PHARMACOGENETICS OF ANTIHYPERTENSIVE DRUGS AND THE RISK OF DIABETES

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Colofon
Picture on cover with permission from the Australian artist Steven Dix. Impression of the artist: 'Rose Garden' is a work reminding me of the roses from my childhood home in Toowoomba. The blended tones, shapes and colour of a delicate flower, grown from the rich volcanic red soils of the earth provide a striking modern vision of any rose garden, yet through different eyes.'
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Rosegarden Gülizar
For me, the picture on the cover stands for my grandmother named Gülizar which means rosegarden in Old Turkish. Even though my grandmother did not have the opportunity to finish primary school, she was my biggest example in life. She concoured everyones heart with her love and understanding and she kept supporting me in all that I did, she had faith in me and always encouraged me to go further. I am proud to be her grandchild. This is for my grandmother. This is for Gülizar.

PHARMACOGENETICS OF ANTIHYPERTENSIVE DRUGS AND THE RISK OF DIABETES

FARMACOGENETICA VAN ANTIHYPERTENSIVA EN HET RISICO OP DIABETES

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 30 maart 2011 des middags te 12.45 uur

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ÖZLEM BOZKURT

geboren op 17 augustus 1981 te Genk, België

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Voor oma

Anneanneme

Contents

CHAPTER ONE

General introduction

The incidence of diabetes is increasing worldwide at an alarming rate. The leaders of the current diabetes epidemics are Southeast Asia and the Western Pacific region with India and China facing the greatest challenges[1]. The most striking issue in these countries is the worsening trend for the disease to affect younger age groups. While in developed countries the biggest increases are seen over 65s, in developing countries most new cases are occurring between 44 and 65 years of age. In all parts of the world, type II diabetes is also now emerging in children and adolescents, thereby raising the threat of the onset of cardiovascular disease at an earlier age[2].

Diabetes mellitus is a chronic disease in which there is an imbalance of blood glucose. Though the precise mechanism remains unknown, diabetes can arise as a result of an insufficiently working pancreas caused by destruction of the beta cell of the islets of Langerhans or by an auto-immune response of the body against the beta cells. This type of diabetes is classified as type I diabetes mellitus and most subjects are diagnosed at an early stage of life and should be put on insuline therapy immediately.

The second type of diabetes mellitus, type 2 diabetes mellitus (T2DM), is more common and is caused by a state called 'insulin resistance'. In this case the pancreas is not able to produce enough insulin, or the body is not making proper use of the insulin to reduce the excess glucose in the bloodstream. This form of diabetes is frequently undiagnosed for many years while hyperglycaemia is often not severe enough to cause symptoms of diabetes, the onset of this type occurs later in life and explains the name "adult-onset diabetes". The current WHO diagnostic criteria for T2DM is fasting plasma glucose $\geq$ 7.0mmol/l (126mg/dl) or 2-h value of plasma glucose after administration of 75g oral glucose $\geq$ 11.1mmol/l (200mg/dl)[3].

Several DNA mutations have also been linked to diabetes mellitus. These can be divided into monogenic and polygenic. Monogenic forms of diabetes mellitus which cause severe defects in insulin secretion are grouped under maturity onset diabetes of the young (MODY1-6), others such as mitochondrial mutations cause maternally inherited diabetes with deafness (MIDD) and even some rare mutations in the insulin gene (PPAR-g, insulin receptors) exist. Monogenic forms of T2DM have a high phenotypic penetrance, are diagnosed at early age and frequently lead to severe clinical features[4].

While monogenic forms of T2DM have a clear inheritance model with the environment inserting almost no effect on the clinical manifestation, polygenic forms of diabetes are much more difficult to identify. These forms of diabetes are the result of the interaction between the

environment and genetic background, are characterised by a later onset and can cause both impaired insulin secretion and insulin resistance. Many different genes contribute to these forms of diabetes and the alleles of these polymorphisms are present in healthy as well as type 2 diabetic patients. Examples of genes that cause polygenic forms of diabetes are calpain 10, KCJN11, adiponectin and IRS-1.[4] Recently, several genome-wide association studies (GWA) have confirmed previously found association and also led to the discovery of associated variants in unsuspected genes outside coding regions[5-7].

Diabetes: epidemiology, risk factors and comorbidity

The prevalence of diabetes worldwide in the year 2000 was estimated to be 2.8%[8]. This number is expected to double in 2030. The increase in the number of persons with diabetes is linked to the overall increase in population size, ageing of the population and increase in detection awareness. However changes in risk factor profile, such as lifestyle (e.g. sedentary lifestyle, obesity, and caloric excess) and comorbidity, can explain the worldwide increase of diabetes in the coming decades. In the Netherlands approximately 600,000 people were suffering from diabetes in 2003[9]. In the same year the incidence of diabetes was 72,500. Independent risk factors for type 2 diabetes include BMI, physical activity and alcohol use. These risk factors may be modified through lifestyle changes (e.g. weight loss and exercise) or pharmacologically (e.g. orlistat, thiazolidinediones, metformin, and acarbose) [10-12].

People with diabetes are at higher risk of developing cardiovascular disease than those without diabetes[13]. Increasing levels of glycosylated hemoglobin in persons with diabetes mellitus moderately increase cardiovascular risk[14], for each 1% increase in HbA1c the risk of cardiovascular events increases with 18%[15]. The coexistence of diabetes mellitus in hypertensive patients doubles the risk of cardiovascular events, cardiovascular mortality and total mortality[16, 17].

Both diabetes and hypertension are components of the metabolic syndrome[3]. Any treatment, through lifestyle or pharmacologically, which aims to reduce components of the metabolic syndrome, can affect the onset and severity of diabetes.

The metabolic syndrome is a clustering of several risk factors[3, 18]. Subjects with the metabolic syndrome have a doubling of mortality and three times higher risk of morbidity from a myocardial infarction or stroke compared to subjects without the syndrome. These subjects have also a fivefold higher risk of developing type II diabetes[11].

When a patient is diagnosed with metabolic syndrome the next step which should be taken is to aggressively treat the risk factors in order to reduce the risk of cardiovascular disease and the development of type II diabetes[10]. Risk factors for the development of complications of diabetes can be divided in non-modifiable and (potentially) modifiable. Susceptibility genes and ethnic origin are examples of non-modifiable risk factors for diabetes. Glycaemic control, blood pressure, blood lipids, smoking and BMI are modifiable risk factors for diabetes. Besides these risk factors, diabetes itself is one of the major risk factors of cardiovascular disease (CVD).

Pharmacologic treatment of diabetes

The treatment of diabetes consists of reducing blood glucose levels to normal. The National Dutch General Practitioners Union (Nederlands Huisartsen Genootschap- NHG) has developed a step-by-step treatment plan for the start of pharmacologic treatment in patients diagnosed with T2DM and where lifestyle changes were not sufficient to achieve normal blood glucose levels.

Treatment should be started with metformin, when this fails, a Sulfonylureum derivate (for BMI<27 or BMI≥27 and no indication of cardiovascular disease or heartfailure) or pioglitazone (for BMI≥27 and no indication of heartfailure) should be added. If this is not sufficient to lower blood glucose levels to normal, insulin should be added to this regimen. First once a day together with the oral antidiabetics, then twice a day NPH-insulin or mix-insulin and finally insulin 4-times a day. No single drug treatment leads to optimal blood glucose for all patients because interindividual variation in response to glucose lowering drugs is very common[12, 19].

Since diabetes is identified by blood glucose, the treatment of both types of diabetes mainly focused on reducing the blood glucose levels to the normal range. Poor glycaemic control has already been linked to the onset and progression of both microvascular[20,21] and macrovascular[22-24] outcomes. Intensive treatment with insulin or oral anti-diabetics, reduces microvascular complications by 25%. However, up to 80% of all patients diagnosed with T2DM will eventually develop or die from macrovascular disease[25, 26]. Some controversy exists about aggressive blood glucose lowering and the reduction in macrovascular disease in diabetics[27]. Therefore, the treatment of diabetes and prevention of its macrovascular complications requires a multifactorial approach[28] taking into account major modifiable risk factors such as serum lipid lowering[29] and blood pressure lowering[30-32].

Risk factor management; the role of antihypertensive drugs

The mechanism underlying the association between blood pressure and diabetes mellitus is not clear yet[33]. Besides aging and increased body weight, hypertension may lead to the onset of diabetes through endothelial dysfunction[34], increased vasoconstriction, deficiency in the insulin signalling pathway or through reduced insulin sensitivity of the skeletal muscle[35, 36].

Antihypertensive drugs have been found to modify the onset of diabetes. Studies have shown that the choice of antihypertensive drug therapy may also play a role in the development of diabetes since antihypertensive agents can be diabetogenic as well as anti-diabetogenic[37, 38].

Certain classes of antihypertensive drugs can cause significant elevations in glucose concentrations. These include β-blockers and thiazide diuretics[39, 40]. Though the precise mechanisms are not clear yet, it is suggested that diuretics impair carbohydrate mechanism through the depletion of potassium which in turn decreases the insulin secretory response to glucose[41] while β-blockers are thought to decrease the pancreatic β-cell insulin release[42]. Blockers of the renin-angiotensin system (RAS) and calcium channel blockers have shown a beneficial or no effect on the development of diabetes, respectively.

The RAS controls cardiovascular, renal and adrenal functions which play a role in fluid and electrolyte balance and subsequently blood pressure regulation (figure 1). The blockade of the RAS plays an important role in cardiovascular pharmacology (figure 1). Blockers of this system have been linked to a reduction in cardiovascular disease mortality and morbidity with diabetic patients having the most benefit[43, 44]. Possible mechanisms through which RAS inhibition may lead to the prevention of diabetes include a preservation of β-cell function and/or an enhancement of insulin sensitivity, leading to a decreased need for pancreatic insulin secretion[45]. Targeting RAS may also lead to alterations in microcirculation and changes in ionic status which could in turn affect islet insulin secretion and cellular insulin action[46].

Inter-individual response to antihypertensive drugs with regard to blood pressure lowering is common, and is partly determined by variation in common genetic variants[47]. These genetic variations may also determine the response to antihypertensive drugs with regard to adverse effects such as an increased risk of diabetes mellitus.

Aim and outline of this thesis

The aim of this thesis is to gain a greater insight into the interaction between antihypertensive drugs and genetic polymorphisms on the development of diabetes.

Besides lowering blood pressure, antihypertensive drugs can also alter blood glucose metabolism. In **Chapter 2.1**, the risk of T2DM associated with thiazide diuretic therapy alone or in combination with potassium sparing agents or other antihypertensive drug therapies was assessed.

In **Chapter 2.2** variations in the renin-angiotensin system (RAS, ACE G4656C, AGT M235T, AGTR1 A1166C) and salt sensitivity genes (GNB3 C825T and ADD G460T) and the risk of diabetes mellitus associated with the use of thiazide diuretics were examined.

In **Chapter 3.1** the modifying effect of genetic polymorphisms in the RAS (ACE I/D, AGT M235T and AGTR1 A1166C) on the use of Angiotensin Converting Enzyme inhibitors and the incidence of treated diabetes mellitus in two population based studies was assessed.

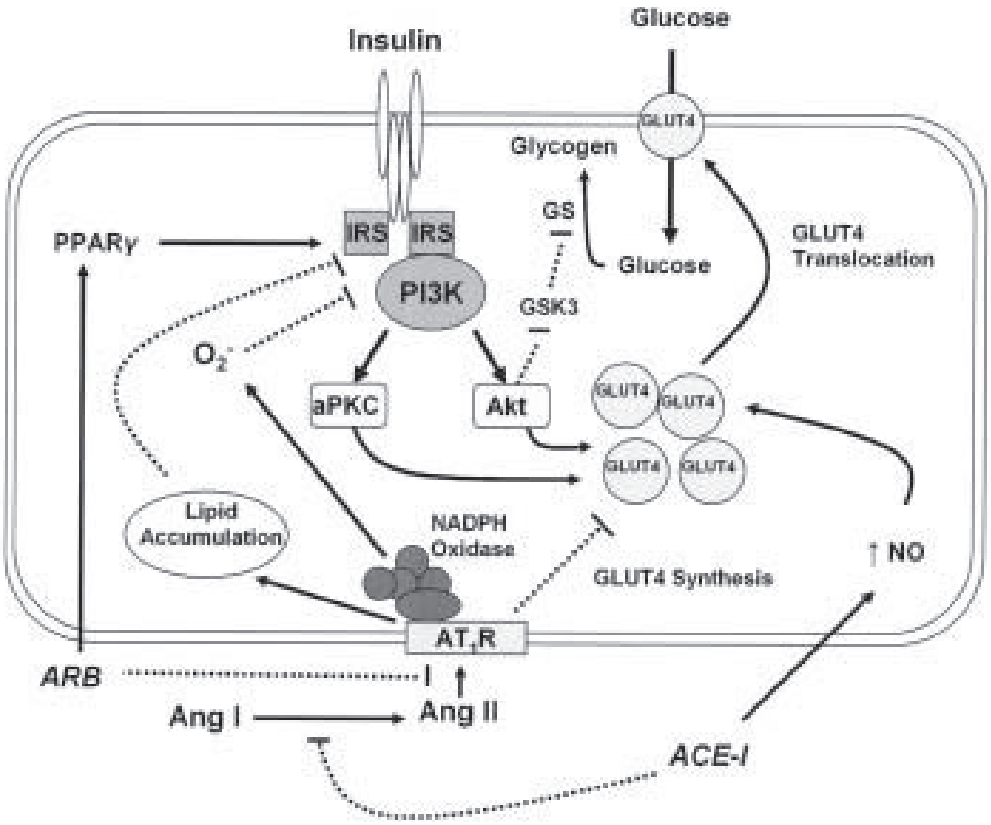
Chapter 3.2 describes the interaction between polymorphisms in the RAS (ACE G4656C, AGT M235T and AGTR1 A1166C) and the use of RAS blockers, such as ACE inhibitors and Angiotensin Receptor Blockers on the risk of diabetes.

Chapter 4 gives an overview of studies that investigated the influence of genetic variants in genes either in the causal pathway or genes affecting the pharmacokinetics or pharmacodynamics of antidiabetic drugs on the response to antidiabetic drugs.

Finally, the main findings, conclusions and possible clinical implications are discussed and future perspectives are given in **Chapter 5**.

Our studies focus on T2DM and it is in this light that from now on when we state diabetes in this thesis we refer to T2DM.

Figure 1. Potential mechanism by which the renin-angiotensin system affects insulin sensitivity and glucose transport in skeletal muscle tissue[36].



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CHAPTER TWO

Thiazide diuretics and diabetes

CHAPTER 2.1

Use of thiazide diuretics and the risk of type 2 diabetes

Abstract

Aim

To assess the risk of type 2 diabetes mellitus associated with thiazide diuretic therapy and the influence of combination therapy with potassium sparing agents and other antihypertensive drug.

Methods

A cohort of patients treated with antihypertensive drugs was selected from the PHARMO-RLS. Data on potential risk factors emerged from a pharmacogenetic study. The outcome of interest was incident type 2 diabetes. The index date for cases was defined by the date the first anti-diabetic drug prescription was dispensed or the date of self-reported diagnosis of diabetes mellitus. For each case up to 5 controls were sampled.

Results

612 incident cases of type 2 diabetes mellitus and 2633 controls, all using antihypertensive drugs at the index date, were selected. Thiazide users receiving ≥ 1 DDD/day had a significantly higher risk of type 2 diabetes compared to no thiazide users (adj. OR 1.84 [95%CI: 1.34-2.54]). Combined with a potassium sparing drug or a potassium supplement, this increase was also statistically significant for doses >1 DDD/day (adj. OR 1.65 [95%CI: 1.10-2.47]). Compared to calcium antagonist monotherapy, the combination of thiazide with ACEi or AII and the combination of thiazide without ACEi/AII antihypertensive drug was not associated with an increased risk (resp. adj. OR; 1.41 [95%CI: 0.79-2.51] and 1.49 [95%CI: 0.88-2.54]). Thiazide diuretic use < 1 DDD/day in combination with ACEi or ARB had the lowest risk of type 2 diabetes associated with thiazide therapy.

Conclusions

Thiazide use alone and in combination with potassium sparing agents or potassium supplement was significantly associated with the risk of developing diabetes in a dose-dependent way.

Introduction

Essential hypertension is closely associated with type 2 diabetes mellitus. Both elevated blood pressure and impaired glucose tolerance are key components of the metabolic syndrome, a major cause of cardiovascular morbidity and mortality[1]. The effects of treatment with antihypertensive drugs on glucose metabolism may be negative (thiazide diuretics, beta-blockers), neutral (calcium antagonists) or positive (angiotensin-converting-enzym (ACE) inhibitors, angiotensin II type 1 receptor antagonists (ARB))[2]. A recent meta-analysis assessed that the odds ratio of type 2 diabetes mellitus by diuretic and β-Blocker were 1.34 and 1.25, for ACE inhibitor and ARB these odds ratio were respectively 0.90 and 0.84 and calcium antagonist users had an odds ratio of 1.05 when compared to placebo[3]. Only initial thiazide therapy and initial beta-blocker therapy were statistically significantly associated with type 2 diabetes mellitus.

Thiazide diuretics impair carbohydrate metabolism through potassium depletion which decrease the insulin secretory response to glucose[4]. Potassium supplementation may fully prevent thiazide-induced glucose tolerance and insulin hyposecretion[5]. However, randomised controlled trials have shown a similar increased risk of diabetes mellitus in users of thiazide diuretics combined with potassium sparing agents[3]. A direct comparison of thiazide with combined thiazide/potassium sparing diuretic therapy has however not been made. Furthermore, in clinical practice many hypertensive patients require more than 1 antihypertensive drug to adequately control blood pressure. The effect of thiazide diuretic therapy in combination with other antihypertensive drug therapies that affect the risk of type 2 diabetes mellitus is unknown. The aim of our study was to assess the risk of type 2 diabetes mellitus associated with thiazide diuretic therapy and to assess the influence of combined use of potassium sparing agents and other antihypertensive drug therapies.

