetics: progress, pitfalls, and promise. *Clin Pharmacol Ther* 2007;81(2):265-9.
31. Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics* 2008;9(1):105-27.
32. Aarnoudse AJ, Dieleman JP, Visser LE, et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. *Pharmacogenet Genomics* 2008;18(4):299-305.
33. Aarnoudse AL, van Schaik RH, Dieleman J, et al. MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine. *Clin Pharmacol Ther* 2006;80(4):367-74.
34. Fellay J, Marzolini C, Meaden ER, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002;359(9300):30-6.
35. 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(4):299-305.
36. Cummins CL, Wu CY, Benet LZ. Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein. *Clin Pharmacol Ther* 2002;72(5):474-89.
37. Parkinson A, Mudra DR, Johnson C, Dwyer A, Carroll KM. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* 2004;199(3):193-209.
38. Tanaka E. Gender-related differences in pharmacokinetics and their clinical significance. *J Clin Pharm Ther* 1999;24(5):339-46.

39. Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* 1995;275(2):1011-8.
40. Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* 2004;308(1):2-9.
41. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 2007;24(7):1227-51.
42. Gomes P, Serrao MP, Viera-Coelho MA, Soares-da-Silva P. Opossum kidney cells take up L-DOPA through an organic cation potential-dependent and proton-independent transporter. *Cell Biol Int* 1997;21(4):249-55.
43. Pinto-do OP, Soares-da-Silva P. Studies on the pharmacology of the inward transport of L-DOPA in rat renal tubules. *Br J Pharmacol* 1996;118(3):741-7.
44. Drug evelopment and drug interactions: table of substrates, inhibitors and inducers. U.S. Food and Drug Administration. Available from http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm. Accessed 20 December 2008.
45. Leabman MK, Giacomini KM. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* 2003;13(9):581-4.
46. Kalow W, Endrenyi L, Tang B. Repeat administration of drugs as a means to assess the genetic component in pharmacological variability. *Pharmacology* 1999;58(6):281-4.
47. Kalow W, Tang BK, Endrenyi L. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics* 1998;8(4):283-9.
48. Weinshilboum R. Inheritance and drug response. *N Engl J Med* 2003;348(6):529-37.
49. Contopoulos-Ioannidis DG, Kouri I, Ioannidis JP. Pharmacogenetics of the response to beta 2 agonist drugs: a systematic overview of the field. *Pharmacogenomics* 2007;8(8):933-58.
50. Taylor MR. Pharmacogenetics of the human beta-adrenergic receptors. *Pharmacogenomics J* 2007;7(1):29-37.
51. Arbouw ME, van Vugt JP, Egberts TC, Guchelaar HJ. Pharmacogenetics of antiparkinsonian drug treatment: a systematic review. *Pharmacogenomics* 2007;8(2):159-76.
52. Aarnoudse AL, Dieleman JP, Newton-Cheh C, et al. Common NOS1AP variants potentiate digoxin induced risk of sudden cardiac death. Submitted 2008.
53. Lee CG, Ramachandra M, Jeang KT, Martin MA, Pastan I, Gottesman MM. Effect of ABC transporters on HIV-1 infection: inhibition of virus production by the MDR1 transporter. *Faseb J* 2000;14(3):516-22.
54. Tirona RG, Lee W, Leake BF, et al. The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat Med* 2003;9(2):220-4.
55. Lee SS, Cha EY, Jung HJ, et al. Genetic polymorphism of hepatocyte nuclear factor-4alpha influences human cytochrome P450 2D6 activity. *Hepatology* 2008;48(2):635-45.
56. Freeman JL, Perry GH, Feuk L, et al. Copy number variation: new insights in genome diversity. *Genome Res* 2006;16(8):949-61.
57. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5(1):6-13.
58. Feinberg AP. Epigenetics at the epicenter of modern medicine. *JAMA* 2008;299(11):1345-50.
59. Ptashne M. On the use of the word 'epigenetic'. *Curr Biol* 2007;17(7):R233-6.
60. Service RF. Proteomics. Proteomics ponders prime time. *Science* 2008;321(5897):1758-61.
61. Unwin RD, Whetton AD. How will haematologists use proteomics? *Blood Rev* 2007;21(6):315-26.
62. Hegedus CM, Gunn L, Skibola CF, et al. Proteomic analysis of childhood leukemia. *Leukemia* 2005;19(10):1713-8.

63. 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.
64. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22(11):819-29.
65. Schwartz JB. The current state of knowledge on age, sex, and their interactions on clinical pharmacology. *Clin Pharmacol Ther* 2007;82(1):87-96.
66. Evans WE. Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. *Ther Drug Monit* 2004;26(2):186-91.
67. Roden DM, Altman RB, Benowitz NL, et al. Pharmacogenomics: challenges and opportunities. *Ann Intern Med* 2006;145(10):749-57.
68. The International HapMap Project. *Nature* 2003;426(6968):789-96.
69. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002;359(9312):1121-2.
70. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;359(9308):727-32.
71. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008;358(6):568-79.
72. Link E, Parish S, Armitage J, et al. SLCO1B1 variants and statin-induced myopathy-a genomewide study. *N Engl J Med* 2008;359(8):789-99.

Chapter 7.

Summary

Chapter 7.1.

Summary

The aim of drug therapy is in general to cure diseases or reduce symptoms. However, drug therapy is ineffective in 30 to 60 percent of the patients and, on the other hand, two to four percent of all hospital admissions result from adverse drug reactions. A better prediction which patients will not respond to drug therapy or will develop adverse drug reactions may avoid these events (**chapter 1**). In this thesis, we analyzed both the effect of co-prescribed drugs and genetic variation on drug response.