Methods

Design and setting: We performed a nested case-control study among treated hypertensive patients. First the Pharmaco-Morbidity Record Linkage System (PHARMO-RLS) was used to identify a cohort of patients treated with antihypertensive drugs. PHARMO-RLS links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (LMR) from 1985 onwards. Currently, the base population of PHARMO-RLS covers about 2 million community-dwelling inhabitants of

about 50 population-defined areas in the Netherlands. Secondly data on potential risk factors for diabetes mellitus such as age, BMI and alcohol use was collected through a pharmacogenetic study of antihypertensive drugs. In this pharmacogenetic study, which comprised 5,140 treated hypertensive subjects, specific genetic polymorphisms are examined for the modification of the effect of antihypertensive drugs on the risk of myocardial infarction in a selection of PHARMO-RLS participants[6].

Data collection: The PHARMO-RLS provides records containing the name of the dispensed drugs, day of dispensing, number of units dispensed, prescribed daily dose and the Anatomical Therapeutical Chemical (ATC) code of the drug. In the pharmacogenetic study of antihypertensive drugs questionnaire information was used to obtain data on demographic variables, cardiovascular diseases and risk factors.

Outcome and exposure definition: The outcome of interest was incident type 2 diabetes mellitus. Pharmacy records and self-reported questionnaire information were used to assess incident diabetes (cases). Incident cases of diabetes were defined as those having a first prescription for oral anti-diabetic drugs or insulin at least one year after the date of entry in PHARMO-RLS or a self-reported diagnosis of diabetes mellitus. Cases were assigned an index date, which was defined as either the date the first anti-diabetic drug prescription was dispensed or the date of self-reported diagnosis of diabetes mellitus, whichever came first. For each case we selected up to 5 controls who also received antihypertensive drugs but who had not (yet) a diagnosis of diabetes mellitus at the index date of the case.

In a small validation study among 83 subjects for whom general practitioner records were available (24 cases and 59 controls) we found that 91.7% (22) of the cases could be confirmed according to WHO criteria for a diagnosis of type 2 diabetes mellitus (either a fasting plasma glucose>7.0 mmol/l and/or random (non-fasting) blood glucose>11.1 mmol/l, and/or use of oral antidiabetic medication and/or use of insulin and/or treatment by diet and registered by a general practitioner as having type 2 diabetes mellitus)[7], whereas 100.0% (59) of the controls had no diagnosis of type 2 diabetes mellitus.

Exposure to antihypertensive drugs was ascertained from pharmacy records and was defined as current (at the index-date), past (before the index-date) or never use. For each subject we calculated the duration of use according to the number of units dispensed and the dosing instructions. To take irregular refilling into account we extended the duration of use with

10%[8]. Use of potassium-sparing diuretics or potassium supplements was defined as current or not current (past and non-users combined).

Statistical analysis: We used student t-tests and chi-square statistics to compare characteristics between cases and controls. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CI) and to adjust for confounding factors. In each model the index-date was included to take the matching on index-date into account.

Results

From a total of 5140 antihypertensive drug users we identified 612 incident cases of type 2 diabetes mellitus and 2633 controls, who were all using antihypertensive drugs at the index date. Incident cases of diabetes were more likely to be obese, had a sedentary lifestyle and were more often treated for high cholesterol with diet. Daily alcohol consumption was higher among cases than among controls (Table 1)

Cases received significantly more often thiazide diuretics (7.6%) than controls, while controls were more likely users of calcium antagonists (3.1%).

Thiazide diuretic use and potassium sparing drugs:

As shown in table 2 thiazide use was associated with a significantly increased risk of developing diabetes compared to no thiazide use (adj. OR 1.61 [95%CI: 1.20-2.14]). Taking into account the dose, only thiazide users receiving ≥ 1 DDD/day had a significantly higher risk (adj. OR 1.84 [95%CI: 1.34-2.54]). When thiazide use was combined with a potassium sparing drug or a potassium supplement, this increase in risk remained but was also statistically significant for doses higher than 1 DDD/day (adj. OR 1.65 [95%CI: 1.10-2.47]).

Thiazide diuretic use and combination with other antihypertensive drugs:

When compared to calcium antagonist monotherapy, the risk of developing diabetes was significantly higher for thiazide monotherapy (table 3). Again this increased risk was seen only for doses ≥ 1DDD (adj. OR 2.20 [95%CI: 1.23-3.91]). Thiazide diuretic plus Angiotensin Converting Enzyme Inihibitor (ACEi) or Angiotensin Receptor Blocker (AII) (adj. OR 1.41 [95%CI: 0.79-2.51]) or thiazide diuretic plus non ACEi/AII antihypertensive drug (adj. OR 1.49 [95%CI: 0.88-2.54]) were not associated with a significantly increased risk compared to calcium antagonist monotherapy. Subjects who used thiazides at doses < 1 DDD/day in combination with ACEi or ARB had the lowest risk of type 2 diabetes associated

with thiazide therapy. Potassium sparing agents did not influence the association between thiazides in combination with other antihypertensives and the risk of type 2 diabetes (data not shown). Other combination therapies without thiazides showed no increase in diabetes risk either (adj. OR 1.02[95%CI: 0.62-1.68]).

Discussion

Our study did not show a protective effect of potassium supplementation or potassium sparing drugs on the risk of developing diabetes among thiazide diuretic treated hypertensives. Thiazide diuretic use was significantly associated with the risk of developing diabetes in a dose-dependent way and was lower for subjects who use thiazides in combination with other antihypertensive drugs.

The tendency of diuretics to precipitate diabetes has long been known. Impairment of carbohydrate metabolism through potassium depletion which decreases the insulin secretory response to glucose seems to be the main mechanism[4]. Both reduced insulin release and decreased insulin sensitivity have been demonstrated, but the findings were not consistent[9-12]. Although potassium supplementation may help in preventing thiazide-induced glucose intolerance and insulin hyposecretion[5], we could not demonstrate such a protective effect on clinically manifest type 2 diabetes mellitus. The need for randomized clinical trials that will test dysglycemia prevention by adequate potassium management remains.

When compared to calcium antagonist monotherapy, only thiazide monotherapy users showed a significantly higher risk of developing diabetes. In our study the combination of thiazides with ACEi or AII had a risk of developing diabetes that was not statistically different from calcium antagonist monotherapy, although sample size was limited to test this with sufficient power. A recent study by Burke et al.[13] showed that antihypertensive drug combinations including an ACEi had a significantly lower risk of new-onset diabetes than antihypertensive drug combinations without an ACEi.

In this study we showed that the thiazide induced risk of diabetes was dose-dependent. In a recent study by Shargorodsky et al.[14], it was shown that an increase of thiazide diuretic dose did not only worsen parameters of glucose metabolism but it did not further decrease blood pressure or improve arterial elasticity. These findings might be clinically relevant since thiazide diuretic monotherapy has been recommended by the fifth report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC-V) as a

preferred initial treatment for hypertension. Low-dose thiazide treatment seems to be well-tolerated, and an excellent first-line choice for hypertensive patients, especially in elderly[15].

Some limitations of our study need to be discussed. First, our case definition was based on self-reported diagnosis of diabetes mellitus or the use of glucose lowering medication which might have misclassified case and control subjects. However, our validation showed that our case definition was highly accurate compared to diagnostic information from general practitioner records. Second, although we adjusted for many important potential confounding factors, we cannot exclude residual confounding due to unmeasured or inaccurately measured factors. For instance we had no information on blood pressure or cholesterol levels and could not adjust for these factors. However, established risk factors for diabetes were confirmed in our study suggesting the validity of our data collection. Finally, our analysis on the influence of potassium sparing agents may be biased as patients who receive potassium sparing agents may have a higher risk to have decreased serum potassium levels or to develop hypokalemia.

Strengths of our study include the relatively large sample size, and the availability of complete and accurate data on drug exposure and risk factors for diabetes mellitus.

Thiazide diuretics are considered first line antihypertensive agents based on their superior efficacy in the reduction of the risk of cardiovascular morbidity and mortality[16]. However, the increased risk of type 2 diabetes mellitus due to thiazide diuretic use is often mentioned as a downside of diuretics and may prevent physicians to prescribe thiazide diuretics as a first choice antihypertensive agent. Our findings suggest that the effect of thiazide diuretics on the risk of diabetes may be less when used in combination with other antihypertensive drugs such as ACE inhibitors or ARBs. Regardless of whether patients need combination therapy, low-dose thiazide therapy is safest with regard to the risk of diabetes.

In conclusion, thiazide use alone and in combination with potassium sparing agents or potassium supplement was significantly associated with the risk of developing diabetes in a dose-dependent way.

Table 1 Characteristics of type 2 diabetes mellitus cases and controls.

	Cases	Controls
Number	612	2633
Age (y)	65.2	65.5
Female	36.3%	29.2%*
Obesity (BMI>30 kg/m^2)	36.3%	17.0%*
Sedentary lifestyle (<4 hours/day)	31.2%	20.7%*
High cholesterol		
Diet	21.8%	15.7%*
Drugs	44.4%	44.8%
Smoking		
Current	17.4%	15.6%
Past	50.0%	49.5%
Use of alcohol		
<6 g/day	34.1%	31.1%*
6-12 g/day	11.6%	15.4%*
12-24 g/day	16.9%	23.1%*
>24 g/day	15.5%	15.6%*
Number of antihypertensive drugs		
Current use of antihypertensive drugs		
Thiazide diuretics	37.2%	29.6%*
Beta-blockers	48.9%	49.1%
ACE inhibitors	27.0%	28.3%
AT-II antagonists	15.3%	16.1%
Calcium-antagonists	18.6%	21.7%*

BMI: Body Mass Index

* $p<0.05$

Table 2 Association between current use of thiazide diuretics, K sparing agents and the risk of type 2 diabetes mellitus.

	Cases	Controls	OR [95%CI]	OR [95%CI]*
No Thiazide	368	1840	1.0 (reference)	1.0 (reference)
Thiazide	188	639	1.55 [1.27-1.90]	1.61 [1.20-2.14]§
<1 DDD	73	307	1.30 [0.98-1.73]	1.31 [0.92-1.88]
≥1 DDD	115	332	1.77 [1.39-2.26]	1.84 [1.34-2.54]§
Thiazide+ K-sparing agent	56	154	1.80 [1.29-2.50]	1.65 [1.11-2.44]§
<1 DDD	4	14	1.42 [0.46-4.43]	1.62 [0.51-5.21]
≥1 DDD	52	140	1.83 [1.30 [2.58]	1.65 [1.10-2.47]§

* adjusted for index-date, Body Mass Index, smoking, high cholesterol, use of beta-blockers, ACE inhibitors Angiotensin Receptor blockers, physical activity, daily alcohol use, sex, loop diuretics, age, number of antihypertensive drugs

§ $p<0.05$

Table 3 The risk of type 2 diabetes stratified by anthiypertensive monotherapy use and thiazide combination therapy.

	Cases	Controls	OR [95%CI]	OR [95%CI]*
Ca-antagonist mono	34	185	1.0 (reference)	1.0 (reference)
ACE-I mono	55	296	0.99 [0.62-1.58]	1.01 [0.62-1.62]
Bblo mono	115	592	1.03 [0.67-1.56]	1.09 [0.71-1.67]
AII mono	28	147	1.13 [0.65-1.96]	1.17 [0.67-2.04]
Other mono	4	18	0.97 [0.30-3.08]	1.21 [0.37-3.98]
Thia mono	35	98	1.95 [1.14-3.32] §	2.05 [1.18-3.57] §
<1 DDD	4	16	1.37 [0.43-4.31]	1.35 [0.41-4.43]
≥1 DDD	31	82	2.06 [1.18-3.58] §	2.20 [1.23-3.91] §
Thia+ACE-i/AII	127	452	1.69 [1.11-2.57] §	1.41 [0.79-2.51]
<1 DDD	54	236	1.41 [0.88-2.27]	1.17 [0.63-2.18]
≥1 DDD	73	216	1.98 [1.25-3.12] §	1.65 [0.90-3.02]
Thia+nonACEi/AII	82	243	1.85 [1.18-2.89] §	1.49 [0.88-2.54]
<1 DDD	19	69	1.61 [0.86-3.01]	1.40 [0.70-2.81]
≥1 DDD	63	174	1.94 [1.21-3.10] §	1.52 [0.87-2.65]
Non thia combination	132	602	1.28 [0.84-1.93]	1.02 [0.62-1.68]

DDD: Daily Defined Dose

* adjusted for index-date, Body Mass Index, smoking, high cholesterol, physical activity, daily alcohol use, sex, loop diuretics, age, number of antihypertensive drugs

§ $p<0.05$

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CHAPTER 2.2

Variation in Renin–Angiotensin System and Salt-Sensitivity Genes and the Risk of Diabetes Mellitus Associated With the Use of Thiazide Diuretics

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Abstract

Aim

Variation in the renin–angiotensin system (RAS) and salt-sensitivity genes may influence the effect of thiazides on the risk of diabetes. We assessed whether polymorphisms in RAS and salt-sensitivity genes influenced the risk of diabetes associated with thiazides.

Methods

Nested case-control study was conducted among antihypertensive drug users. Pharmacy records and questionnaires were used to assess new onset diabetes (cases), to ascertain antihypertensive use and risk factors for diabetes. Cases were matched to controls (up to five) who were not (yet) diagnosed with diabetes mellitus. We genotyped angiotensin-converting enzyme (ACE) (G4656C), angiotensinogen (AGT) (M235T), angiotensin II type 1 receptor, (AGTR1) (A1166C), adducin 1 (alpha) (ADD1) (G460T), guanine nucleotide binding protein (G protein), β-polypeptide 3 (GNB3) (C825T).

Results

Among 497 incident cases of type 2 diabetes and 2,633 controls, AGTR1 CC genotype carriers had no increased risk of diabetes due to thiazides (odds ratio (OR) 0.63 (95% confidence interval (CI): 0.28–1.40)) compared to AGTR1 1166A allele carriers (OR 1.79 (95% CI: 1.43–2.23)) receiving thiazides (synergy index (SI) for interaction 0.32 (95% CI: 0.15–0.68)). Although homozygous ACE GG subjects and ACE C allele carriers both had an increased risk of diabetes associated with thiazide use, this risk was more increased for ACE GG subjects (SI 1.70 (95% CI: 1.08–2.66)), particularly at doses ≥1 daily defined dose (DDD) (=25 mg hydrochlorothiazide)/day (SI 2.0 (95% CI: 1.20–3.32)). Among GNB3 T allele carriers, the risk of diabetes due to thiazide use was less increased than among homozygous GNB3 CC subjects (SI 0.62 (95% CI: 0.41–0.93)).

Conclusion

The risk of diabetes due to thiazide use was not increased among AGTR1 1166 CC homozygous subjects and less increased among GNB3 T allele carriers. The ACE 4656 GG genotype enhanced the risk of diabetes due to thiazides.

Introduction

Diabetes mellitus and hypertension are both major risk factors for cardiovascular morbidity and mortality. The coexistence of both diabetes and hypertension doubles the risk of cardiovascular events, cardiovascular mortality, and total mortality.[1,2]

The effect of different classes of antihypertensive drugs on incident diabetes mellitus has been described in a recent meta-analysis.[3] When compared to initial diuretic use, the relative risk reduction of diabetes was the highest for angiotensin receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, and calcium-channel blockers (38%, 33%, and 21%, respectively). β-Blockers did not significantly change this risk. Compared to placebo, only the use of thiazides and β-blockers were significantly associated with the risk of diabetes.

The mechanisms through which thiazide diuretics impair glucose tolerance have not been fully elucidated yet. Thiazides block the renal sodium chloride channel and thereby increase sodium and chloride excretion. In the distal convoluted tubule, thiazides deliver a high sodium load with a resulting increase in potassium excretion. In addition, thiazides activate the renin–angiotensin system (RAS) through volume depletion, which leads to increased aldosterone secretion, further enhancing potassium excretion. One of the hypotheses is that this diuretic-induced hypokalemia leads to a higher secretion ratio of proinsulin to insulin that is biologically less active compared to insulin,[4] which impairs glucose homeostasis.[5] Potassium supplementation may help in preventing thiazide-induced glucose intolerance and insulin hyposecretion.[6] A recent meta-analysis of randomized trials of thiazide diuretic therapy by Zillich *et al.* supports this hypothesis.[7] Increases in glucose were strongly related to thiazide-induced hypokalemia. Investigation of mechanisms of thiazide induced diabetes mellitus and identifying subgroups (e.g., based on genetic constitution) with more or less susceptibility to this side effect may help to identify strategies to prevent thiazide-induced diabetes as was recently acknowledged by a National Heart, Lung, and Blood Institute working group on thiazide-induced dysglycemia.[8] Genes involved in the RAS and salt-sensitivity system can be considered candidate genes for thiazide-induced type 2 diabetes mellitus. Whether variation in these candidate genes influences the response to thiazide diuretics with regard to the risk of diabetes is unknown. The study of interactions between genetic variation in candidate genes of thiazide diuretic response may eventually lead to an individualized treatment approach that determines which patients should be given diuretics and which patients not.

The aim of the present study was to assess whether the risk of diabetes associated with the use of thiazide diuretics was modified by variation in RAS and salt-sensitivity genes.