In chapter two, we studied the exposure to and clinical consequences of drug-drug interactions (DDI). The exposure to DDIs in the elderly general population (≥70 years of age) has almost doubled between 1992 and 2005 from ten to nineteen percent (**chapter 2.1**). Also the exposure to potentially life threatening DDIs almost doubled from 1.5 percent in 1992 to 2.9 percent in 2005. In the Netherlands, pharmacists are obliged to intervene prescriptions that imply a high risk for the patient. In **chapter 2.2 and 2.3** we studied which factors were involved in the dispensing of prescriptions which involved a DDI with a high risk for patient harm. In the literature, the relationship with the prescriber, the medication surveillance software and pharmacy organization were described as factors associated with these dispensings. In a subsequent study, we analyzed whether these factors were associated with the dispensing of high-risk DDIs in community pharmacies in the Netherlands. Pharmacies using the Euroned medication surveillance program and pharmacies that were part of a health care centre dispensed one high risk DDI more often. The clinical consequences of DDIs were studied in a literature review (**chapter 2.4)**. About half a percent of all hospital admissions were due to DDIs. In the elderly this proportion was about five percent.

In chapter three we studied the effect of genetic variation on the response to drugs used in the treatment of type 2 diabetes mellitus. The antidiabetic drug tolbutamide, one of the drugs in the sulfonylurea group, is metabolized by CYP2C9. Incident tolbutamide users with a CYP2C9*3 variant allele were prescribed lower doses than users with the wildtype genotype, most likely due to a decrease in tolbutamide metabolism (**chapter 3.1**). In **chapter 3.2 and 3.3** the antidiabetic drug metformin was studied. Metformin is not metabolized, but genetic variation in transporters involved in the carriage of metformin may affect the glucose lowering effect. Metformin is a substrate for the organic cation transporter 1 (OCT1), encoded by the *SLC22A1* gene, and the multidrug and toxin extrusion 1 **(**MATE1) transporter, encoded by the *SLC47A1* gene. Both OCT1 and MATE1 are located in the hepatocyte, OCT1 transports metformin into the hepatocyte and MATE1 transports metformin out of the hepatocyte into the bile. We studied whether genetic variation in these genes is associated with the change in HbA1c level in incident metformin users. The rs622342 minor C allele in the *SLC22A1* gene was associated with a 0.3 % smaller reduction in HbA1c level and the rs2289669 minor A allele in the *SLC47A1* gene with a 0.3 % larger reduction. Most likely, the rs622342 C allele codes for a crippled OCT1 transporter, and the rs2289669 A allele for a crippled MATE1 transporter. In **chapter 3.4** we describe an interaction between these polymorphisms. The effect of the rs2289669 polymorphism is stronger in patients with the rs622342 CC genotype than

in patients with the AA or AC genotype. In patients with a crippled OCT1 influx transporter (rs622342 CC genotype) and a normal functioning MATE1 efflux transporter (rs2289669 GG genotype), the MATE1 efflux transporter will outperform the OCT1 influx transporter, resulting in low intracellular metformin levels and a hampered glucose lowering effect. In other patients, OCT1 will outperform MATE1, and the glucose lowering effect will be normal.

Recently, the rs10494366 SNP in the *NOS1AP* gene was associated with an increased QTc interval. Most likely, this SNP regulates intracellular calcium levels through an effect on the inward calcium channel currents. Sulfonylurea, a group of antidiabetic drugs, indirectly trigger the opening of voltage dependent calcium channels. In view of these similarities, we studied in **chapter 3.5** whether this SNP is associated with response to sulfonylurea. In glibenclamide users, the rs10494366 TG and GG genotype are associated with a reduced response and higher mortality rates than in glibenclamide users with the TT genotype. In tolbutamide and glimepiride users these genotypes were associated with lower mortality rates.

The effects of genetic variation on two groups of cardiovascular drugs, the statins and calcium channel blockers (CCB), were studied in chapter four. The statins simvastatin and atorvastatin are substrates for the P-glycoprotein (P-gp) transporter, encoded by the *ABCB1* gene, and the CYP3A4 metabolizing enzyme. In **chapter 4.1**, the association between the 1236/2677/3435 haplotypes in the *ABCB1* gene and the cholesterol lowering effect of simvastatin was studied. In men, the TTT and CGT haplotype were associated with a 0.40 to 0.53 mmol/l larger reduction in total and LDL cholesterol levels than the reference CGC haplotype. In women, no significant associations were found. In **chapter 4.2** we studied whether the polymorphisms C1236T, G2677AT and C3435T in the *ABCB1* gene and the polymorphism CYP3A4*1B were associated with a decrease of the prescribed dose or a switch to another cholesterol lowering drug during simvastatin and atorvastatin therapy, possibly indicating adverse drug reactions or a too strong reduction in cholesterol level. Simvastatin and atorvastatin users with the variant CYP3A4*1B variant G allele had a two times lower risk for a dose decrease or switch to another cholesterol lowering drug. No associations were found for the *ABCB1* polymorphisms or haplotypes. Women with the CYP3A4*1B variant G allele had a three times lower risk than women with the CYP3A4*1B reference A allele and in the group of *ABCB1* CT or TT genotype carriers the CYP3A4*1B variant G was associated with 2.5 times lower risk.