Methods

Design and setting: Among treated hypertensive patients, we performed a nested case-control study. Patients treated with antihypertensive drugs were identified from the Pharmaco-Morbidity Record Linkage System (PHARMO-RLS). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (Landelijke Medische Registratie) from 1985 onwards. Currently, the base population of PHARMO covers about 2,000,000 community-dwelling inhabitants of about 50 population-defined areas in the Netherlands. Data from a pharmacogenetic study of antihypertensive drug treatment including 5,140 antihypertensive drug users were used to collect data on genotypes and potential risk factors for diabetes mellitus. In this pharmacogenetic study, specific genetic polymorphisms are examined with regard to antihypertensive drug response in a selection of PHARMO-RLS participants.[9] This study was approved by the Medical Ethical Committee of the University Medical Center Utrecht and all subjects have signed written informed consent.

Data collection: The PHARMO-RLS provides records containing the name of the dispensed drugs, day of dispensing, number of units dispensed, prescribed daily dose, and the anatomical therapeutical chemical code of the drug. Questionnaire information was used to collect data on demographic variables, cardiovascular diseases, and risk factors, whereas buccal swabs were used to collect DNA.

Outcome and exposure definition: The outcome of interest was incident diabetes. Pharmacy records were used to identify incident diabetes (cases). Incident cases of diabetes were defined as those having a first prescription for oral antidiabetic drugs or insulin at least 1 year after the date of entry in PHARMO-RLS. To assess the date of first diagnosis of diabetes mellitus, we used both pharmacy records and self-reported questionnaire information. All subjects reported on whether they had diabetes mellitus and on which date this was first diagnosed by a physician. Cases were assigned an index date, which was defined as either the date the first antidiabetic drug prescription was dispensed or the date of self-reported diagnosis of diabetes mellitus, whichever came first. For each case, we selected up to five controls who also received antihypertensive drugs but who were not (yet) diagnosed with diabetes mellitus at the index date of the case.

In a small validation study among 83 subjects for whom general practitioner records were available (24 cases and 59 controls), we found that 91.7% (22) of the cases could be confirmed according to World Health Organization criteria for a diagnosis of type 2 diabetes mellitus (either a fasting plasma glucose >7.0 mmol/l and/or random (nonfasting) blood glucose >11.1 mmol/l, and/or use of oral antidiabetic medication and/or use of insulin and/or treatment by diet and registered by a general practitioner as having type 2 diabetes mellitus),[10] whereas 100.0% (59) of the controls had no diagnosis of type 2 diabetes mellitus.

Exposure to antihypertensive drugs was ascertained from pharmacy records. All subjects were current users of at least one antihypertensive drug. Thiazide diuretic use was defined as current (at the index date) or not current (before the index date). We also calculated the dose of current use of thiazides according to the defined daily dose (DDD), which is the average daily dose of a drug for its main indication in adults, and is recommended by the World Health Organization for drug utilization studies.[11] For the diuretics that were used in our study, the DDDs were bendroflumethiazide (2.5 mg), hydrochlorothiazide (25 mg), chlorthiazide (25 mg), epitizide (4 mg), clopamide (10 mg), chlorthalidone (25 mg), mefruside (25 mg), and indapamide (2.5 mg).

Genotyping: Genomic DNA was isolated from buccal swabs according to standard procedures. Genotyping was determined using a multiplex single base extension method. Multiplex single base extension was performed using SNaPshot as described by the manufacturer (Applied Biosystems, Foster City, CA). This method was described earlier,[12] but adapted to a new set of polymorphisms for the pharmacogenetic study of antihypertensive drug therapy. The multiplex assay was validated on a set of 100 DNA samples that had previously been genotyped with an alternative technique; this technique concerned a multilocus genotyping assay for candidate markers of cardiovascular disease risk (Roche Molecular Systems, Basel, Switzerland) and has been described in detail previously.[13] The ACE (I/D) polymorphism was determined by detecting ACE4656+CT observed on the SNaPshot as ACE G4656C (NCBI single-nucleotide polymorphism (SNP) database nr: rs4341). The ACE4656+CT polymorphism in the 3'-untranslated region of the ACE gene consists of a repetition of two or three CT dinucleotides and is in complete linkage disequilibrium with ACE (I/D).[12,14] The G detected on the SNaPshot corresponds with the deletion allele and the C corresponds with the insertion allele of the ACE gene. Furthermore, we assessed angiotensin II type 1 receptor (AGTR1) A1166C (NCBI SNP database: rs5186), angiotensinogen (AGT) Met235Thr (further denoted as M235T, NCBI SNP database nr:

rs699), guanine nucleotide binding protein (G protein), β -polypeptide 3 (GNB3) C825T (NCBI SNP database nr: rs5443), and adducin 1 (alpha) (ADD1) Gly460Trp (further denoted as G460T, NCBI SNP database nr: rs4961).

Analysis: Analysis was performed using SPSS (version 12.1, SPSS, Chicago, IL). Continuous variables are presented as mean ± s.d. Deviations from Hardy–Weinberg equilibrium were considered using chi-square tests. Unconditional logistic regression analysis was used to study the association between thiazide diuretic use and the incidence of new onset of diabetes. All analyses were stratified by ACE (G4656C), AGT (M235T), AGTR1 (A1166C), GNB3 (C825T), and ADD1 (G460T) genotype to study effect modification. We considered gender, smoking, physical activity, body mass index, alcohol use, calendar year, self-reported high blood pressure, and drug treated hypercholesterolemia as potential confounders.

Based on the results of preliminary analyses, we chose to combine homozygous and heterozygous 4656C allele carriers of the ACE gene, homozygous and heterozygous 1166A allele carriers of the AGTR1 gene, and homozygous and heterozygous 235T allele carriers of the AGT gene. These genotype groups showed similar effects of thiazides on the risk of diabetes. Because of small numbers of subjects, homozygous for the less common alleles of the GNB3 and ADD1 polymorphisms, these individuals were grouped together with heterozygotes.

To assess potential interactions, we estimated the odds ratio (OR) and confidence intervals for diabetes in strata defined by genotype. To test for drug–gene interaction, we computed the synergy index (SI) by adding an interaction term (THIAZIDE X GENOTYPE) to the logistic regression model. The SI can be interpreted as the ratio of the OR in subjects with a susceptible genotype and the OR in subjects without this genotype. A SI of 1 indicates no interaction on the multiplicative scale.[15]

In sensitivity analyses, we also distinguished between thiazide diuretic use with or without a RAS inhibitor and thiazide diuretic use with or without a potassium sparing diuretic.

Results

Among 5,140 antihypertensive drug users, we identified 497 incident cases of type 2 diabetes mellitus and 2,633 controls. **Table 1** shows the baseline characteristics of cases and controls.

The common risk factors for type 2 diabetes mellitus such as obesity, sedentary lifestyle, and hypercholesterolemia were significantly more common among cases than controls. Of the

antihypertensive drugs studied, only the frequency of thiazide diuretic use differed significantly between cases and controls. Hydrochlorothiazide was the most frequently used thiazide diuretic, followed by indapamide and chlorthalidone.

Overall thiazide diuretic use was associated with an increased risk of diabetes mellitus in a dose-dependent way (OR $_{<1\ DDD/day}$ 1.30 (95% confidence interval (CI): 0.95–1.77), OR$_{\geq 1\ DDD/day}$ 1.82 (95% CI: 1.43–2.31), see **Table 2**, *P* for trend <0.001). For the largest groups of thiazide diuretics such as hydrochlorothiazide, epitizide, chlorthalidone, and indapamide the increased risk of diabetes mellitus was similar. The increased risk did not change when thiazides were used in combination with potassium sparing diuretics (adjusted OR 1.53 (95% CI: 1.05–2.23)) or in combination with RAS inhibitors (adjusted OR 1.70 (95%CI: 1.27–2.27)).

In the analysis stratified by polymorphisms in the RAS and salt-sensitivity genes, the dose dependent risk of diabetes due to thiazide use remained for all genotypes, except for the AGTR1 CC homozygotes (**Table 3**).

For the polymorphisms in the salt-sensitivity genes, ADD1 T allele carriers had a slightly higher risk of diabetes due to thiazide use (adjusted OR 1.81 (95% CI: 1.28–2.58)) compared to ADD1 GG heterozygotes (adjusted OR 1.48 (95% CI: 1.13–1.93)). This interaction was however not statistically significant (SI 1.34 (95% CI: 0.88–2.04)). Among subjects using thiazides at doses ≥1 DDD/day, this interaction was slightly higher, though not statistically significant (SI 1.53 (95% CI: 0.95–2.46)). Thiazide users carrying the GNB3 CC genotype had a higher risk of diabetes (adjusted OR 2.01 (95% CI: 1.49–2.72) compared to T allele carriers who used thiazides (adjusted OR 1.36 (95% CI: 1.01–1.84)). The SI for the interaction between the GNB3 T allele and thiazide use was 0.62 (95% CI: 0.41–0.93) (*P* = 0.021).

With regard to the polymorphisms in the RAS, both ACE 4656C allele carriers and ACE 4656 GG homozygotes had a significantly increased risk of developing diabetes while using thiazides (OR 1.38 (95% CI: 1.08–1.78) and 2.49 (95% CI: 1.65–3.75), respectively). However, carriers of the ACE GG genotype had a more increased risk while on thiazide therapy compared to C allele carriers (SI 1.70 (95% CI: 1.08–2.66), *P* = 0.022). This interaction was not present at doses <1 DDD/day (SI 1.18 (95% CI: 0.58–2.38)), whereas among subjects who used thiazides at doses ≥1 DDD/day the interaction was strongest (SI 2.00 (95% CI: 1.20–3.32)).

Carriers of the AGTR1 1166 A allele had an increased risk of diabetes due to thiazide use (OR 1.79 (95% CI: 1.43–2.23)). Carriers of the AGTR1 CC genotype had no increased risk of diabetes while using thiazides (OR 0.63 (95% CI: 0.28–1.40)), not even at doses ≥ 1DDD/day (OR 0.57 (95% CI: 0.23–1.41)). The SI for the interaction between thiazide use and the AGTR1 CC genotype was 0.32 (95% CI: 0.15–0.68) ($P = 0.003$).

The AGT M235T polymorphism did not modify the association between thiazide use and diabetes mellitus.

In sensitivity analyses, we did not observe any influence of the combined use of thiazides and potassium sparing diuretics, nor of the combined use of thiazides and RAS inhibitors on the interaction between thiazides and the genetic polymorphisms of interest. We also studied the interaction between individual types of thiazide diuretics and found no major differences. The numbers for these analyses were quite small making definite inferences not possible. Finally, we also investigated all combinations of two genes. None of these combinations resulted in statistically significantly interactions with thiazide diuretic use.

Discussion

Our study suggests that the risk of diabetes associated with the use of thiazides is strongly modified by the GNB3 C825T, ACE G4656C, and AGTR1 A1166C polymorphisms. Carriers of the ACE 4656 GG genotype were most susceptible to the detrimental effect of thiazides on the risk of diabetes, whereas carriers of the AGTR1 CC genotype had no increased risk of diabetes and carriers of the GNB3 825T allele who used thiazides had a less increased risk of diabetes. The increased susceptibility of ACE 4656 GG homozygote subjects to the effect of thiazides on the risk of diabetes was only present among subjects who used doses ≥1 DDD/day.

The strengths of this observational study are the relatively large sample size and the complete and accurate information on drug exposure and most risk factors that are important when considering possible antihypertensive drug–gene interactions. The limitations of our study are first of all our case definition that was based on the use of glucose lowering medication and the use of self-reported information on diagnosis dates. This method may have lead to a misclassification of cases and controls. Our small validation study however showed that our case definition was highly accurate compared to diagnostic information from general practitioner records. Second, we did not have information on serum potassium levels. The review by Zillich *et al.* showed that the use of potassium sparing agents was associated with

half the increase in glucose compared with no use of potassium sparing agents.[7] Third, we used the World Health Organization DDD classification to analyse dose–response relationships. This classification implies that a dose of 25 mg of chlorthalidone is equivalent to a dose of 25 mg hydrochlorothiazide. This assumption is probably not valid. A recent cross-over trial suggests that chlortalidone is about 1.5–2 times as potent as hydrochlorothiazide with regard to antihypertensive efficacy.[16] Nonetheless, in this study no differences in potassium levels were observed between chlorthalidone and hydrochlorothiazide. Therefore, in our study on the risk of diabetes mellitus the use of the DDD for dose–response relationships was appropriate.

Fourth, a limitation of this study is that despite almost complete information on important potential confounders, we cannot fully exclude residual confounding due to unmeasured or inaccurately measured factors.

Fifth, our study was restricted to only one polymorphism per gene, although the polymorphisms were selected because of a biologically plausible role in response to thiazides. Finally, although the sample size of our study was relatively large, it was too small to assess drug–gene–gene interactions and to assess the effect of potassium sparing agents. When a more stringent significance level of 0.01 would have been applied, only the interaction between AGTR1 and thiazides remained significant. Therefore, our findings should be considered as hypothesis generating and need replication in other studies. To our knowledge, this is the first study that investigated the influence of variation in RAS genes and salt-sensitivity genes on the risk of diabetes associated with thiazide use.

One might speculate on explanations for our findings. On the one hand, one could entertain the hypothesis that genotypes that may be related to an increased RAS activity (ACE GG genotype, AGTR1 CC genotype, and AGT T allele) would be associated with a smaller response to thiazide diuretics (more counterregulation, less potassium excretion, and smaller risk of diabetes).[17] However, on the other hand, increased activation of the RAS due to thiazides among subjects with a genetically already increased RAS activity might further increase angiotensin II signaling, which diminishes insulin signaling and glucose transport and thereby increase the risk of diabetes.[18,19,20] Our finding that carriers of the ACE GG genotype (which corresponds to the DD genotype of the ACE insertion/deletion polymorphism) had a twofold increased risk of developing diabetes while receiving thiazide diuretics in doses ≥1 DDD compared to C allele carries (I allele) suggests that the deleterious effects of increased RAS activity on glucose metabolism might be the dominating

mechanism. However, the AGTR1 CC genotype that is associated with a greater sensitivity for angiotensin-II through a greater number of receptor binding sites and affinity for angiotensin-II[21] was associated with a reduced risk of diabetes due to thiazides. This finding suggests that more counterregulation of the thiazide induced sodium-volume depletion and related potassium excretion might explain the reduced risk of diabetes among these subjects. The sample size of our study was not large enough to investigate whether the ACE G4656C or the AGTR1 A1166C polymorphism is more important in determining the effect of thiazides on the risk of diabetes.

The GNB3 825T allele has been associated with an increased blood pressure response to thiazide diuretic therapy[22]. Interestingly, in a small trial the GNB3 825T allele was not associated with increased thiazide-induced potassium excretion,[23] whereas in another trial the 825T allele was associated with a greater angiotensin-II responsiveness[24]. These experimental findings would suggest that a greater RAS activity of GNB3 825T allele carriers may result in a stronger counterregulation of thiazide induced volume depletion and related potassium excretion. This increased potassium excretion probably explains the lower risk of thiazide induced diabetes among GNB3 825T allele carriers. Increased potassium excretion resulting in hypokalemia increase glucose levels and probably the risk of type 2 diabetes mellitus as was suggested by the National Heart, Lung, and Blood Institute working group on thiazide-induced dysglycemia and further supported by the analysis of Zillich *et al.*[7,8] Moreover, Shafi *et al.* demonstrated in a *post hoc* analysis of the Systolic Hypertension in the Elderly Program trial comparing chlorthalidone with placebo that the risk of diabetes was mediated for about 41% by decreased serum potassium levels[25].

Although our findings need confirmation in other studies, in the future an individualized treatment approach in which the choice for thiazide diuretic therapy depends on the genotype of a patient might be feasible. To appreciate the potential impact of a genotype-based treatment with thiazides, we calculated the number of diabetes mellitus patients that might be prevented if for instance only subjects with the GNB3 T allele would be given thiazides and that homozygous wild type subjects would be given other antihypertensive agents. Based on an absolute risk of incident diabetes mellitus of about 6.8% during an average follow-up of approximately 3–4 years in the placebo groups of randomized controlled trials of antihypertensive drug treatment, a prevalence of the GNB3 T allele of 69%, this strategy would prevent about 25 cases of diabetes mellitus for every 1,000 patients who were going to be treated with thiazide diuretics. In an overall treatment strategy, the beneficial effects of

thiazides on the risk of cardiovascular disease should be weighed against side effects such as an increased risk of diabetes mellitus.

In conclusion, the risk of diabetes associated with the use of thiazide diuretics in hypertensive subjects was modified by polymorphisms in the RAS and one salt-sensitivity gene. Carriers of the AGTR1 1166 CC had a significantly lower risk of diabetes compared to A allele carriers, while carriers of the ACE 4656 GG genotype who used thiazides at doses ≥1 DDD/day had a significantly higher risk of diabetes compared to C allele carriers. GNB3 T allele carriers of the C825T polymorphism had a significantly decreased risk of diabetes due to thiazides compared to CC genotype carriers.

Table 1 Baseline characteristics of cases and controls.