As mentioned before, the rs10494366 SNP in the *NOS1AP* gene was associated with an increased QTc interval, most likely due to an effect on the inward calcium channel currents. CCB affect the voltage dependent calcium channels. We studied in **chapter 4.3** the effect of this polymorphism on the incidence of diabetes mellitus in calcium channel blocker users, because insulin release is triggered by an influx of calcium in the pancreatic beta-cells. CCB users with the rs10494366 TG or GG genotype had a two times lower risk of diabetes mellitus than users with the TT genotype, although small numbers preclude definitive statements and replication of these results is indicated. In **chapter 4.4** we studied the effect of the rs10494366

SNP on cardiovascular mortality in calcium channel blocker users. Dihydropyridine CCB users with the TG genotype had a 3.5 times higher cardiovascular mortality risk and users with the GG genotype a 6 times higher cardiovascular mortality risk than users with the TT genotype. In the non-dihydropyridine CCB users, no associations with cardiovascular mortality were found. Also in this study, replication of the results is indicated.

In chapter 3.2 we found that the rs622342 polymorphism in the *SLC22A1* gene, coding for the OCT1 transporter is associated with metformin response. Also the anti-Parkinson drugs pramipexole, amantadine and, possibly, levodopa are substrates for OCT1. The rs622342 variant C allele was associated with higher prescribed doses of anti-Parkinson drugs, especially amantadine and selegiline, and a shorter survival time (**chapter 5.1**). After start of levodopa therapy, patients with the CC genotype had a two times higher mortality risk and had lived on average 2.5 years shorter than patients with the AA genotype.

In the general discussion (**chapter 6**), the results are summarized and discussed. Apart from the identification of polymorphisms not previously associated with drug response, the most important result is that the interaction between individual polymorphisms, between polymorphisms and gender and, possibly, between polymorphism and co-prescribed drugs, do add substantially to the prediction in drug response. Whether genotyping is useful in individualizing pharmacotherapy depends on the possibility to prevent either adverse drug reactions or increased costs due to ineffective therapy, weighed against the costs of genotyping.

Chapter 7.2.

Samenvatting voor niet ingewijden

Een behandeling met geneesmiddelen heeft vaak als doel om een ziekte te genezen of om symptomen te onderdrukken. In veel gevallen zullen deze doelen niet gehaald worden. Enerzijds is dertig tot zestig procent van de behandelingen niet effectief, en anderzijds worden veel behandelingen gestopt in verband met bijwerkingen. Een groot aantal factoren bepaalt bij wie een behandeling effectief is, en bij wie bijwerkingen zullen optreden (**hoofdstuk 1**). Hoe meer men weet over deze factoren, des te beter men dit kan voorspellen. Deze kennis kan in de praktijk gebruikt worden om geneesmiddelen efficiënter en veiliger in te zetten. In dit proefschrift kijken we naar twee factoren, namelijk de invloed van geneesmiddelen die gelijktijdig worden gebruikt en de invloed van erfelijke factoren.

Geneesmiddelen kunnen de werking van andere geneesmiddelen beïnvloeden, zogenaamde geneesmiddel-geneesmiddel interacties. Dit kan bijvoorbeeld gebeuren als geneesmiddel A de afbraak van geneesmiddel B remt of juist versnelt, waardoor de concentraties in het bloed van geneesmiddel B respectievelijk hoger en lager zullen zijn indien beide geneesmiddelen tegelijkertijd worden gebruikt. Hogere concentraties kunnen leiden tot bijwerkingen, en lagere concentraties tot een verminderde effectiviteit.

Ook erfelijke factoren kunnen van invloed zijn op de werking van geneesmiddelen. De erfelijke informatie is opgeslagen in het DNA. Het DNA codeert voor de opbouw van eiwitten, waaronder de eiwitten die betrokken zijn bij de afbraak van geneesmiddelen (enzymen), en de eiwitten die een pompfunctie hebben en geneesmiddelen in en uit cellen transporteren. Kleine veranderingen in het DNA, zogenaamde polymorfismen, kunnen leiden tot eiwitten die minder goed of juist beter werken. In het geval van enzymen die geneesmiddelen afbreken, kunnen polymorfismen leiden tot een versnelde of vertraagde afbraak van geneesmiddelen, soortgelijk als met de bovengenoemde geneesmiddel-geneesmiddel interacties. Het gevolg van veranderingen in de werking van pompen is een verhoging of een verlaging van de geneesmiddelconcentratie in de cellen van het betrokken orgaan, en dit kan leiden tot een veranderde effectiviteit of tot het ontstaan van bijwerkingen.

In **hoofdstuk 2** hebben we onderzoek gedaan naar de blootstelling aan en de gevolgen van geneesmiddel-geneesmiddel interacties, combinaties van geneesmiddelen die elkaars werking beïnvloeden. De blootstelling aan geneesmiddel-geneesmiddel interacties, of kortweg interacties, in de bevolking van 70 jaar en ouder is bijna verdubbeld tussen 1992 en 2005. In 1992 was tien procent van deze groep blootgesteld aan een interactie tegen negentien procent in 2005. Hierbij zijn ook alle interacties inbegrepen die maar een kleine invloed en daardoor weinig klinische relevantie hebben. In dezelfde periode steeg de blootstelling aan potentieel levensbedreigende interacties van 1,5 naar 2,9 procent. Hoewel deze percentages een groot gevaar suggereren, zijn de meeste risico's beperkt als de richtlijnen worden gevolgd. Aan de hand van eerder verschenen studies onderzochten we welk deel van de ziekenhuisopnamen wordt veroorzaakt door interacties. Bij naar schatting een half procent van alle ziekenhuisopnamen was een interactie de oorzaak of medeoorzaak van de

opname. Bij ouderen van boven de 65 jaar, werd vijf procent van de ziekenhuisopnamen (mede) veroorzaakt door interacties.

In Nederland hebben apothekers de taak om het afleveren van interacties, die een hoog risico met zich meebrengen, te voorkomen. In dit hoofdstuk hebben we onderzocht welke factoren invloed hebben op het goed uitvoeren van deze taak. Apotheken die gebruik maken van het computersysteem Euroned, leveren interacties met een hoog risico vaker af dan apotheken die één van de andere systemen gebruiken. Ook apotheken die onderdeel zijn van een gezondheidscentrum leveren deze interacties vaker af.

In **hoofdstuk 3** hebben we gekeken welke invloed erfelijke factoren hebben op de werking van geneesmiddelen voor de behandeling van type II suikerziekte (diabetes mellitus type II, voorheen ook bekend als ouderdomsdiabetes). Tolbutamide behoort tot de groep van sulfonylureum derivaten en is een geneesmiddel dat wordt gebruikt voor de behandeling van type II suikerziekte. Tolbutamide wordt in het lichaam afgebroken door het enzym CYP2C9. Sommige mensen hebben door een verandering in het DNA een minder goed functionerend CYP2C9 enzym, en de lever van deze mensen breekt tolbutamide minder goed af. We vonden dat deze mensen inderdaad lagere doseringen tolbutamide kregen voorgeschreven dan mensen met een normaal werkend CYP2C9 enzym.

We hebben ook het geneesmiddel metformine onderzocht. Metformine is het meest gebruikte geneesmiddel voor de behandeling van type II suikerziekte, en zorgt ervoor dat de aanmaak van glucose in de levercellen wordt geremd zodat de glucose spiegels in het bloed dalen. Voor een goede werking zijn voldoende hoge concentraties van metformine in de levercel nodig. Metformine wordt door de pomp OCT1 van het bloed naar de levercel getransporteerd, en door de pomp MATE1 de levercel uit naar de gal. Al eerder was beschreven, dat veranderingen in het voor OCT1 coderende DNA, zorgen voor een minder goed functionerende OCT1 pomp. Wij vonden een andere, niet eerder beschreven verandering in het DNA die leidt tot het minder goed functioneren van de OCT1 pomp. In personen met een slecht functionerende OCT1 pomp, die met metformine startten, daalde de glucose spiegel nauwelijks, terwijl in patiënten met een goed functionerende OCT1 pomp de glucose spiegel wel duidelijk daalde.

We waren de eersten die beschreven dat ook veranderingen in het voor MATE1 coderende DNA invloed hebben op het glucose verlagend effect van metformine. MATE1 is de pomp die metformine uit de levercel naar de gal transporteert. Bij patiënten die startten met metformine, leidde een slecht functionerende MATE1 pomp tot een sterker glucose verlagend effect dan een goed functionerende MATE1 pomp. De verschillen tussen metformine gebruikers in het glucose verlagend effect werd nog beter verklaard als naar beide pompen samen werd gekeken. Op basis van het functioneren van beide pompen kunnen we de bevolking indelen in negen groepen met een aflopende respons op metformine. Bij acht procent van de mensen, met een goed functionerende OCT1 pomp en een slecht functionerende MATE1 pomp is metformine erg effectief in het verlagen van de glucose spiegels. Daar staat tegen-

over dat ongeveer vijf procent van de bevolking een slecht functionerende OCT1 pomp en een goed functionerende MATE1 pomp heeft, waardoor metformine slecht de levercel wordt ingepompt en goed de levercel uit. Bij deze mensen geeft metformine een minimale verlaging van de glucose spiegels. Het is de vraag of het zinvol is om deze groep mensen met metformine te behandelen.

Het DNA dat codeert voor het eiwit NOS1AP speelt een belangrijke rol in de calciumhuishouding van cellen. Calcium zorgt voor een groot aantal celfuncties, zoals het samentrekken van de cellen in de hartspier, en het afgeven van insuline door alvleeskliercellen. Eerder was aangetoond dat een verandering in het DNA, dat codeert voor NOS1AP, kan leiden tot hartritmestoornissen. We onderzochten of dezelfde verandering ook invloed heeft op de effectiviteit van sulfonylureum derivaten, die worden gebruikt voor de behandeling van type II suikerziekte. Sulfonylureum derivaten zorgen voor extra afgifte van insuline door een effect op de calciumhuishouding in de alvleeskliercellen. Voor gebruikers van glibenclamide, één van de sulfonylureum derivaten, vonden we dat mensen met de verandering in het DNA dat codeert voor NOS1AP, minder respons hebben op glibenclamide en een hogere kans om te overlijden. Voor twee andere sulfonylureum derivaten, glimepiride en het al eerder beschreven tolbutamide, vonden we dat deze verandering leidt tot een lagere kans om te overlijden, omgekeerd aan het effect van glibenclamide. Sulfonylureum derivaten verschillen in de invloed die ze hebben op het hart, en deze verschillen kunnen mogelijk het omgekeerde effect op sterfte verklaren.

In **hoofdstuk 4** van dit proefschrift hebben we gekeken naar de invloed van erfelijke factoren op de werking van geneesmiddelen, die voor de behandeling van hart- en vaatziekten worden gebruikt. De calciumkanaal blokkers worden onder andere gebruikt voor de behandeling van hoge bloeddruk en pijn op de borst. De geneesmiddelen in deze groep remmen de instroom van calcium in de cellen van het hart en de bloedvaten en hebben dus invloed op de calciumhuishouding. We onderzochten of de hierboven genoemde verandering in het DNA, dat codeert voor NOS1AP, ook invloed heeft op het effect van calciumkanaal blokkers. We vonden dat gebruikers van calciumkanaal blokkers met deze verandering in het DNA een kleinere kans hebben op het ontwikkelen van suikerziekte en een grotere kans om te overlijden.