	Cases	Controls	p-value
Number	497	2633	
Age	65.8	65.7	0.954
Sex (Female)	170 (34.2%)	818 (31.1%)	0.167
BMI>30 kg/m^2	102 (34.8%)	311 (17.9%)	<0.001[§]
High blood pressure	375 (88.9%)	1999 (88.3%)	0.804
High cholesterol			0.002[§]
Normal			
High, diet	113 (29.5%)	815 (38.0%)	
High, drugs	71 (18.5%)	299 (13.9%)	
	199 (52.0%)	1030 (48.0%)	
Smoking			0.034[§]
Never			
Current	109 (36.2%)	626 (35.1%)	
Past	61 (20.3%)	267 (15.0%)	
	131 (43.5%)	888 (49.9%)	
Sedentary (<4 hour/week)	100 (30.8%)	409 (21.7%)	<0.001[§]
Use of alcohol			0.088
Never use			
Past use or <6g/day	66 (20.6%)	331 (17.8%)	
6-12g/day	116 (36.3%)	574 (30.8%)	
12-24g/day	42 (13.1%)	284 (15.2%)	
≥24g/day	66 (20.6%)	412 (22.1%)	
	30 (9.4%)	263 (14.1%)	
Current use antihypertensive drugs			
Thiazide diuretics	198 (39.8%)	793 (30.1%)	<0.001[§]
Betablockers	288 (57.9%)	1430 (54.3%)	0.14
ACE inhibitors	168 (33.8%)	888 (33.7%)	1.000
AT2 blockers	89 (17.9%)	471 (17.9%)	1.000
Calcium antagonists	122 (24.5%)	667 (25.3%)	0.736
Miscellaneous	18 (3.6%)	82 (3.1%)	0.577

[§] p<0.05

Table 2 Overall analysis between thiazide diuretic use and the risk of type 2 diabetes mellitus.

	Cases	Controls	OR*	OR**
Other AH	299	1840	1.0 (reference)	1.0 (reference)
Thiazide diuretics	198	793	1.58 [1.30-1.93]§	1.61 [1.31-1.99]§
< 1 DDD	62	321	1.28 [0.95-1.73]	1.30 [0.95-1.77]
≥ 1 DDD	136	472	1.78 [1.42-2.24]§	1.82 [1.43-2.31]§
Other AH	299	1840	1.0 (reference)	1.0 (reference)
Thiazide alone	156	639	1.57 [1.26-1.94]§	1.64 [1.31-2.05]§
Thiazide + potassium sparing diuretic	42	154	1.65 [1.15-2.38]§	1.53 [1.05-2.23]§
Other AH	153	948	1.0 (reference)	1.0 (reference)
Thiazide alone	97	341	1.67 [1.25-2.23]§	1.66 [1.22-2.25]§
Thiazide + RAS inhibitor	107	452	1.62 [1.23-2.13]§	1.70 [1.27-2.27]§
RAS inhibitor	146	892	1.08 [0.84-1.38]	1.08 [0.84-1.39]

Other AH: Other antihypertensives including calcium-antagonists, betablockers and miscellaneous antihypertensive drugs; DDD: Daily Defined Dose

* Adjusted for calendaryear

** Adjusted for calendaryear, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity and smoking

§ $p<0.05$

Table 3 Stratified analysis of genetic polymorphisms in the RAS, GNB3 and ADD1 and the risk of type 2 diabetes mellitus associated with the use of thiazides.

	Cases	Controls	OR*	OR**	SI*	SI**
ADD						
1460GG						
Other AH	192	1092	1.0 (reference)	1.0 (reference)		
Thiazide	121	500	1.43 [1.11-1.84]§	1.48 [1.13-1.93]§		
< 1 DDD	42	197	1.32 [0.91-1.91]	1.34 [0.91-1.97]		
≥ 1 DDD	79	303	1.49 [1.11-2.00]§	1.56 [1.15-2.13]§		
1460GT/TT						
Other AH	107	748	1.0 (reference)	1.0 (reference)		
Thiazide	77	293	1.88 [1.36-2.59]§	1.81 [1.28-2.58]§	1.34 [0.89-2.03]	1.34 [0.88-2.04]
< 1 DDD	20	124	1.18 [0.70-1.98]	1.16 [0.67-2.00]	0.94 [0.50-1.78]	0.99 [0.52-1.91]
≥ 1 DDD	57	169	2.36 [1.64-3.39]§	2.26 [1.52-3.34]§	1.59 [1.00-2.54]§	1.53 [0.95-2.46]
GNB3						
825CC						
Other AH	138	875	1.0 (reference)	1.0 (reference)		
Thiazide	109	363	1.98 [1.49-2.63]§	2.01 [1.49-2.72]§		
< 1 DDD	32	143	1.56 [1.02-2.40]§	1.66 [1.06-3.60]§		
≥ 1 DDD	77	220	2.23 [1.62-3.06]§	2.22 [1.58-3.11]§		
825CT/TT						
OA	161	965	1.0 (reference)	1.0 (reference)		
Thiazide	89	430	1.27 [0.96-1.69]	1.36 [1.01-1.84]§	0.64 [0.43-0.96]	0.62 [0.41-0.93]§
< 1 DDD	30	178	1.06 [0.70-1.62]	1.07 [0.69-1.66]	0.68 [0.37-1.24]	0.61 [0.33-1.12]
≥ 1 DDD	59	252	1.41 [1.01-1.96]§	1.58 [1.11-2.24]§	0.63 [0.40-1.00]§	0.64 0.40-1.02]
ACE						
4656 CC/CG						
Other AH	224	1300	1.0 (reference)	1.0 (reference)		
Thiazide	137	584	1.39 [1.10-1.76]§	1.38 [1.08-1.78]§		
< 1 DDD	47	234	1.24 [0.87-1.75]	1.22 [0.85-1.75]		
≥ 1 DDD	90	350	1.49 [1.14-1.96]§	1.49 [1.12-1.99]§		
4656 GG						
Other AH	75	540	1.0 (reference)	1.0 (reference)		
Thiazide	61	209	2.25 [1.53-3.29]§	2.49 [1.65-3.75]§	1.55 [0.99-2.41]	1.70 [1.08-2.66]§
< 1 DDD	15	87	1.38 [0.75-2.53]	1.61 [0.85-3.03]	1.04 [0.52-2.08]	1.18 [0.58-2.38]
≥ 1 DDD	46	122	2.84 [1.86-4.34]§	3.07 [1.94-4.84]§	1.86 [1.13-3.06]§	2.00 [1.20-3.32]§

Table 3 continued

AGTR1						
1166AA/AC						
Other AH	255	1679	1.0 (reference)	1.0 (reference)		
Thiazide	186	717	1.76 [1.43-2.17]§	1.79 [1.43-2.23]§		
< 1 DDD	59	301	1.38 [1.01-1.88]§	1.39 [1.01-1.92]§		
≥ 1 DDD	127	416	2.02 [1.59-2.57]§	2.07 [1.61-2.66]§		
1166CC						
Other AH	43	156	1.0 (reference)	1.0 (reference)		
Thiazide	11	75	0.53 [0.26-1.09]	0.63 [0.28-1.40]	0.30 [0.14-0.64]§	0.32 [0.15-0.68]§
< 1 DDD	3	20	0.61 [0.17-2.18]	0.84 [0.21-3.43]	0.43 [0.12-1.57]	0.45 [0.12-1.68]
≥ 1 DDD	8	55	0.50 [0.22-1.15]	0.57 [0.23-1.41]	0.25 [0.11-0.60]§	0.27 [0.11-0.63]§
AGT						
235MM						
Other AH	94	667	1.0 (reference)	1.0 (reference)		
Thiazide	66	298	1.63 [1.15-2.30]§	1.71 [1.18-2.48]§		
< 1 DDD	18	136	0.99 [0.58-1.70]	1.04 [0.59-1.84]		
≥ 1 DDD	48	162	2.13 [1.45-3.15]§	2.25 [1.48-3.42]§		
235MT/TT						
Other AH	205	1173	1.0 (reference)	1.0 (reference)		
Thiazide	132	495	1.55 [1.21-1.98]§	1.57 [1.21-2.03]§	0.93 [0.61-1.42]	0.92 [0.60-1.41]
< 1 DDD	44	185	1.46 [1.01-2.09]§	1.46 [1.0-2.13]§	1.40 [0.73-2.67]	1.31 [0.68-2.52]
≥ 1 DDD	88	310	1.61 [1.21-2.1]§	1.63 [1.21-2.9]§	0.74 [0.46-1.20]	0.75 [0.46-1.23]

DDD: Daily Defined Dose; Other AH: other antihypertensives including calcium-antagonists, betablockers and miscellaneous antihypertensive drugs

* Adjusted for calendaryear

** Adjusted for calendaryear, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity and smoking

§ $p<0.05$

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CHAPTER THREE

Genetic polymorphisms in RAAS genes, association between use of ACE Inhibitors and ARB and the incidence of diabetes

CHAPTER 3.1

Genetic variation in the Renin-Angiotensin-System modifies the beneficial effects of ACE inhibitors on the risk of diabetes mellitus among hypertensives

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Abstract

Aim

The aim of this study was to assess whether the association between ACE inhibitor use and the incidence of treated diabetes mellitus is modified by genetic polymorphisms in the RAS.

Methods

In a nested case-control study, treated hypertensive patients were genotyped for angiotensin converting enzyme (ACE; insertion(I)/deletion(D)), angiotensinogen (AGT; M235T), and Angiotensin II type-1-receptor (AGTR1; A1166C). Cases of newly treated diabetes were identified based on pharmacy records and controls were not yet drug-treated for diabetes (case:control ratio 1:10). Self-administered questionnaires and physical examinations were used to assess risk factors for diabetes mellitus. Logistic regression was used to calculate the relative risk of diabetes associated with ACE inhibitor use relative to other antihypertensive treatment, stratified by the RAS genotypes.

Results

Among 205 cases and 2050 controls, homozygous 1166A-carriers of the AGTR1 gene had a significantly decreased incidence of diabetes associated with current use of ACE inhibitors (OR 0.47[95%CI:0.26-0.84]), whereas this incidence was increased among 1166C-allele carriers (OR 1.32[95%CI: 0.81-2.14]). The interaction OR was 3.21 [95%CI:1.53-6.75]. ACE-I allele carriers had a significantly reduced incidence of diabetes associated with ACE inhibitors use (OR 0.63 [95%CI:0.41-0.98]), whereas DD homozygotes had no reduced risk (OR 0.95 [95%CI:0.46-1.96]. The risk of diabetes associated with ACE inhibitor use was not significantly modified by the AGT-M235T polymorphism.

Conclusions

Treatment with ACE inhibitors in hypertensive subjects significantly reduces the occurrence of diabetes in homozygous 1166A-carriers of the AGTR1 gene and carriers of the ACE-I allele, but not in 1166C-allele carriers of the AGTR1 gene and in homozygous ACE-D allele carriers.

Introduction

Essential hypertension is closely associated with type 2 diabetes mellitus. Both elevated blood pressure and impaired glucose tolerance are key components of the metabolic syndrome, a major cause of cardiovascular morbidity and mortality [1].

The metabolic effects of treatment with antihypertensive agents may be negative (diuretics, beta-blockers), neutral (calcium antagonists), or positive (ACE inhibitors and angiotensin II type 1 receptor blockers (ARBs)). A recent meta-analysis assessed that the relative risk reduction of type 2 diabetes mellitus by ACE inhibitors or ARB therapy compared to diuretic therapy was 23% and 43%, respectively[2]. The mechanisms through which ACE inhibitors and ARBs reduce the risk of diabetes mellitus remain uncertain, although several mechanisms, such as decreased renal potassium wasting, and improved islet blood flow and pancreatic beta-cell perfusion by reducing angiotensin II-mediated vasoconstriction in the pancreas, have been proposed[3]. Polymorphisms in three genes that code for major components of the renin-angiotensin-system (RAS), angiotensinogen (AGT) M235T, angiotensin-converting enzyme (ACE) insertion/deletion (I/D), and angiotensin II type 1 receptor (AGTR1) A1166C, may influence the response to these antihypertensive agents and thus the incidence of diabetes. The aim of this study was to assess whether the association between the use of ACE inhibitors and the incidence of diabetes is modified by genetic polymorphisms in RAS genes.

Methods

Design and setting: We performed a nested case-control study among treated hypertensive patients. The Pharmaco-Morbidity Record Linkage System (PHARMO-RLS) was used to identify patients treated with antihypertensive drugs. PHARMO-RLS links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (LMR) from 1985 onwards. Currently, the base population of PHARMO-RLS covers about 2 million community-dwelling inhabitants of about 50 population-defined areas in the Netherlands. As data from two population-based studies overlap with the PHARMO-RLS population, these could be used to collect data on genotypes and potential risk factors for diabetes mellitus (see Figure 1).

The Monitoring Project on Cardiovascular Risk Factors was a population-based cross-sectional study of cardiovascular disease risk factors conducted in three Dutch cities

(Amsterdam, Maastricht and Doetinchem). In Doetinchem, 12,449 men and women between the age of 20 and 59 from a total population of circa 40,000 inhabitants during 1987 to 1991, were examined. Details have been published elsewhere[4]. Of these Doetinchem subjects, 9,336 were also linked to the PHARMO-RLS and of 571 subjects both pharmacy records and genotype data were available for treated hypertensive subjects.

In a pharmacogenetic study including 4,985 treated hypertensive subjects specific genetic polymorphisms were examined for the modification of the effect of antihypertensive drugs on the risk of myocardial infarction in a selection of PHARMO-RLS participants(5). Of these 4,985 subjects, 1275 had both pharmacy records available and genotype data were available for treated hypertensive subjects.

In total, 1,846 treated hypertensive patients with both pharmacy records and genotype data were available from both populations.

Data collection: The PHARMO-RLS provides records containing the name of the dispensed drugs, day of dispensing, number of units dispensed, prescribed daily dose and the Anatomical Therapeutical Chemical (ATC) code of the drug. In both Monitoring Project on Cardiovascular Risk Factors and the pharmacogenetic study, data were obtained on demographic variables, cardiovascular diseases and risk factors. In the Monitoring Project on Cardiovascular Risk Factors Study, venous blood samples were obtained for DNA extraction whereas in the pharmacogenetic study buccal swabs were used to collect DNA.

Genotyping: Genomic DNA was isolated from peripheral blood or buccal swabs according to standard procedures. The genotyping procedure of the AGT M235T[6], ACE I/D[7] and AGTR1 1166A/C[8] polymorphisms, in the Monitoring Project on Cardiovascular Risk Factors Study were previously described. Genotyping in the pharmacogenetic study was determined using a multiplex Single Base Extension (SBE) method. Multiplex SBE was performed using SNaPshotTM as described by the manufacturer (Applied Biosystems). This method was described earlier[9], but adapted to a new set of polymorphisms for the pharmacogenetic study. The multiplex assay was validated on a set of 100 DNA samples that had previously been genotyped with an alternative technique; this technique concerned a multilocus genotyping assay for candidate markers of cardiovascular disease risk (Roche Molecular Systems Inc) and has been described in detail previously[10]. All three RAS polymorphisms were concordant. In the Monitoring Project on Cardiovascular Risk Factors Study, the ACE I/D polymorphism was directly assessed while in the pharmacogenetic study

the ACE I/D polymorphism was determined by detecting ACE 4656+CT observed on the SNaPshot as ACE (G4656C). The G4656C polymorphism in the 3'-untranslated region of the ACE gene consists of a repetition of 2 or 3 CT dinucleotides, which is in complete linkage disequilibrium with ACE (I/D)[9, 11].

Outcome and exposure definition: The outcome of interest was incident diabetes. Pharmacy records were used to assess incident diabetes (cases). Incident cases of diabetes were defined as those having a first prescription for oral anti-diabetic drugs or insulin at least one year after the date of entry in PHARMO-RLS. Cases were assigned an index date, which was defined as the day the first anti-diabetic drug prescription was dispensed. For each case we selected 10 controls who also received antihypertensive drugs but who were not (yet) treated with anti-diabetic drugs at the index date of the case. We used risk-set sampling of cases and controls which means that one subject may serve as control multiple times for different cases and also that a control may become a case later on [12].

Exposure to antihypertensive drugs was ascertained from pharmacy records and was defined as current, past or never use. We also calculated the dose of current use of ACE inhibitors according to the defined daily dose (DDD), which is the average daily dose of a drug for its main indication in adults, and is recommended by the World Health Organization for drug utilization studies [11].

In sensitivity analyses we also categorized subjects as current users of ACE inhibitors, ARB, thiazides, and other antihypertensives (e.g. beta-blockers, calcium-antagonists). In these analyses the group of other antihypertensives served as the reference group.

Statistical analysis: Analyses were performed using SPSS (version 12.1, SPSS Inc., Chicago). Continuous variables are presented as mean ± standard deviation. Deviations from Hardy-Weinberg equilibrium were checked using chi-square tests. Unconditional logistic regression analysis was used to study the association between ACE INHIBITOR use and incident diabetes. Then, all analyses were stratified by ACE (I/D), AGT (M235T), and AGTR1 (A1166C) genotypes to study interaction. We considered age, gender, smoking, physical activity, body mass index and alcohol use as potential confounders. Because of the assumed association of the ACE DD genotype with higher incidence of diabetes, the II and ID genotypes were grouped together in the analysis, and crude and adjusted OR were computed for both groups separately. For the analysis of the AGT gene the TT and the MT genotypes

were analyzed together. Similarly, the AGTR1 CC and CA genotypes were grouped together and compared with AA genotype.

The presence and statistical significance of interaction OR was tested by adding an interaction term (ACE INHIBITOR x GENOTYPE) to the logistic regression model. The interaction OR can be interpreted as the ratio of the OR in subjects with a susceptible genotype and the OR in subjects without this genotype. An interaction OR of 1 indicates no interaction on the multiplicative scale[14]. We also calculated the relative excess risk due to interaction (RERI) according to Rothman[12]. A RERI of 0 indicates no interaction on the additive scale.

Results

Table 1 shows the baseline characteristics of the 205 cases and 2050 controls. Cases were obese significantly more often than controls. Of the antihypertensive drugs studied, only the frequency of thiazide diuretic use differed significantly between cases and controls. All polymorphisms were in Hardy-Weinberg equilibrium. The ACE, AGT and AGTR1 genotype frequencies did not differ significantly between cases and controls.