Statinen zijn geneesmiddelen, die de cholesterol spiegels verlagen en daarmee het risico op hart- en vaatziekten verkleinen. We onderzochten de effecten van twee statinen, namelijk simvastatine en atorvastatine. Deze statinen worden in de levercellen afgebroken door het CYP3A4 enzym. In onder andere de levercellen, bevindt zich ook de P-gp pomp, die deze statinen de cel uitpompt. Er zijn veranderingen in het voor de P-gp pomp coderende DNA bekend, die invloed hebben op de effectiviteit van deze pomp. We vonden dat deze veranderingen ook invloed hebben op het cholesterolverlagend effect van simvastatine. Het is bekend dat in mannelijke levercellen de P-gp pomp meer voorkomt dan in vrouwelijke levercellen. In ons onderzoek was het effect inderdaad sterker in mannen dan in vrouwen.

We hebben ook de invloed van een verandering in het DNA dat codeert voor CYP3A4 onderzocht op het optreden van bijwerkingen tijdens het gebruik van simvastatine en atorvastatine. Deze verandering zorgt ervoor dat het CYP3A4 enzym sterker gaat werken, waardoor de concentraties van deze statinen in het bloed lager worden. We vonden dat mensen met deze verandering in het DNA, een twee keer kleinere kans hebben op bijwerkingen. Dit effect werd sterker als we ook keken naar de P-gp pomp. Mensen met een sterker werkend CYP3A4 enzym en een slechter werkende P-gp pomp, hadden een zeven keer kleinere kans op bijwerkingen dan mensen met een normaal werkend CYP3A4 enzym. Omdat de P-gp pomp in deze mensen slechter werkt en dus minder statine uit de levercel pompt, zal er meer statine beschikbaar zijn voor het CYP3A4 enzym om af te breken. Daarnaast werkt het CYP3A4 enzym in deze mensen beter, waardoor simvastatine en atorvastatine zeer snel worden afgebroken en weinig bijwerkingen zullen veroorzaken. Onduidelijk is of in deze groep mensen het cholesterolverlagend effect ook verminderd is.

Behalve metformine, worden ook andere geneesmiddelen door de OCT1 pomp getransporteerd, waaronder geneesmiddelen die gebruikt worden voor de behandeling van de ziekte van Parkinson. In **hoofdstuk 5** hebben we de invloed onderzocht van de eerder beschreven verandering in het DNA dat codeert voor de OCT1 pomp, op de geneesmiddelen die worden gebruikt voor de behandeling van de ziekte van Parkinson. We vonden dat mensen met deze verandering in het DNA een minder goede respons hebben op deze geneesmiddelen. We onderzochten daarnaast de tijd tussen starten met geneesmiddelbehandeling en het tijdstip van overlijden. Parkinson patiënten met deze verandering in het DNA leefden gemiddeld 2,5 jaar korter dan patiënten zonder deze verandering.

In dit proefschrift hebben we de invloed van geneesmiddel-geneesmiddel interacties en de invloed van erfelijke factoren op geneesmiddel respons onderzocht. Het blijkt dat geneesmiddel-geneesmiddel interacties met name bij ouderen een belangrijke oorzaak zijn van ziekenhuisopnamen. We hebben een aantal nieuwe en niet eerder beschreven associaties gevonden, zoals de associaties tussen het DNA dat codeert voor NOS1AP en het effect van calciumkanaal blokkers, MATE1 en het glucose verlagend effect van metformine en OCT1 en de werking van geneesmiddelen voor de behandeling van de ziekte van Parkinson. Daarnaast hebben we een aantal associaties bevestigd, namelijk die tussen veranderingen in het voor CYP2C9 en P-gp coderende DNA, en het effect van respectievelijk tolbutamide en statinen. Deze associaties kunnen helpen bij het individualiseren van farmacotherapie, omdat mogelijk bijwerkingen en ineffectief gebruik van geneesmiddelen voorspeld en daarmee voorkomen kunnen worden (**hoofdstuk 6**). Daarnaast is een belangrijke uitkomst dat niet alleen factoren op zich een belangrijke rol spelen, maar dat effectiviteit van geneesmiddelen en het ontstaan van bijwerkingen veel beter voorspeld kunnen worden als naar de samenhang tussen factoren wordt gekeken. Zo hangt de effectiviteit van metformine samen met zowel veranderingen in het DNA dat codeert voor de OCT1 pomp als veranderingen in het DNA dat codeert voor de MATE1 pomp. De werking van simvastatine hangt samen met

geslacht en veranderingen in het DNA dat codeert voor de P-gp pomp en het enzym CYP3A4. Het is te verwachten dat ook de samenhang met gelijktijdig gebruikte geneesmiddelen een belangrijke rol speelt.

Abbreviations

ABC	ATP binding cassette
ACE	angiotensin converting enzyme
ADE	adverse drug event
ADR	adverse drug reaction
ANOVA	analysis of variance
ATC	anatomical therapeutical chemical
ATP	adenosine-5′-triphosphate
AUC	area under curve
BMI	body-mass index
Ca	calcium
CCB	calcium channel blocker
CCKL	coördinatie commissie ter bevordering van de kwaliteitsbeheersing op het gebied van laboratoriumonderzoek in de gezondheidszorg
CI	confidence interval
CNV	copy number variation
COX	cyclo-oxygenase
COXIB	cyclo-oxygenase 2 selective inhibitor
CYP	cytochrome P450
DDD	defined daily doses
DDI	drug-drug interaction
DNA	deoxyribonucleic acid
DRA	drug related admission
ECG	electrocardiogram
ED	emergency department
eGFR	estimated glomerular filtration rate
FTE	full-time equivalent
GI	gastro-intestinal
GP	general practitioner
GWA	genome wide association
HbA1c	glycosylated hemoglobin
HERG	human ether-a-go-go related gene
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPLC	high-performance / pressure liquid chromatography
HR	hazard ratio
HWE	hardy-weinberg equilibrium
ICD	international classification of diseases

IHC	inspectorate for health care
IPA	international pharmaceutical abstracts
kbp	kilobasepairs
LDL	low-density lipoprotein
MAF	minor allele frequency
MATE	multidrug and toxin extrusion
MDC	medisch diagnostisch centrum
MDR	multidrug resistance
MeSH	medical subject heading
NA	not available
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NOS1AP	nitric oxide synthase 1 adaptor protein
NSAID	non-steroid anti-inflammatory drug
OCT	organic cation transporter
OR	odds ratio
PCR	polymerase chain reaction
P-gp	P-glycoprotein
RAAS	renin-angiotensin-aldosterone system
RALES	randomized aldactone evaluation study
R_c	slope
SAM	S-adenosyl methionine
SD	standard deviation
SLC	solute carrier
SNP	single nucleotide polymorphism
SR	sarcoplasmic reticulum
SSRI	selective serotonin reuptake inhibitor
STAR	stichting trombosedienst en artsenlaboratorium rijnmond
SUR	sulfonylurea receptor
TF	therapeutic failure
UGDP	university group diabetes program
TPMT	thiopurine methyltransferase
UGT	UDP glucoronosyltransferases
UKPDS	united kingdom prospective diabetes study
WHO	world health organization

Dankwoord

Drie jaar werk zit er tussen de kaften van dit boekje, waarbij de eerste stappen al zeven jaar geleden gezet zijn. Veel mensen hebben mij terzijde gestaan tijdens mijn promotieonderzoek, en deze mensen ben ik veel dank verschuldigd.

Veel dank gaat uit naar mijn promotoren, Prof. dr. Bruno Stricker en Prof. dr. Arnold Vulto.

Bruno, je hebt me begeleid vanaf mijn afstudeeronderzoek bij het RIVM. Ik waardeer je grote mate van betrokkenheid, en de vrijheid die je me hebt gegeven om mijn eigen weg te gaan. De frequente besprekingen die we hadden waren een stimulans om verder te gaan. Je hebt me altijd goed geholpen op de momenten dat het nodig was, en je adviezen waren vaak even simpel als doeltreffend. Daarnaast kon ik alle mogelijke ideeën inbrengen en heb ik ook altijd de mogelijkheden gekregen om mijn eigen ideeën uit te voeren.

Arnold, je hebt mijn onderzoek in een klinisch perspectief kunnen zetten. Wetenschappelijk onderzoek focust soms op p-waarden en betrouwbaarheidsintervallen maar negeert daarmee vaak de betekenis voor de individuele patiënt. De combinatie van enerzijds onderzoek en anderzijds klinisch werk is in mijn ogen een vruchtbare tijd geweest.

Veel dank ben ik ook verschuldigd aan mijn copromotor, Dr. Loes Visser. Loes, je kon altijd goed tegenwicht bieden aan 'de hoge heren' door op de rem trappen als ze met onmogelijke ideeën kwamen of anderszins tot last waren. Dank ook voor het nauwkeurig lezen van de manuscripten en je gedetailleerde commentaar.

Prof. dr. André Uitterlinden, Prof. dr. Ton de Boer en Prof. dr. Paul Smits wil ik bedanken voor hun bereidheid om zitting te nemen in de kleine commissie en voor de inhoudelijke beoordeling van dit proefschrift.

In een groot aantal hoofdstukken heb ik gebruik mogen maken van de gegevens van de ERGO studie ('Rotterdam Study'). De ERGO studie en daarmee de hoofdstukken in dit proefschrift waren niet mogelijk zonder de deelnemers die in de wijk Ommoord in Rotterdam wonen. De deelnemers van de ERGO studie, medewerkers, huisartsen, openbaar apothekers en de Stichting Trombosedienst en Artsenlaboratorium Rijnmond ben ik zeer erkentelijk voor hun bijdrage.

Prof. dr. Bert Hofman, bedankt dat ik, samen met veel andere promovendi, gebruik heb mogen maken van de data van de ERGO studie. Hoewel farmacogenetica niet tot de primaire onderzoeksgebieden van de ERGO studie behoorde, zijn de data uiterst bruikbaar voor dit onderzoek.

Prof. dr. André Uitterlinden, dank voor de genome-wide genotyperingen van de ERGO deelnemers, die ik heb mogen gebruiken. Deze data waren voor mij de slagroom op de taart en hebben veel nieuw onderzoek in dit proefschrift mogelijk gemaakt. Het eind van de mogelijkheden op farmacogenetisch gebied is nog lang niet in zicht.

Dr. Ron van Schaik en Dr. Teun van Gelder, dank dat jullie willen opponeren tijdens mijn verdediging. Ron, je enthousiasme voor de farmacogenetica heb ik altijd erg kunnen waarderen. Teun, als arts heb je vaak een andere kijk op zaken dan ik als apotheker. Ondanks de drukte neem je altijd ruim de tijd om vragen te beantwoorden en ik heb de afgelopen jaren veel van je geleerd.