Overall use of ACE inhibitors was not statistically significantly associated with a reduced incidence of diabetes (adjusted OR 0.74 [95%CI: 0.51-1.06]). When stratified on dose of ACE inhibitor we found that a dose of < 1 DDD per day was not associated with the incidence of diabetes (adjusted OR 1.08 [95%CI:0.61-1.93], whereas current use of a dose ≥ 1 DDD per day was significantly associated with a reduced risk of diabetes (adjusted OR: 0.64 [0.42-0.97]). **Table 2** shows the associations between use of ACE inhibitors and incidence of diabetes stratified by genetic polymorphisms in the RAS. Subjects carrying the AA genotype of the AGTR1 gene had a significantly lower incidence of diabetes when they were current users of ACE inhibitors (adjusted OR 0.47 [95%CI: 0.26-0.84]). Subjects with the AA genotype who used ≥ 1 DDD per day had a lower relative risk of diabetes compared to subjects who used < 1 DDD per day. This association was not seen in carriers of the C allele (adjusted OR 1.32 [95%CI: 0.81-2.14]). C allele carriers who used ≥ 1 DDD per day had a higher relative risk of diabetes compared to subjects who used < 1 DDD per day. The incidence of diabetes was also lower in current users of ACE inhibitors carrying the I allele of the ACE gene (adjusted OR 0.63 [95%CI: 0.41-0.98]). The incidence of diabetes decreased with increasing dose among ACE I allele carriers. No significant association was found with regard to ACE inhibitor use in ACE DD subjects (adjusted OR 0.95 [95%CI: 0.46-1.96]). The AGT M235T polymorphism did not modify the association between ACE inhibitor use and

the risk of diabetes (adjusted OR T allele 0.81[95%CI: 0.55-1.19]; adjusted OR MM 0.41[95%CI: 0.13-1.23]).

We also assessed the interaction between use of ACE inhibitors and polymorphisms in the RAS on the incidence of diabetes **(Table 3)**. The use of ACE inhibitors in AGTR1 C allele carriers was associated with significantly higher incidence of diabetes compared to AA genotype (adjusted interaction OR 3.21 [95%CI: 1.53-6.75]). This interaction was particularly present among those who used ≥ 1 DDD per day (adjusted interaction OR 8.30 [95%CI: 3.27-21.1]. ACE inhibitor users with the ACE DD genotype had a non significant higher incidence of diabetes compared to ACE I allele carriers (adjusted interaction OR 2.03 [95%CI: 0.92-4.46]). Current users of ACE inhibitors carrying the T allele of the AGT gene polymorphism had a 2-fold higher incidence (adjusted interaction OR 2.03 [95%CI: 0.63-6.50]) of diabetes in comparison to MM carriers, though not statistically significant.

In sensitivity analyses, adjustment for the combined use of thiazides or beta-blockers did not change the results (interaction OR for AGTR1 C allele: 3.23 [95%CI:1.53-6.82], ACE DD genotype (adjusted interaction OR: 1.98 [95%CI:0.90-4.36]), and AGT T allele (adjusted interaction OR: 2.07 [95%CI:0.64-6.66]). Furthermore, separate classification of subjects as current users of ACE inhibitors, ARB, thiazides, and other antihypertensives also did not change the results (adjusted interaction for AGTR1 C allele: 2.73 [95%CI:1.20-6.18]), ACE DD genotype (adjusted interaction OR: 1.59 [95%CI:0.63-4.01]), and AGT T allele (adjusted interaction OR: 2.00 [95%CI:0.53-7.62]).

On an additive scale the relative excess risk due to interaction (RERI) was significantly increased for ACE inhibitor users with the AGTR1 C allele (RERI 0.85 [95%CI:0.31-1.37]) and subjects with the ACE DD genotype (RERI 0.63 [95%CI:0.16-1.43]). The RERI for ACE inhibitors users with the T allele of the AGT gene was 0.31 [95%CI:-0.39-1.03].

Discussion

In this study, the risk of diabetes associated with the use of ACE inhibitors was strongly modified by the AGTR1 A1166C and modestly by the ACE I/D polymorphism. Subjects with the AGTR1 AA genotype who used ACE inhibitors had a 53% decreased risk of diabetes compared to users of other antihypertensive drugs while among subjects carrying the AGTR1 C allele no such reduction was observed. Carriers of the insertion allele of the ACE gene who used ACE inhibitors had a reduction in their risk for diabetes of 37%. No reduction was seen in DD homozygous subjects.

To appreciate the findings in this study, some aspects need to be discussed. Confounding is always an issue when causal analyses are conducted using observational data. The interactions that we found are probably less vulnerable to confounding because physicians prescribe ACE inhibitors without knowledge of the genotype. Although we adjusted for many important potential confounding factors, we cannot exclude residual confounding due to unmeasured or inaccurately measured factors. Another limitation of the current study is the genotyping of the ACE gene polymorphism in the pharmacogenetic study which was not assessed directly. However, the G4656C SNP of the ACE gene is almost in complete linkage disequilibrium with the ACE I/D polymorphism and would not affect our results in a major way. Our analyses were limited to one polymorphism per gene and we could not take other possible variations in these genes into account. Furthermore, our sample size was not large enough to study gene-gene-drug interactions. Finally, there are other genes of the RAS which might play a role in modifying the response to ACE INHIBITOR such as the aldosterone synthase (CYP11B2) gene and angiotensin II type 2 receptor gene [15, 16]. In this study we considered receiving a first time oral anti-diabetic prescription as a reliable proxy for incident diabetes although the time of onset may be much earlier than the first prescription. Furthermore, we will have missed incident diabetes cases that were not severe enough to be treated. We do not expect this to be differential for the various antihypertensive drug classes, and therefore the influence on our results will probably be a small underestimation of the effect of ACE inhibitors on the incidence of diabetes mellitus and also of its interaction with the genetic polymorphisms we studied.

The strengths of this study are that we had complete information on most risk factors that are important when considering possible antihypertensive drug-gene interactions and we also had a reasonable sample size.

This is the first study into the influence of genetic polymorphisms on the risk of diabetes associated with ACE INHIBITOR therapy. The mechanism through which inhibition of ACE leads to a significant higher risk of diabetes among C allele carriers of the AGTR1 remains unclear. The AGTR1 A1166C polymorphism has previously been associated with both an increased and decreased blood pressure response to ACE inhibitors[17]. Although the DD polymorphism of the ACE gene is associated with higher circulating ACE plasma levels in Caucasians[18], no study has focused on the modifying effect of the RAS genes on the response to ACE inhibitors with regard to the incidence of diabetes. Almost 50% of the variance of serum ACE levels can be predicted by the ACE insertion/deletion

polymorphism[7] and ACE inhibitors may improve insulin-stimulated glucose uptake of the whole organism by increasing the glucose uptake by the skeletal musculature[19]. Perhaps that the DD genotype carriers compared to I allele carriers need higher doses of ACE inhibitors to achieve a similar effect [20].

The TT genotype of the AGT gene polymorphism has been associated with elevated AGT levels, hypertension, increased heart disease risk, and improved blood pressure in response to ACE inhibitors[21]. The homozygous TT state was associated with approximate 20% increase in plasma AGT and elevated plasma AGT levels have been demonstrated in insulin-resistant states[22]. Consequently subjects with the AGT T allele might need higher doses of ACE inhibitors to achieve a similar effect on the incidence of diabetes compared to AGT MM homozygous subjects. However we were not able to show this in our study.

In conclusion, treatment with ACE inhibitors in hypertensive subjects significantly reduces the occurrence of diabetes in homozygous 1166A carriers of the AGTR1 gene and carriers of the ACE insertion allele, but not in 1166C allele carriers of the AGTR1 gene and in homozygous ACE deletion allele carriers.

Table 1 Baseline characteristics of cases and controls

		Cases	Controls	P-value
Number		205	2050	
Age (y, SD)		62.5 (±9.0)	62.1(±9.8)	0.48
Male		57.1 (117)	61.8 (1267)	0.19
Smoking				
	Never smoking	27.8 (57)	29.6 (607)	
	Past smoking	22.0 (45)	24.3 (499)	0.71
	Current smoking	22.0 (45)	20.4 (418)	
Obesity (BMI>30 kg/m^2)		33.7 (69)	21.9 (449)	0.01[§]
Physical activity				
	Low (≤ 4 hours/week)	42.9 (88)	50.9 (1043)	0.09
	Regularly (> 4 hours/week)	31.2 (64)	25.9 (531)	
Alcohol use				
	No use of alcohol	25.4 (52)	11.4 (233)	
	1-2 glasses/week	42.4 (87)	53.8 (1102)	0.01[§]
	> 2 glasses/week	8.8 (18)	12.3 (252)	
Current use of antihypertensive drugs				
	ACE inhibitors	23.4 (48)	24.1 (494)	0.07
	ARB	8.8 (18)	9.1 (187)	0.98
	Thiazides	25.9 (53)	19.7 (403)	0.01[§]
	α blockers	0.0 (0)	0.0 (1)	0.46
	β blockers	47.3 (97)	44.4 (910)	0.63
	K^+-sparing diuretics	2.0 (4)	1.3 (26)	0.22
	Ca-antagonists	23.9 (49)	23.1 (473)	0.61
	Combination preparations	12.7 (26)	9.6 (196)	0.16
	Other antihypertensives	0.6 (1)	0.3 (5)	0.46
AGTR1	CC	11.2 (23)	8.5 (175)	
	CA	40.5 (83)	44.0 (902)	0.49
	AA	47.8 (98)	46.6 (956)	
ACE	II	23.9 (49)	20.7 (425)	
	ID	46.3 (95)	50.6 (1037)	0.26
	DD	28.8 (59)	28.4 (582)	
AGT	TT	30.2 (62)	28.2 (578)	
	TM	55.1 (113)	50.8 (1042)	0.1
	MM	14.6 (30)	21.0 (430)	

RAS: renin-angiotensin system; AGTR1: angiotensin II type 1 receptor; ACE: angiotensin converting enzyme; AGT: angiotensinogen

[§] $p<0.05$

Table 2 Association between the use of ACE-inhibitors and the incidence of diabetes stratified by genetic polymorphisms in angiotensin II receptor type 1(AGTR1), angiotensin converting enzyme(ACE) and angiotensinogen (AGT)

Genotype		ACE inhibitor use	Cases	Controls	OR [95% CI]	OR [95%CI]*
AGTR1	CC/AC	Never	61	595	1.0 (reference)	1.0 (reference)
		Past	14	240	0.57 [0.31-1.04]	0.53 [0.28-1.00]
		Current	31	242	1.25 [0.79-1.97]	1.32 [0.81-2.14]
		< 1 DDD	6	75	0.78 [0.33-1.87]	0.65 [0.26-1.61]
		≥1 DDD	25	167	1.46 [0.89-2.40]	1.73 [1.02-2.95] §
	AA	Never	69	568	1.0 (reference)	1.0 (reference)
		Past	12	145	0.68 [0.36-1.29]	0.65 [0.33-1.23]
		Current	17	243	0.58 [0.33-1.00] §	0.47 [0.26-0.84] §
		< 1 DDD	9	36	2.06 [0.95-4.45]	1.80 [0.81-4.01]
		≥1 DDD	8	207	0.32 [0.15-0.67] §	0.24 [0.11-0.52] §
ACE	DD	Never	39	379	1.0 (reference)	1.0 (reference)
		Past	5	99	0.49 [0.19-1.28]	0.48 [0.17-1.34]
		Current	15	104	1.40 [0.74-2.64]	0.95 [0.47-1.96]
		< 1 DDD	7	29	2.35 [0.96-5.71]	1.55 [0.57-4.23]
		≥1 DDD	8	75	1.04 [0.47-2.31]	0.71 [0.29-1.74]
	II/ID	Never	90	788	1.0 (reference)	1.0 (reference)
		Past	21	287	0.64 [0.39-1.05]	0.63 [0.38-1.05]
		Current	33	387	0.75 [0.49-1.13]	0.63 [0.41-0.98] §
		< 1 DDD	8	88	0.80 [0.37-1.70]	0.77 [0.36-1.65]
		≥1 DDD	25	299	0.73 [0.46-1.16]	0.59 [0.36-0.97] §
AGT	TT/MT	Never	111	925	1.0 (reference)	1.0 (reference)
		Past	20	326	0.51 [0.31-0.84] §	0.57 [0.34-0.95] §
		Current	44	369	0.99 [0.69-1.44]	0.81 [0.55-1.19]
		< 1 DDD	14	85	1.37 [0.75-2.50]	1.28 [0.70-2.37]
		≥1 DDD	30	284	0.88 [0.58-1.35]	0.67 [0.43-1.06]
	MM	Never	20	245	1.0 (reference)	1.0 (reference)
		Past	6	60	1.23 [0.47-3.18]	1.17 [0.44-3.11]
		Current	4	125	0.39 [0.13-1.17]	0.41 [0.13-1.23]
		< 1 DDD	1	32	0.38 [0.05-2.95]	0.41 [0.05-3.22]
		≥1 DDD	3	93	0.40 [0.12-1.36]	0.40 [0.12-1.41]

* Adjusted for index date, obesity, use of alcohol

§ $p<0.05$

Table 3 Interactions between polymorphisms in the RAS and ACE-inhibitors on a multiplicative scale.

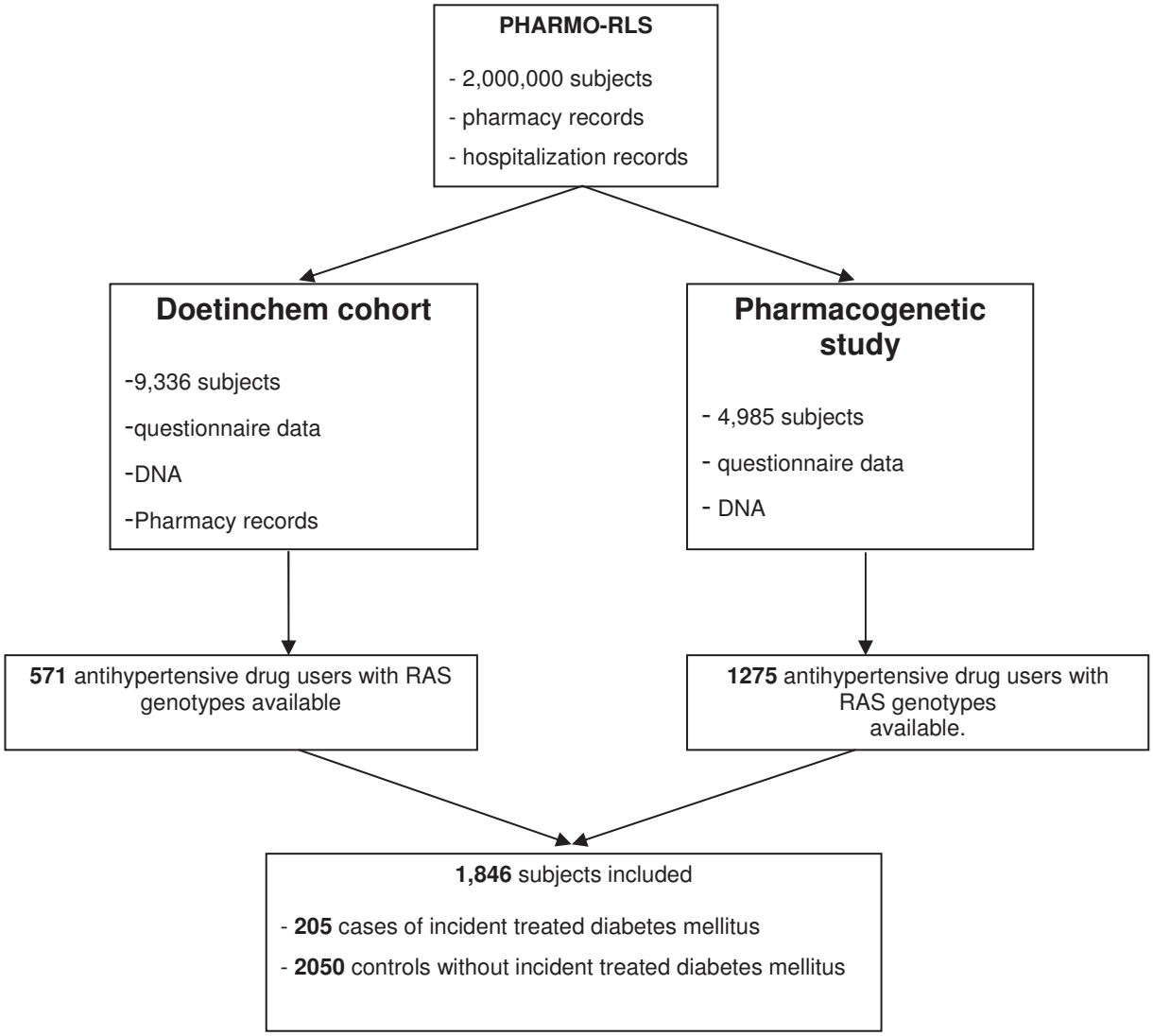
	IOR (95%CI)	IOR (95% CI)*
AGTR1 CC/AC vs AA		
Past ACE inhibitor use	0.84[0.35-2.01]	0.78 [0.32-1.92]
Current ACE inhibitor use	2.17 [1.06-4.44] §	3.21 [1.53-6.75] §
< 1 DDD	0.38 [0.12-1.22]	0.32 [0.10-1.06]
≥ 1 DDD	4.59 [1.87-11.3] §	8.30 [3.27-21.1] §
ACE DD vs II/ID		
Past ACE inhibitor use	0.77 [0.26-2.25]	0.83 [0.28-2.48]
Current ACE inhibitor use	1.88 [0.88-4.01]	2.03 [0.92-4.46]
< 1 DDD	2.95 [0.92-9.46]	2.66 [0.81-8.80]
≥ 1 DDD	1.42 [0.56-3.57]	1.63 [0.63-4.23]
AGT TT/MT vs MM		
Past ACE inhibitor use	0.42 [0.14-1.22]	0.50 [0.17-1.50]
Current ACE inhibitor use	2.54 [0.80-8.05]	2.03 [0.63-6.50]
< 1 DDD	3.59 [0.43-30.1]	3.52 [0.41-30.0]
≥ DDD	2.23 [0.60-8.24]	1.65 [0.44-6.18]

IOR: interaction OR

* adjusted for index date, obesity, use of alcohol

§ $p < 0.05$

Fig 1 Flowdiagram of study population.