De start van mijn promotieonderzoek ligt, achteraf gezien, bij het RIVM. Ik wil Dr. Marjon Kallewaard, Peter Caspers en André van der Veen bedanken voor de goede begeleiding en de mogelijkheid die ik heb gekregen om mijn onderzoek voort te zetten als promotieonderzoek. Jullie enthousiasme is een belangrijke stimulans geweest om aan dit promotieonderzoek te beginnen.

Beste collegae van de afdelingen Epidemiologie en Medische Informatica. Jullie zijn met teveel om allemaal persoonlijk te bedanken op deze pagina's. Jullie bijdrage aan dit boekje is groot en varieert van kritisch commentaar op concept-manuscripten tot gezelligheid op het terras van Coenen en het Wester Paviljoen. Dank voor jullie steun, zowel op momenten van 'wind-mee' als momenten van 'wind-tegen'.

Ik heb mijn promotieonderzoek gecombineerd met het werk als apotheker en later als ziekenhuisapotheker in opleiding in de apotheek van het Erasmus MC. Hoewel het werk in de apotheek en het werk aan mijn promotieonderzoek altijd gescheiden waren, was er zeker sprake van kruisbestuiving. Ik heb veel geleerd tijdens het werk in de apotheek, dat nuttig was voor mijn onderzoek en omgekeerd. Mijn collegae dank ik voor de prettige samenwerking in de apotheek. Met name wil ik de 'jonge apothekers' noemen; dank voor jullie steun, deze is op sommige momenten van onschatbare waarde voor mij geweest.

Ik ben blij dat mijn paranimfen, Michiel Schaap en Monique Bijl, mij willen bijstaan tijdens mijn verdediging. Michiel, we begonnen ongeveer tegelijkertijd aan onze promotieonderzoeken. Onze onderwerpen zijn erg verschillend, maar juist daardoor had je een frisse blik op mijn onderzoek. Ik kijk met plezier terug naar de discussies die we hebben gehad. Monique, we hebben lange tijd op dezelfde (warme) kamer gezeten en promoveren allebei op het gebied van de farmacogenetica. Daardoor heb ik veel steun gehad aan het lief en leed dat we hebben kunnen delen. De samenwerking zal ook na onze promoties een vervolg krijgen, met de opleiding tot ziekenhuisapotheker die we allebei volgen. Dank ook voor je suggestie voor de voorkant van dit boekje, ook al was dat waarschijnlijk onbewust.

Beste pa en ma, Michiel en Francine, vrienden en familie. Jullie hebben mijn onderzoek vooral vanaf de zijlijn gevolgd. Dank voor jullie steun en ook voor het begrip in drukke tijden. Pap, door jou heb ik 'de liefde voor het vak' al vroeg meegekregen. Mam, jij hebt dat mooi aangevuld met je analytische achtergrond. Beide zijn denk ik erg belangrijk geweest voor mijn werk. Ik ben blij dat ik de mogelijkheid van jullie heb gekregen om minder logische stappen te nemen, zoals mijn tweede studie en mijn bestuursjaar bij Orca.

Lieve Marije, dank voor je liefde en begrip bij het schrijven van dit proefschrift, ook in perioden dat ik, om jouw woorden te gebruiken, 'in volledige symbiose met mijn computer leefde'. Het leven gaat te snel voorbij om vaak genoeg te zeggen hoe blij ik ben dat ik met jou mag samenleven, zo blij dat het ook niet mogelijk is om dit vaak genoeg te zeggen.

Bibliography

MANUSCRIPTS BASED ON THIS THESIS

2.1 Becker ML, Visser LE, van Gelder T, Hofman A, Stricker BH. Increasing exposure to drug-drug interactions between 1992 and 2005 in people aged > or = 55 years. *Drugs Aging* 2008;25(2):145-52.

2.2 Becker ML, Kallewaard M, Caspers PW, Schalekamp T, Stricker BH. Potential determinants of drug-drug interaction associated dispensing in community pharmacies. *Drug Saf* 2005;28(5):371-8.

2.3 Becker ML, Caspers PW, Kallewaard M, Bruinink RJ, Kylstra NB, Heisterkamp S, de Valk V, van der Veen AA, Stricker BH. Determinants of potential drug-drug interaction associated dispensing in community pharmacies in the Netherlands. *Pharm World Sci* 2007;29(2):51-7.

2.4 Becker ML, Kallewaard M, Caspers PW, Visser LE, Leufkens HG, Stricker BH. Hospitalisations and emergency department visits due to drug-drug interactions: a literature review. *Pharmacoepidemiol Drug Saf* 2007;16(6):641-51.

3.1 Becker ML, Visser LE, Trienekens PH, Hofman A, van Schaik RH, Stricker BH. Cytochrome P450 2C9 *2 and *3 polymorphisms and the dose and effect of sulfonylurea in type II diabetes mellitus. *Clin Pharmacol Ther* 2008;83(2):288-92.

3.2 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J* 2009;9(4):242-7.

3.3 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose lowering effect of metformin in patients with diabetes mellitus: a preliminary study. *Diabetes* 2009;58(3):745-9.

3.4 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. Submitted.

3.5 Becker ML, Aarnoudse AJ, Newton-Cheh C, Hofman A, Witteman JC, Uitterlinden AG, Visser LE, Stricker BH. Common variation in the NOS1AP gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea. *Pharmacogenet Genomics* 2008;18(7):591-7.

4.1 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Common genetic variation in the ABCB1 gene is associated with the cholesterol lowering effect of simvastatin in males. *Pharmacogenomics*. Accepted

4.2 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Influence of genetic variation in CYP3A4 and ABCB1 on dose decrease or switching during simvastatin and atorvastatin therapy. *Pharmacoepidemiol Drug Saf*. Accepted.

4.3 Becker ML, Visser LE, Newton-Cheh C, Witteman JC, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the NOS1AP gene is associated with the incidence of diabetes mellitus in users of calcium channel blockers. *Diabetologia* 2008;51(11):2138-40.

4.4 Becker ML, Visser LE, Newton-Cheh C, Hofman A, Uitterlinden AG, Witteman JC, Stricker BH. A common NOS1AP genetic polymorphism is associated with increased cardiovascular mortality in users of dihydropyridine calcium channel blockers. *Br J Clin Pharmacol* 2009;67(1):61-7

5.1 Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. The OCT1 polymorphism rs622342 A>C is associated with decreased drug response and shorter survival time in Parkinson's disease. Submitted.

OTHER MANUSCRIPTS

o Becker ML, Vermes A. Vernakalant (Kynapid) - Een atriumselectief antiarrhythmicum. *Pharmaceutisch Weekblad* 2008;143(21):28-31.

o Becker ML, Visser LE, Uitterlinden AG, van Schaik RH, Hofman A, Stricker BHC. Farmacogenetisch onderzoek in The Rotterdam Study - Op weg naar antidiabetica op maat. *Pharmaceutisch Weekblad* 2007;142(36):38-39.

o Becker ML, de Langen-Wouterse JJ / LAREB. SSRI's en gynaecomastie. *Geneesmiddelenbulletin* 2006; 40(9):106-107.

o Becker ML, Brouwer MS, Donkers AW, Schobben AF. Veiligheid van anti-arrhythmica tijdens de zwangerschap. *Pharmaceutisch Weekblad* 2005;140(10):325-326

o Becker ML, Caspers PW, Kallewaard M, Heisterkamp SH, van der Veen AA. Medicatie-veiligheid in de openbare apotheek: Naar een risicomodel op basis van indicatoren voor het afleveren van interacterende medicatie. Bilthoven: RIVM; 2005.

PhD portfolio

RESEARCH SKILLS AND IN-DEPTH COURSES

Master of Science in Clinical Epidemiology, NIHES, Rotterdam, the Netherlands	2006-2009
Clinical Trials and Drug Risk Assessment, Utrecht University, Utrecht, the Netherlands	2007
Repeated Measurements in Clinical Studies, NIHES, Rotterdam, the Netherlands	2007
SNPs and Human Diseases, Molecular Medicine, Rotterdam, the Netherlands	2007

(INTER)NATIONAL CONFERENCE PRESENTATIONS

Nederlandse Vereniging voor Klinische Farmacologie & Biofarmacie – Voorjaarsdag, Utrecht, the Netherlands. *Oral presentation*	2007
Nederlandse Vereniging van Ziekenhuisapothekers - Tweede Nederlandse Ziekenhuisfarmaciedag, Leiden, the Netherlands. *Poster presentation*	2007
American Society for Clinical Pharmacology and Therapeutics – Annual Meeting, Orlando, U.S.A. *Poster presentation*	2008
International Society for Pharmacoepidemiology - International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Copenhagen, Denmark. *Oral presentation*	2008
Nederlandse Vereniging voor Klinische Farmacologie & Biofarmacie – Voorjaarsdag, Utrecht, the Netherlands. *Oral presentation*	2009

TEACHING

Supervising and teaching medical and pharmacy students, Erasmus MC, Rotterdam, the Netherlands	2007-

OTHER

NIHES award: Best research paper 2008-2009	2009
Referee activities for various international scientific journals	2008-
Reviewer for Medisch-Farmaceutische Mededelingen	2006-

About the author

Matthijs Lambertus Becker, was born on August 20th 1978 in Nijmegen. After finishing VWO education at Canisius College-Mater Dei in Nijmegen in 1996, he started studying Pharmacy at the Utrecht University. Three years later, he started, beside the study Pharmacy, the study Health Policy and Management at the Erasmus University Rotterdam. In 2004, he finished the Master of Science in Health Policy and Management, and in 2006, he finished the Master of Science in Pharmacy.

In 2002 and 2003, as part of the Master of Science in Pharmacy, he performed a research project at the National Institute for Public Health and the Environment (RIVM) in Bilthoven. Risk factors were assessed for the dispensing of potential drug-drug interactions in community pharmacies. Subsequently, he worked as a researcher at the RIVM and at the Erasmus MC to measure the actual numbers of high risk drug-drug interactions and to assess the associated risk factors. In 2004 and 2005, he worked as an employee for the Order of Medical Specialists in Utrecht.

During his study time, he was an active member of the rowing club A.U.S.R. Orca in Utrecht. During the first few years as a race-rower and later as coach. During the academic year 2001-2002 he acted as member of the board of A.U.S.R. Orca as a treasurer.

After finishing his Master of Science in Pharmacy in 2006, he started his PhD project at the Department of Epidemiology of the Erasmus MC in Rotterdam. At the same time, he started the Master of Science in Clinical Epidemiology at NIHES in Rotterdam. After half a year, he combined his PhD project with the work as a pharmacist at the hospital pharmacy of the Erasmus MC. In 2008, at the end of his PhD project, he started his training as a hospital pharmacist at the Erasmus MC. In 2009, he finished his Master of Science in Clinical Epidemiology.