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CHAPTER 3.2

Renin-angiotensin-system polymorphisms and the association between use of angiotensin II receptor blockers or ACE inhibitors and the risk of diabetes

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Abstract

Aim

To assess the influence of genetic polymorphisms in the renin-angiotensin-system (RAS) on the risk of diabetes associated with the use of angiotensin-receptor blockers (ARB) and ACE inhibitors.

Methods

Matched case-control study among antihypertensive drug users. Pharmacy records and questionnaires were used to ascertain incident diabetes (cases), antihypertensive drug use and risk factors. Controls did not (yet) have diabetes. We genotyped ACE (G4656C which is in complete LD with the ACE insertion/deletion polymorphism), angiotensinogen (M235T), and Angiotensin II type 1 receptor (A1166C).

Results

Among 495 cases of incident diabetes and 2624 controls, homozygous 1166C carriers of AGTR1 who used ARBs had an increased risk of diabetes compared to AGTR1 1166A carriers (interaction OR 5.3 [95%CI: 1.8-16.1]). Homozygous ACE GG subjects who used ACE inhibitors ≥1 DDD/day had a higher risk of diabetes compared to subjects with the ACE C allele (interaction OR 2.3 [95%CI:1.2-4.5].

Conclusion

ARBs increase the occurrence of diabetes in homozygous 1166C carriers of AGTR1, but not in 1166A carriers. ACE inhibitors at doses ≥ 1 DDD/day increase the risk of diabetes among homozygous ACE GG carriers, but not in 4656 C carriers.

Introduction

Diabetes is a major risk factor for cardiovascular morbidity and mortality and the coexistence of diabetes mellitus in hypertensive patients doubles the risk of cardiovascular events, cardiovascular mortality and total mortality[1, 2]. A recent meta-analysis assessed that the relative risk reductions of type 2 diabetes mellitus by angiotensin converting enzyme inhibitor (ACE inhibitors), angiotensin receptor blocker (ARB)compared to diuretic therapy were 33%, and 38%, respectively[3]. Although a pooled analyses showed that compared to placebo these risks were not significantly reduced, individual trials have demonstrated small decreased risks of diabetes associated with ARB and ACE inhibitors[4]. Even if there would be no overall beneficial effect of ACE inhibitors and ARBs on the risk of diabetes, subgroups of patients defined by for instance genetic variation might experience more or less benefit from these drugs with regard to the risk of diabetes.

The mechanisms through which ACE inhibitors and ARBs reduce the risk of diabetes mellitus remain uncertain, although several mechanisms, such as decreased renal potassium wasting, and improved islet blood flow and pancreatic beta-cell perfusion by reducing angiotensin II-mediated vasoconstriction in the pancreas, have been proposed[5]. Polymorphisms in genes that code for components of the RAS may influence the response to these agents and risk of diabetes. Spiering et al.[6] found that the CC genotype of the angiotensin II receptor type 1 (AGTR1) A1166C gene was associated with increased sensitivity to angiotensin II. In another study by the same authors[7], the renal hemodynamic effects of ARB blockade in patients with the CC genotype were smaller compared to patients with the AA genotype.

These findings support the idea that the response to ARBs could be genetically determined. However, not all studies confirm the AGTR1 A1166C polymorphism as an important modulator of the response to angiotensin II[8]. The D allele of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism was found to be associated with higher levels of circulating ACE[9,10], which has been suggested to impair glucose tolerance and elevate the risk of developing diabetes[11].

Previously we found in a small study among treated hypertensives that the AGTR1 C allele and the ACE insertion/deletion polymorphism modified the risk of diabetes associated with ACE inhibitors[12]. Whether the risk of diabetes associated with ARB use is influenced by genetic polymorphisms in RAS-genes is unknown.

The aim of this study was 1) to assess whether the association between the use of ARB and the incidence of new onset diabetes is modified by genetic polymorphisms in RAS genes such as angiotensinogen (AGT), angiotensin converting enzyme (ACE) and angiotensin II receptor type 1(AGTR1) and 2) to confirm our initial findings with regard to ACE inhibitors.

Methods

Design and setting: We performed a nested case-control study among treated hypertensive patients. The Pharmaco-Morbidity Record Linkage System (PHARMO-RLS) was used to identify patients treated with antihypertensive drugs. PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registrations of hospital discharges (LMR) from 1985 onwards. Currently, the base population of PHARMO covers about 2,000,000 community-dwelling inhabitants of about 50 population-defined areas in The Netherlands. Data from a pharmacogenetic study of antihypertensive drug treatment including 5,140 antihypertensive drug users was used to collect data on genotypes and potential risk factors for diabetes mellitus. In this pharmacogenetic study, specific genetic polymorphisms are examined for the modification of the effect of antihypertensive drugs on the risk of myocardial infarction in a selection of PHARMO-RLS participants[13]. Approval for this study was obtained from the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands.

Data collection: The PHARMO-RLS provides records containing the name of the dispensed drugs, day of dispensing, number of units dispensed, prescribed daily dose and the Anatomical Therapeutical Chemical (ATC) code of the drug. Questionnaire information was used to collect data on demographic variables, cardiovascular diseases and risk factors, whereas buccal swabs were used to collect DNA.

Outcome and exposure definition: The outcome of interest was incident diabetes. Pharmacy records and self-reported questionnaire information were used to assess incident diabetes (cases). Incident cases of diabetes were defined as those having a first prescription for oral anti-diabetic drugs or insulin at least one year after the date of entry in PHARMO-RLS. Cases were assigned an index date, which was defined as either the date the first anti-diabetic drug prescription was dispensed or the date of self-reported diagnosis of diabetes mellitus, whichever came first. For each case we selected up to 5 controls who also received antihypertensive drugs but who had not (yet) a diagnosis of diabetes mellitus at the index date of the case. Subjects who already had a diagnosis of diabetes mellitus (either based on self-

report or antidiabetic medication use) before they started antihypertensive drug use were excluded.

In a small validation study among 83 subjects for whom general practitioner records were available (24 cases and 59 controls) we found that 91.7% (22) of the cases could be confirmed according to WHO criteria for a diagnosis of type 2 diabetes mellitus (either a fasting plasma glucose>7.0 mmol/l and/or random (non-fasting) blood glucose>11.1 mmol/l, and/or use of oral antidiabetic medication and/or use of insulin and/or treatment by diet and registered by a general practitioner as having type 2 diabetes mellitus)[14], whereas 100.0% (59) of the controls had no diagnosis of type 2 diabetes mellitus.

Exposure to antihypertensive drugs was ascertained from pharmacy records. All subjects were current users of at least one antihypertensive drug. Thiazide diuretic use was defined as current (at the index-date) or not current (before the index-date). ARB use was defined as current or not current (excluding current thiazide diuretic users and current ACE inhibitor users). ACE inhibitor use was defined as current or not current (excluding current thiazide diuretic and current ARB users). Subjects who were current users of both ACE inhibitor and ARB were excluded. Subjects who did not currently use a thiazide, ARB, or ACE inhibitor, but were currently using another antihypertensive drug such as beta-blockers and calcium-antagonists were used as the reference category. We also calculated the dose of current use of ARB and ACE inhibitors according to the defined daily dose (DDD), which is the average daily dose of a drug for its main indication in adults, and is recommended by the World Health Organization for drug utilization studies[15].

Genotyping: Genomic DNA was isolated from buccal swabs according to standard procedures. Genotyping was determined using a multiplex Single Base Extension (SBE) method. Multiplex SBE was performed using SNaPshotTM as described by the manufacturer (Applied Biosystems). This method was described earlier[16], but adapted to a new set of polymorphisms for the PHARMO-genetics study. The multiplex assay was validated on a set of 100 DNA samples that had previously been genotyped with an alternative technique; this technique concerned a multilocus genotyping assay for candidate markers of cardiovascular disease risk (Roche Molecular Systems Inc) and has been described in detail previously[17]. The ACE (I/D) polymorphism was determined by detecting ACE4656+CT observed on the SNaPshot as ACE (G4656C). The ACE4656+CT polymorphism in the 3'-untranslated region of the ACE gene consists of a repetition of 2 or 3 CT dinucleotides and is in complete linkage

disequilibrium with ACE (I/D)[16, 18]. The G detected on the SNaPshot resembles the deletion allele and the C resembles the insertion allele of the ACE gene.

Analysis: Analysis was performed using SPSS (version 12.1, SPSS Inc., Chicago). Continuous variables are presented as mean ± standard deviation. Deviations from Hardy-Weinberg equilibrium were considered using chi-square tests. Unconditional logistic regression analysis was used to study the association between ARB use and the incidence of new onset of diabetes. All analyses were stratified by ACE (G4656C), AGT (M235T), and AGTR1 (A1166C) genotype to study effect modification. We considered age, gender, smoking, physical activity, body mass index, alcohol use, index date, self reported high blood pressure and hypercholesterolemia as potential confounders. Based on our previous findings we assumed a recessive model for the interaction with the AGTR1 and ACE gene and a dominant model for the AGT gene[12]. The interaction OR was calculated by adding an interaction term (ARB (or ACE inhibitor) x GENOTYPE) to the logistic regression model. The interaction OR can be interpreted as the ratio of the OR in subjects with a susceptible genotype and the OR in subjects without this genotype. An interaction OR of 1 indicates no interaction on the multiplicative scale[19].

Results

From a total of 5140 antihypertensive drug users, 493 incident cases of type 2 diabetes mellitus and 2618 controls who were current users of antihypertensive drugs were identified. **Table 1** shows the charactistics of case and control subjects. Risk factors for type 2 diabetes mellitus such as obesity, sedentary lifestyle, and hypercholesterolemia were significantly more common among cases than controls. Of the antihypertensive drugs studied, only the frequency of thiazide diuretic differed significantly between cases and controls. About 49% of the cases started antidiabetic medication at their date of diagnosis, whereas 51% of the cases did not start antidiabetic medication immediately. However, due to the method of our selection of cases, all incident cases used antidiabetic medication at some point after their diagnose date.

The incidence of diabetes was increased among thiazide diuretic users compared to users of other antihypertensive drugs (adjusted OR 1.68 [95%CI: 1.31-2.16]). No significant associations were found for current use of ARB and ACE inhibitors (**Table 2**).

Table 3 shows the association between use of ARBs, ACE inhibitors and the incidence of diabetes stratified by the genetic polymorphisms in the RAS. Subjects carrying the CC

genotype of the AGTR1 gene had a significantly higher incidence of diabetes when they used ARBs (adjusted OR 7.71 [95%CI: 2.28-26.1]). The risk of diabetes increased from 2.72 for current users of ≤1 DDD/day ARB to 24.8 for current users of >1 DDD/day ARB. CC homozygous subjects had a 5 times higher risk of developing diabetes while using ARBs compared to A allele carriers who users ARBs (adjusted SI 5.34 [95%CI: 1.77-16.1]). This interaction was most pronounced for subjects who used >1 DDD/day ARB (adjusted SI 15.5 [95%CI: 3.23-74.2]).

The association between ACE inhibitor use and incidence of diabetes was not statistically significantly modified by the AGTR1 polymorphism.

Tables 4 and 5 show that the association between ARB use and the incidence of diabetes was not statistically significantly modified by the ACE G4656C or the AGT M235T polymorphism. The association between current use of ACE inhibitors and the incidence of diabetes was statistically significantly modified by the ACE G4656C polymorphism for subjects who use ≥ 1 DDD/day (SI 2.29 [95%CI: 1.17-4.47]).

Discussion

Our study suggests that the risk of diabetes associated with the use of ARBs is strongly modified by the AGTR1 CC genotype. Subjects with the AGTR1 CC genotype who used ARB had a 5 times higher risk of developing diabetes compared to carriers of the A allele. This interaction was most pronounced for subjects who used > 1 DDD/day. Subjects with the ACE GG genotype who used ≥ 1 DDD/day ACE inhibitors had a 2-times increased risk of diabetes compared to subjects with the ACE C allele, which confirms our initial finding of an interaction between the ACE insertion/deletion polymorphism and the use of ACE inhibitors on the risk of diabetes[12]. We could not confirm our initial finding of an interaction between the AGTR1 C allele and the use of ACE inhibitors.

The results of our study should be interpreted with respect to the limitations. First, our case definition was based on self-reported diagnosis of diabetes mellitus or the use of glucose lowering medication which might have misclassified case and control subjects. However, all incident cases of diabetes were required to use antidiabetic medication at some point after their initial diagnosis of diabetes mellitus. This methodology would minimize misclassification of cases and controls. This was confirmed in our small validation that showed that our case definition was highly accurate compared to diagnostic information from general practitioner records. Second, when causal analyses are conducted in observational

studies, confounding will always be a issue. Since physicians prescribe ARBs without knowledge of the patients genotype we do not expect confounding to play an important role for the interactions we found. Despite adjustments for important potential confounders, residual confounding cannot be fully excluded due to unmeasured or inaccurately measured factors. However, established risk factors for diabetes were confirmed in our study suggesting the validity of our data collection.

Other limitations of our study are the genotyping of the polymorphisms in the ACE gene, which was not assessed directly, and the restriction of our analysis to only one polymorphism per gene. Unfortunately we were not able to take other possible variations in these genes into account nor did we look at other genes of the RAS which might play a role in modifying the response to ARBs, such as the aldosterone synthase (CYP11B2), renin and angiotensin II type 2 receptor gene [20,21]. Our findings should be considered as hypothesis generating and need replication in other studies.

The strengths of this study include the relatively large sample size and the complete and accurate information on drug exposure and most risk factors that are important when considering possible antihypertensive drug-gene interactions. Furthermore, our confirmation of the results from a meta-analysis indicating that overall ARB and ACE inhibitors are not associated with a decreased risk of diabetes and that thiazides significantly increase the risk of diabetes suggests that our observational data are valid[4].

In a recent review several mechanisms through which inhibition of the RAAS by ACE inhibitor or ARB therapy may decrease the risk of diabetes have been suggested[22]. Hemodynamic effects of RAS inhibition may improve metabolic control by reducing angiotensin II mediated vascular resistance and thereby increasing the perfusion of skeletal muscle and/or pancreatic islet beta-cells. Activation of the RAAS in the pancreas may impair first phase insulin release by beta-cells, possibly via changes in intra-islet blood flow. Inhibition of pancreatic RAS may therefore be an independent mechanism for slowing the progression of islet cell damage. At the cellular level, angiotensin II increases expression of glucose transporter, GLUT-4 and the activity of hexokinase, which is a key enzyme in glucose metabolism on skeletal muscle. Some of the effects of ACE inhibition may also be mediated by increased levels of bradykinin which can enhance insulin signalling and translocation of GLUT-4.

The mechanisms through which thiazide diuretics impair glucose tolerance have not been fully elucidated yet. Thiazides block the renal sodium chloride channel (NCC) and thereby increase sodium and chloride excretion. In the distal convoluted tubule, thiazides deliver a high sodium load with a resulting increase in potassium excretion. In addition, thiazides activate the RAAS through volume depletion which leads to increased aldosterone secretion, further enhancing potassium excretion. One of the hypotheses is that this diuretic-induced hypokalemia leads to a higher secretion ratio of proinsulin to insulin which is biologically less active compared to insulin[23], which impairs glucose homeostasis[24]. Potassium supplementation may help in preventing thiazide-induced glucose intolerance and insulin hyposecretion[25].

This is the first study into the influence of genetic polymorphisms on the risk of diabetes associated with ARB therapy. The mechanism through which inhibition of angiotensin II leads to a significant higher risk of diabetes among C allele carriers of the AGTR1 remains to be established. The AGTR1 CC genotype is associated with a greater sensitivity for angiotensin-II through a greater number of receptor binding sites and affinity for angiotensin-II[26]. One study suggested that the A1166C polymorphism of the AGTR1 gene could be in linkage disequilibrium with a mutation that dynamically increases the responsiveness to angiotensin II, on top of changes that are induced by exogenous stimuli. In the case of patients carrying the CC genotype, who already have an increased responsiveness to angiotensin II, this would lead to a further increase in their responsiveness and a blunted response to ARBs[27]. The deletion allele of the ACE gene has been associated with greater activity of ACE and explains up to 50% of variation in ACE levels[10]. Furthermore, the deletion allele of the ACE gene has been associated with an increased of type 2 diabetes mellitus[28]. Perhaps that the beneficial effects of ACE inhibitors on the risk of diabetes are diminished in subjects with the ACE 4656 GG genotype (similar to ACE DD).

In conclusion, treatment with ARBs in hypertensive subjects significantly increases the occurrence of diabetes in homozygous 1166C carriers of the AGTR1 gene. Treatment with ACE inhibitors at doses ≥1 DDD/day increases the risk of diabetes in homozygous 4656 G allele carriers of the ACE gene.

Table 1 Baseline characteristics of cases and controls.

	Cases	Controls	p-value
Number	495	2624	
Age	65.8	65.7	0.902
Sex (Female)	169 (34.1%)	816 (31.1%)	0.185
BMI>30 kg/m^2	102 (35.1%)	310 (18.0%)	<0.001[§]
High blood pressure	373 (88.8%)	1991 (88.3%)	0.775
High cholesterol			0.002[§]
Normal	113 (29.7%)	813 (38.1%)	
High, diet	71 (18.6%)	298 (14.0%)	
High, drugs	197 (51.7%)	1021 (48.0%)	
Smoking			0.031[§]
Never	108 (36.1%)	624 (35.2%)	
Current	61 (20.4%)	266 (15.0%)	
Past	130 (43.5%)	884 (49.8%)	
Sedentary (<4 hours of exercise/week)	100 (31.0%)	409 (21.8%)	<0.001[§]
Use of alcohol			0.102
Never use	66 (20.8%)	330 (17.8%)	
Past use or <6g/day	115 (36.2%)	570 (30.7%)	
6-12g/day	42 (13.2%)	284 (15.3%)	
12-24g/day	65 (20.4%)	409 (22.0%)	
≥24g/day	30 (9.5%)	262 (14.2%)	
Current use antihypertensive drugs			
Thiazide diuretics	198 (40.0%)	793 (30.2%)	<0.001[§]
Betablockers	286 (57.8%)	1425 (54.3%)	0.173
ACE inhibitors	166 (33.5%)	879 (33.5%)	0.972
AT2 blockers	87 (17.6%)	462 (17.6%)	0.924
Calcium antagonists	122 (24.6%)	664 (25.3%)	0.786
Miscellaneous	18 (3.7%)	81 (3.1%)	0.518

[§] $p < 0.05$

Table 2 Association between current use of angiotensin II receptor blockers, ACE inhibitors, thiazide diuretics and the risk of type 2 diabetes mellitus.

	Cases	Controls	OR*	OR**
Other antihypertensives	153	948	1.0 (reference)	1.0 (reference)
Thiazide diuretics	198	793	1.64 [1.30-2.08] §	1.68 [1.31-2.16] §
ARB	44	282	1.08 [0.75-1.55]	1.12 [0.77-1.63]
≤ 1 DDD	29	191	1.04 [0.68-1.60]	1.08 [0.70-1.67]
> 1 DDD	15	29	1.15 [0.64-2.04]	1.21 [0.67-2.19]
ACE inhibitors	100	601	1.07 [0.81-1.40]	1.06 [0.80-1.40]
< 1 DDD	22	156	0.88 [0.54-1.42]	0.85 [0.52-1.38]
≥ 1 DDD	78	445	1.14 [0.85-1.53]	1.14 [0.84-1.55]

Other antihypertensives include calcium-antagonists, betablockers, and miscellaneous antihypertensive drugs.

* Adjusted for calendar year

** Adjusted for calendar year, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity and smoking

§ $p < 0.05$

Table 3 Influence of AGTR1 A1166C polymorphisms on the risk of type 2 diabetes mellitus associated with the use of antihypertensive drugs

	Cases	Controls	OR*	OR**	SI*	SI**
AGTR1 AA/AC						
Other antihypertensives	136	860	1.0 (reference)	1.0 (reference)		
Thiazide diuretics	184	711	1.74 [1.36-2.22]	1.76 [1.36-2.29]		
ARB	34	269	0.88 [0.59-1.32]	0.90 [0.60-1.36]		
≤ 1 DDD	26	183	0.99 [0.63-1.56]	1.01 [0.63-1.60]		
> 1 DDD	8	86	0.65 [0.31-1.38]	0.67 [0.31-1.43]		
ACE inhibitors	83	543	1.00 [0.75-1.35]	0.99 [0.73-1.34]		
< 1 DDD	20	145	0.89 [0.54-1.46]	0.83 [0.50-1.39]		
≥ 1 DDD	63	398	1.05 [0.76-1.45]	1.05 [0.75-1.46]		
AGTR1 CC						
Other antihypertensives	16	85	1.0 (reference)	1.0 (reference)		
Thiazide diuretics	11	75	0.80 [0.35-1.86]	0.97 [0.38-2.48]	0.46 [0.19-1.09]	0.46 [0.19-1.10]
ARB	10	12	5.39 [1.94-15.0]	7.71 [2.28-26.1]	5.60 [1.91-16.5]†	5.34 [1.77-16.1]†
≤ 1 DDD	3	8	2.30 [0.54-9.78]	2.72 [0.54-13.8]	2.15 [0.48-9.67]	1.97 [0.42-9.19]
> 1 DDD	4	7	12.6 [3.17-49.9] †	24.8 [3.90-158.1] †	16.7 [3.58-77.9]†	15.5 [3.23-74.2] †
ACE inhibitors	17	57	1.64 [0.75-3.55]	1.43 [0.60-3.45]	1.60 [0.71-3.65]	1.48 [0.64-3.42]
< 1 DDD	2	11	0.80 [0.15-4.18]	0.90 [0.15-5.25]	0.98 [0.18-5.33]	1.15 [0.21-6.37]
≥ 1 DDD	15	46	1.88 [0.84-4.22]	1.51 [0.59-3.84]	1.72 [0.73-4.07]	1.52 [0.63-3.64]

SI: Synergy index defined as the ratio of the OR in subjects with the susceptible genotype compared to the non-susceptible genotype

* Adjusted for calendar year

** Adjusted for calendar year, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity, and smoking.

† $p<0.05$

Table 4 Influence of AGT M235T polymorphism on the risk of type 2 diabetes mellitus associated with the use of antihypertensive drugs

	Cases	Controls	OR*	OR**	SI*	SI**
AGT MM						
Other antihypertensives	46	322	1.0 (reference)	1.0 (reference)		
Thiazide diuretics	66	296	1.64 [1.09-2.49]	1.66 [1.06-2.60]		
ARB	12	110	0.81 [0.41-1.59]	0.80 [0.40-1.61]		
≤ 1 DDD	7	73	0.71 [0.31-1.64]	0.71 [0.30-1.69]		
> 1 DDD	5	37	1.03 [0.38-2.76]	0.98 [0.35-2.74]		
ACE inhibitors	36	230	1.13 [0.70-1.80]	1.02 [0.62-1.67]		
< 1 DDD	6	63	0.67 [0.27-1.63]	0.60 [0.24-1.50]		
≥ 1 DDD	30	167	1.31 [0.79-2.15]	1.20 [0.71-2.04]		
AGT MT/TT						
Other antihypertensives	107	626	1.0 (reference)	1.0 (reference)		
Thiazide diuretics	130	491	1.62 [1.22-2.16]	1.69 [1.24-2.29]	0.94 [0.57-1.54]	0.92 [0.56-1.53]
ARB	32	172	1.24 [0.80-1.91]	1.31 [0.84-2.05]	1.42 [0.64-3.16]	1.37 [0.61-3.08]
≤ 1 DDD	22	118	1.24 [0.75-2.05]	1.30 [0.78-2.18]	1.65 [0.62-4.37]	1.60 [0.60-4.30]
> 1 DDD	10	54	1.22 [0.60-2.48]	1.31 [0.64-2.72]	1.10 [0.33-3.70]	1.05 [0.31-3.60]
ACE inhibitors	64	371	1.04 [0.74-1.46]	1.08 [0.76-1.53]	0.90 [0.51-1.61]	0.90 [0.50-1.63]
< 1 DDD	16	93	1.02 [0.58-1.81]	1.00 [0.56-1.79]	1.53 [0.53-4.43]	1.45 [0.49-4.25]
≥ 1 DDD	48	278	1.05 [0.73-1.52]	1.11 [0.76-1.63]	0.78 [0.42-1.45]	0.79 [0.42-1.49]

SI: Synergy index defined as the ratio of the OR in subjects with the susceptible genotype compared to the non-susceptible genotype

* Adjusted for calendar year

** Adjusted for calendar year, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity, and smoking.

† $p<0.05$

Table 5 Influence of ACE G4656C polymorphism on the risk of type 2 diabetes mellitus associated with the use of antihypertensive drugs.

	Cases	Controls	OR*	OR**	SI*	SI**
ACE CC/GC						
Other antihypertensives	121	671	1.0 (reference)	1.0 (reference)		
Thiazide diuretics	137	584	1.36 [1.04-1.78]	1.35 [1.01-1.80]		
ARB	35	196	1.07 [0.71-1.61]	1.08 [0.71-1.66]		
≤ 1 DDD	23	126	1.09 [0.67-1.77]	1.09 [0.66-1.80]		
> 1 DDD	12	70	1.03 [0.54-1.97]	1.06 [0.55-2.06]		
ACE inhibitors	68	426	0.92 [0.66-1.26]	0.90 [0.65-1.26]		
< 1 DDD	17	108	0.89 [0.52-1.54]	0.90 [0.51-1.57]		
≥ 1 DDD	51	318	0.92 [0.65-1.32]	0.90 [0.63-1.31]		
ACE GG						
Other antihypertensives	32	277	1.0 (reference)	1.0 (reference)	-	-
Thiazide diuretics	61	209	2.81 [1.75-4.51]	3.17 [1.90-5.29]	1.92 [1.12-3.29] †	2.21 [1.28-3.82] †
ARB	9	86	1.09 [0.50-2.40]	1.28 [0.57-2.89]	0.91 [0.38-2.19]	1.05 [0.43-2.57]
≤ 1 DDD	6	65	0.97 [0.38-2.43]	1.12 [0.43-2.89]	0.79 [0.28-2.22]	0.90 [0.32-2.57]
> 1 DDD	3	21	1.50 [0.42-5.38]	1.97 [0.53-7.32]	1.28 [0.31-5.29]	1.57 [0.37-6.58]
ACE inhibitors	32	175	1.60 [0.94-2.73]	1.55 [0.89-2.69]	1.72 [0.93-3.19]	1.84 [0.98-3.44]
< 1 DDD	5	48	0.83 [0.30-2.26]	0.70 [0.25-1.94]	0.96 [0.31-2.99]	0.87 [0.27-2.75]
≥ 1 DDD	27	127	1.93 [1.10-3.39] †	1.99 [1.10-3.59] †	2.01 [1.04-3.88] †	2.29 [1.17-4.47] †

* Adjusted for calendar year

** Adjusted for calendar year, sex, obesity, use of alcohol, high cholesterol, high blood pressure, physical activity, and smoking.

† $p<0.05$

SI: Synergy index defined as the ratio of the OR in subjects with the susceptible genotype compared to the non-susceptible genotype

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CHAPTER FOUR

Pharmacogenetic studies on glucose-lowering drugs

Pharmacogenetics of glucose lowering drug treatment: a systematic review

Molecular Diagnosis and Therapy 2007: 11(5): 291-302

Abstract

Aim

Intensive blood glucose lowering can significantly reduce the risk of micro- and macrovascular complications in diabetes. However, 30% of all treated patient do not achieve optimal blood glucose levels. Genetic factors may influence the response to glucose lowering medication. This review aimed to summarize available evidence of genetic influence on response to glucose lowering medication.

Methods

Using combinations of the words “gene”, “genotype”, “polymorphism”, “genetics”, “diabetes” , “pharmacogenetics”, “SNP”, “variant” in combination with the names of specific oral anti-diabetic drugs, we searched MEDLINE from 1966 to july 2007. Studies reporting data on response to glucose lowering drugs and genetic polymorphisms were included.

Results

37 studies were identified on the use of oral glucose lowering drugs and genetic polymorphisms. The inwardly rectifying potassium channel (KIR channel) 6.2 and the insulin receptor substrate (IRS-1) polymorphisms were associated with an increased risk of (secondary) failure to SU while the *3 polymorphism in the cytochrome-P450 isoform 2C9 (CYP2C9) was associated with decreased clearance. On the other hand one study reported an increased insulin secretion in carriers of the CYP2C9*3 polymorphism when they using glyburide.

CYP2C9*3 and CYP2C8*3 polymorphisms were found to influence the clearance of meglitinides. Non-carriers of the Gly972Arg polymorphism of the IRS-1 gene showed a decrease in fasting insulin level and insulin response (measured by HOMA) when they used metformin while secondary failure to SU was seen in carriers of this polymorphism. A significant decrease in fasting plasma glucose and HbA1c was seen in subjects carrying the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ gene and receiving rosiglitazone. Conversely carriers of this polymorphism also had a higher

conversion to diabetes mellitus when treated with acarbose and this effect was also seen in adiponectin gene polymorphism carriers.

Conclusion

The Ileu359Leu variant of the CYP2C9 gene showed strong relation with reduced clearance of SU's while polymorphisms in receptor genes (IRS-1, KIR6.2, PPAR-γ) were related to reduced response or failure to treatment.

Introduction

Diabetes is a growing global health burden. Up to 80% of type 2 diabetics die from macrovascular cardiovascular disease[1]. Several trials have demonstrated that achieving near normal glycemic control in type 1 and type 2 diabetes mellitus patients reduces the risk of microvascular complications[1, 2]. Intensive control of blood glucose can significantly reduce and retard the microvascular complications of retinopathy, nephropathy and neuropathy[3]. In practice many patients do not achieve optimal blood glucose levels. According to previous research 30% of patients in general practice do not achieve the targets for good glycaemic control[4]. Inter-individual variation in response to glucose lowering drugs is common and may explain that no single agent leads to optimal blood glucose in all treated patients[5]. Several factors such as obesity, physical activity, diet and genetic risk factors[6] are thought to play a role in the inter-individual variation in response to diabetic medication. A pharmacogenetic approach may help to understand the role of genetics in variable drug response[7].

Pharmacogenetics aims to study the role of genetic variation in inter-individual variation in drug response [8]. The information gathered from pharmacogenetic research may be used to optimize treatment regimens that reduce the risk of adverse drug reactions and improve efficacy in susceptible persons. Genetic variability can influence the response to medication through several pathways: variation in genes involved in pharmacokinetics, pharmacodynamics and in genes that are in the causal pathway of the disease[8, 9].

The aim of this review is to summarize all known genetic variants that have been studied in relation to the response to glucose lowering drug therapy.

Methods

A literature search was conducted in MEDLINE (1966 to july 2007) to identify studies containing information on pharmacogenetics of diabetes. Keywords of the search were “gene”, “genotype”, “polymorphism”, “genetics”, “diabetes”, “pharmacogenetics”, “SNP”, “variant” in combination with the names of specific oral anti-diabetic drugs. The references of all identified articles were checked. All studies reporting data on pharmacokinetic and pharmacodynamic response to anti-diabetic drugs and genetic

polymorphisms were included. Response was not further defined. Case-reports, in vitro studies and animal studies were excluded.

Results

The literature search identified 42 articles of which five were case-reports. These case-reports were excluded[6, 10-13]. The details and main findings of all included studies are summarized in Table I. Table II shows the frequency of the most common polymorphisms studied.

1. Candidate genes affecting pharmacokinetics

1.1. CYP2C9 polymorphisms and response to sulfonylureas

Pharmacokinetics of oral hypoglycemics can be altered through polymorphisms involved in drug metabolisation (CYP2 genes), catalysation (CYP3A5) and transport (OAT and OCT's). CYP2C9, CYP2C8, and CYP2D6 are major cytochrome P450 enzymes that are involved in the metabolic clearance of a wide variety of therapeutic agents[14]. CYP3A5 is a principal catalyst of the biotransformation of repaglinide[15] Important drug transporters include the organic anion transporters (OAT, e.g. SLCO1B1), and organic cation transporters (OCTs) which are involved in the uptake of many hydrophilic organic cations[16].

Most of the studies with the CYP genes have a very small sample size and also a substantial part of them have been performed in healthy subjects.

Healthy carriers of the Ile359Leu polymorphism of the CYP2C9 gene, also referred to as CYP2C9*3, showed decreased clearance of tolbutamide[17-20], glyburide[21-23], glimepiride[21, 24] and chlorpropamide[25]. These findings did not differ substantially between Caucasians[17-19, 21, 22], Koreans[20, 25] nor Chinese[23, 24]. Blood glucose response was not influenced by the CYP2C9 polymorphisms among Caucasians[21, 22] although insulin secretion was increased within 12 hours of ingestion[22]. Among Chinese, 2h blood glucose response and 2h insulin response was more reduced in *3 carriers[23].

The CYP2C9*3/*3 and the *2/*3 genotypes were more common in diabetic patients admitted to the emergency department with severe hypoglycaemia during sulfonylurea

drug treatment compared with a control group of patients with type 2 diabetes but without a history of severe hypoglycaemia[26].

Diabetic patients carrying the CYP2C9*3 polymorphism require lower doses of tolbutamide to regulate serum glucose than carriers with the wild type genotype treated with tolbutamide[27].

No differences related to CYP2C19 polymorphism, tolbutamide[20], glyburide[23], chlorpropamide[25] use were found.

1.2. CYP2C9, CYP2C8, CYP3A5, SLCO1B1 and CYP2D6 polymorphisms and response to meglitinides.

The pharmacokinetics of meglitinides was altered in healthy carriers of the CYP2C9*3[28], CYP2C8*3[29, 30] and SLCO1B1 521T>C[31]. The CYP2D6*4/*5[28] and CYP3A5*1[30] polymorphisms did not change the response of meglitinides. While no statistically significant changes were seen in blood glucose response to meglitinides, the SLCO1B1 -11187G/A[30] single nucleotide polymorphism was associated with an increased glucose lowering effect.

1.3. CYP2C8 and SLCO1B1 polymorphisms and response to thiazolidinediones

The CYP2C8*3[32] and SLCO1B1 521T>C[33] polymorphisms did not affect the pharmacokinetics of thiazolidinediones in healthy volunteers.

1.4. OCT polymorphisms and response to biguanides

The OCT1 and OCT2 polymorphisms did not change the response to metformin[34].

2. Candidate genes affecting pharmacodynamics

2.1. SUR-1 polymorphisms and response to sulfonylureas

SUR-1 is a subunit of the pancreatic beta-cell K (ATP) channel and plays a key role in the regulation of glucose-induced insulin secretion[35], it binds sulfonylureas with different affinity which might explain the difference in response.

The influence of the SUR1 intron 16 –3t→c polymorphism and the impact of sulfonylurea therapy on plasma insulin, glucose, and triglyceride concentrations could not

be detected[36, 37]. However there was a significant reduction of insulin response in diabetic subjects carrying the combined genotype; silent exon 18 Thr775Thr (ACC→ACT) and exon16 (nt-3) of the SUR-1 gene[38], after tolbutamide load. Carriers of the -437a/t polymorphism did not differ in glucose or tolbutamide stimulated insulin response during a glucose tolerance test with intravenous tolbutamide injection[39]. And children with diffuse SUR1-/- hyperinsulinism, characterized by two abnormal SUR1 alleles, showed no acute insulin response to tolbutamide[40].

2.2. IRS-1 polymorphisms and response to sulfonylureas and biguanides

Insulin receptor substrate-1 (IRS-1), a member of the IRS protein substrate family, is considered to play a role in the insulin-signaling pathway[41], this receptor substrate can be activated by several sulfonylureas.

The Gly972Arg variant of IRS-1 was associated with failure to sulfonylureas with carriers having a higher risk of failure[42]. This polymorphism also modified the response to metformin. In Gly972Arg negative subjects, metformin lowered fasting insulin levels and insulin resistance(HOMA) more effectively and significantly than in G972A positive subjects[43].

2.3. PPAR-γ polymorphisms and response to thiazolidinediones and acarbose

Peroxisome proliferator-activated receptor-γ (PPAR-γ) receptors are found in key target tissues for insulin action, such as adipose tissue, skeletal muscle, and liver. Evidence indicates that these receptors are important regulators of adipocyte differentiation, lipid homeostasis, and insulin action[44]. This receptor is the target receptor for thiazolidinediones (TZD) compounds, which are a class of insulin-sensitizing drugs used in the treatment of type 2 diabetes[5]. The PPAR-γ coactivator 1α (PGC-1α) activates PPAR-γ and regulates the determination of muscle fibre type[45], controls insulin sensitive glucose transporter expression in muscle cell[46] and phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the liver[47]. The Gly482Ser polymorphism in this gene has been reported to be associated with T2DM.

The response to pioglitazone[48] and failure to troglitazone[49] was not modified by the Pro12Ala and the Pro12Pro genotypes of the PPAR-γ gene. In contrast, impaired glucose tolerant subjects with the Pro12Pro genotype of the PPAR-γ gene, showed a higher

conversion to T2DM compared to non carriers while treated with troglitazone[50] or acarbose[51]. Meanwhile acarbose also prevented the development of diabetes among carriers of the 482Ser allele of the Gly482Ser polymorphism of the PGC-1α gene. Treatment with rosiglitazone significantly decreased hemoglobin A1c (HbA1c) level in Korean subjects with the Ala12 allele, more than those without this allele[52].

2.4. ACDC polymorphisms and response to thiazolidinediones and acarbose

Adiponectin (ACDC) is a protein secreted by adipocytes and is known to be a potent insulin sensitizer. Low fasting adiponectin concentration is associated with low insulin-stimulated skeletal muscle insulin receptor tyrosine phosphorylation. Although adiponectin gene expression in adipose tissue is associated with obesity, insulin resistance, and type 2 diabetes, hypo adiponectinemia is more strongly related to the degree of insulin resistance than to the degree of adiposity or glucose intolerance[53]. Genetic polymorphisms may be involved in the regulation of adiponectin[54].

Carriers of the GG genotype for +45T/G polymorphism of the adiponectin gene (ACDC) showed smaller reduction in fasting plasma glucose level and HbA1c value after rosiglitazone treatment[55]. No association was found between the +45T/G polymorphism of the ACDC gene and conversion to T2DM among acarbose treated Impaired Glucose Tolerant patients[56]. However the TT genotype of the +276G/T polymorphism was associated with a higher risk of type 2 diabetes than the GG genotype in all subjects treated with acarbose.

2.5. KCNJ11 polymorphisms and response to sulfonylurea and biguanides

The K_{ATP} channel comprise four pore-forming inwardly rectifying potassium channel (Kir channel) 6.2 subunits and four regulatory sulfonylurea receptor (SUR) subunits. Kir6.2 is found in the pancreatic β-cell, cardiac and skeletal muscle and non-vascular smooth muscle. KCNJ11, encoding Kir6.2, has been shown to be associated with both Type 2 diabetes mellitus and cardiovascular disease in several populations[57].

Carriers of the E23K variant of the KCNJ11 more often showed secondary failure to sulfonylurea[58] (secondary failure is defined as requiring insulin due to uncontrolled hyperglycemia after adding metformin in patients whose plasma glucose rose to >300mg/dl after 3 months of SU treatment). K allele carriers had a tendency toward

shorter duration of therapy with oral agents before failure to sulfonylurea compared to EE subjects. Comparably carriers of the K/K polymorphism responded less well to the protective effect of metformin than E/E homozygotes[59].

2.6. TCF7L2 polymorphisms and response to sulfonylureas and biguanides

Transcription factor-7–like 2 (*TCF7L2*) is one of the most important type 2 diabetes susceptibility gene. Genetic variants in the gene encoding *TCF7L2* have been associated with type 2 diabetes (T2D) and impaired ß cell function, but the mechanisms remains unknown. It has been suggested that the risk allele increases *TCF7L2* expression in the pancreatic ß cell, reducing insulin secretion, predisposing the individual to diabetes[60, 61].

Carriers of the risk allele have been found to respond less to sulfonylurea treatment and had a higher risk for failure to sulfonylurea. The response to metformin was not found to be modified by this polymorphism[62].

3. Candidate genes in the causal pathway

3.1. HNF-α polymorphisms and response to sulfonylureas and biguanides

Hepatocyte nuclear factor-1α (HNF-1α) constitutes part of a network of transcription factors controlling organ-specific gene expression during embryonic development and in adult tissues. HNF-1α is expressed in the pancreatic ß-cell, and mutations in this gene lead to ß-cell dysfunction and maturity onset diabetes of the young (MODY3). MODY3 diabetes is the most common form of MODY in many countries[63]. The presence of two defective HNF-1α alleles is assumed to be lethal in humans.

The plasma insulin responses to glucose and tolbutamide in HNF-1α mutation carriers was preserved[64]. Normoglycaemic as well as recently diagnosed diabetic HNF-1α mutation carriers expressed a normal sized response to sulfonylureas.

The response of fasting glucose and fructosamine in patients with mutations in the HNF-1α gene treated with gliclazide was better than in patients with type 2 diabetes[65]. The fall in fasting plasma glucose in response to gliclazide was 3.9-fold larger in HNF-1α diabetic patients than in type 2 diabetic patients. No difference was found in the glucose

lowering response to metformin between HNF-1α diabetes patients and type 2 diabetic patients[65].

3.2. Lipoprotein lipase polymorphism and response to thiazolidinediones

Lipoprotein lipase(LPL) is an enzyme that plays a central role in lipid metabolism which catalyzes the hydrolysis of triglycerides, providing free fatty acids for cells and affecting the maturation of circulating lipoproteins[66, 67]. It has been proposed that the enzyme plays a role in the development of obesity and atherosclerosis[68].

The LPL S447X genotype was associated with lower response rate to pioglitazone. In contrast subjects with the S447S genotype had a more significant decrease in blood pressure after pioglitazone treatment than S447X genotype carriers[69].

Discussion

This review demonstrated that pharmacogenetics could play an important role in treatment of diabetes. Several studies have indicated a pharmacogenetic interaction between blood glucose lowering medication and genetic polymorphisms.

The most popular genes which have been studied for pharmacogenetic effects in relation to treating diabetes, were the pharmacokinetic genes such as the cytochrome complex genes, the organic cation and anion transporters (see table III). Most of these studies reported a decrease in clearance when subjects had the variant gene, whereas no effect was seen on insulin secretion and blood glucose values.

The studies that have been performed focused mainly on a small number of SNP's in a small number of genes. So it is not surprising that appropriate genes, SNP's or haplotypes of major importance have not yet been identified or studied. In many cases it is also unclear in which tissue a given polymorphism exerts its effect to influence the phenotype of interest.

When comparing the study population of the above-mentioned studies questions concerning study power, false positive findings and ethnic-specific effects may rise. Since the majority of the pharmacogenetic studies are very small sized it is not surprising that contradictory results between some polymorphisms exist. While some studies were not able to show an interaction others might suffer from false positive results due to the

small numbers of subjects and the potentially small contribution of any given polymorphism to blood glucose lowering. Except for a few studies all studies were performed among Caucasians. Since the frequency of some polymorphisms is related to ethnicity, the results seen in the study population should be interpreted with care.

Remarkably a lot of pharmacokinetic studies used healthy subjects. One can imagine that certain drugs have different effects in healthy and affected subjects. Also environmental factors and gene-gene interactions were not always taken into account. This is very important since it is known that diabetes is a multifactorial disease and several environmental factors can reveal or facilitate the phenotypic expression of susceptibility genes. These interactions may help to find other possible candidate genes and drug targets.

Unfortunately it is not yet possible to predict the response to blood glucose lowering medicine in diabetic patients given their genetic background. New candidate genes are ready to be investigated and several interactions with other genes and the environment are becoming clearer. But the magnitude of the genetic effects is not known which makes it difficult to calculate the required number of experimental subjects to obtain conclusive results.

Genes involved in pancreatic development and in the control of insulin secretion become more and more interesting to look at when considering the increased risk to develop diabetes. Several loci have been found which contain genes potentially involved in β-cell development or function such as IDE, HHEX and KIF11[70]. Also genes involved in hypertension and obesity such as the APM1, LEPR and GNB3 gene have been shown to play a role in the development of diabetes[71, 72].

Future pharmacogenetic studies should be performed more in diabetic subjects since the response to oral anti-diabetics can be different in non diabetic and diabetic patients. We should also mention population structure which can generate false genotype-phenotype associations. Recently a few solutions have been proposed to deal with this kind of bias such as genomic control and structured association methods[73]. Several approaches are available to perform pharmacogenetic studies. A population based genome wide screen has recently become feasible with advances in genome-wide genotyping[70, 74]. Currently

studies in which several SNP's are typed in multiple candidate genes are most feasible to study interactions between oral anti-diabetics and genetic polymorphisms. These studies should provide a powerful way to the discovery of genes which play a role in anti-diabetic drug response.

Table 1 The influence of genetic polymorphisms on the effect of glucose lowering medicine

Reference	Population	Therapy	Gene	PM	Allelic interaction
Lee et al.(17)	T2DM	Tolbutamide	CYP2C9	Arg144Cys(*2)	↓ clearance: *1/*3: 48%, *1/*2: 29% vs *1/*1 (p< .05)
	n=15			Ile359Leu(*3)	↔ blood glucose lowering
Kirchheiner et al.(18)	T2DM	Tolbutamide	CYP2C9	*2, *3	↓clearance: *1/*2:12 %; *2/*2: 23%; *1/*3: 42%; *2/*3:
	n=23				54%;*3/*3:84% vs *1/*1 ↔blood glucose, plasma insulin
Lee et al.(19)	T2DM	Tolbutamide	CYP2C9	*2, *3	↓ in 24h formation clearance of tolbutamide metabolites:
	n=16				*1/*2: 32%: *1/*3: 42% vs *1/*1
Shon et al.(20)	T2DM	Tolbutamide	CYP2C9	*3	↓ $t_{1/2}$*1/*3: 24% vs *1/*1
	n=18, Korean		CYP2C19	EM/PM	↔ pharmacokinetic or pharmacodynamic parameters
Niemi et al. (21)	T2DM	Glyburide	CYP2C9	*2, *3	t1/2(h) *1/*1:1.7[1.5;1.9], *1/*3 or *2/*3:2.6 [2.3;2.8] (p≤0.05)
	n=29				↔ blood glucose lowering
Kirchheiner et al.(22)	T2DM	Glyburide	CYP2C9	*2, *3	↓ oral clearance *3: 50% vs *1 (p≤0.001)
	n=21				Significant ↑ insulin secretion 12 hours after administration
Yin et al. (23)	T2DM	Glyburide	CYP2C9	*3	↑ $t_{1/2}$ *1/*3: 71% vs *1/*1(p<0.03), Δ glucose (2h) 17.85% more ↓ in *3, Δ insulin (2h) 161.1% more ↑ in *3 vs *1*1
	n=18, Chinese		CYP2C19	EM/PM	↔ pharmacokinetic or pharmacodynamic parameters
Niemi et al. (21)	T2DM	Glimepiride	CYP2C9	*2, *3	t1/2(h) *1/*1:1.9[1.1;2.5], *1/*3 or *2/*3:3.0 [2.5;4.0] (p≤0.01)
	n=29				↔ blood glucose lowering
Wang et al. (24)	T2DM	Glimepiride	CYP2C9	*3	↑ $t_{1/2}$ *1/*3: 163% vs *1/*1 (p<0.05)
	n=19, Chinese				↓ clearance *3/*3: 75% vs *1/*1 (p< 0.05)
Shon et al.(25)	T2DM	Chlorpropamide	CYP2C9	*3	NC *1/*1: 1.8 ± 0.2, *1/*3: 2.4 ± 0.1 ml/h kg p<0.05
	n=21, Korean		CYP2C19	EM/PM	↔ pharmacokinetic nor pharmacodynamic parameters
Holstein et al.(26)	T2DM	SU	CYP2C9	*2, *3	*3 associated with ↑ risk of severe hypoglycemia
	n=20				

Table 1 continued (1)

Reference	Population	Therapy	Gene	PM	Allelic interaction
Becker et al. (27)	T2DM n=172	Tolbutamide	CYP2C9	*2,*3	*3 significantly less ↑ in prescribed daily dose between 1st and 10th prescription
Kirchheiner et al. (28)	T2DM n=24	Nateglinide	CYP2C9 CYP2D6	*2, *3	twofold ↑ median AUC *3/*3 than *1/*1 ↔ pharmacokinetic or pharmacodynamic parameters
Niemi et al. (29)	T2DM n=28	Repaglinide	CYP2C8*3	Arg139Lys Lys399Arg	↓ mean AUC of *1/*3: 45% vs *1/*1 (p<0.05) ↔ blood glucose lowering
Niemi et al. (30)	T2DM n=56	Repaglinide	SLCO1B1	–11187G/A	–11187GA allele associated with ↑ glucose lowering effect, maximum ↓ 1.8 ± 0.9 mmol/L (p<0.05)
Zhang et al. (31)	T2DM n=17	Nateglinide	SLCO1B1	521T>C	↑ t1/2 CC:78% vs TT ↑ AUC of TC:82% and CC:108% vs TT
Kirchheiner et al. (32)	T2DM n=31	Rosiglitazone	CYP2C8	*3	↓ mean AUC of *3: 36% vs *1/*1 ↔ blood glucose
Kalliokoski et al. (33)	T2DM n=16	Rosiglitazone Pioglitazone	SLCO1B1	521T>C	↔ pharmacokinetic parameters of rosiglitazone and pioglitazone by SLCO1B1
Shikata et al.(34)	T2DM n=33, Japanese	Metformin	OCT	Met408Val	↔of OCT polymorphism on response to metformin
Meirhaeghe et al.(36)	T2DM n=70	SU	SUR1	16-3t-->c	-3t allele associated with significant ↓ in plasma [triglycerides] (p=0.026), ↔with plasma insulin and [glucose]
Zychma et al.(37)	T2DM n=68	SU or comb.	SUR1	16-3t-->c	no significant ↔ in allele distribution in T2DM with early failure compared to diabetic patients treated with SU
Hansen et al.(38)	T2DM n=449	Tolbutamide	SUR1	C/T exon 18 -3C/T exon 16	significant ↓ insulin response (19-22min) in carriers of combined exon18/exon16(nt-3): 124±27 vs 231±10min*pmol/l; p=0.045

Table 1 continued (2)

Reference	Population	Therapy	Gene	PM	Allelic interaction
Hansen et al.(39)	T2DM n=233	Tolbutamide	SUR1	-437a/t	↔of tolbutamide stimulated insulin response between carriers and non-carriers of the -437a/t allele
Grimbergen et al.(40)	HI n=24	Tolbutamide	SUR1	SUR1-/-	SUR1-/- no acute insulin response to tolbutamide compared to heterozygote and normal subjects
Sesti et al.(42)	T2DM n=477	SU	IRS-1	Gly 972 Arg	Arg972 IRS-1 associated with failure to sulfonylurea
Ertunc et al.(43)	PCOS n=60	Metformin	IRS-1	Gly 972 Arg	women lacking Arg variant of IRS-1, metformin ↓fasting insulin level and insulin resistance more effectively (both $p<0.001$)
Blüher et al.(48)	T2DM n=131	Pioglitazone	PPAR-γ2	Pro12Ala	↔ blood glucose lowering
Snitker et al.(49)	GDM n=93	Troglitazone	PPAR-γ2	Pro12Ala	↔ blood glucose lowering
Florez et al. (50)	T2DM n=3548	Troglitazone	PPAR-γ2	Pro12Ala	↔ PPAR-γ2 PM on response to troglitazone
Andrulionytè et al.(51)	IGT n=356	Acarbose	PPAR-γ2 PGC-1α	Pro12Ala Gly482Ser	2 .9 times higher conversion to T2DM in women with Pro12Pro vs Pro12Ala, prevention of diabetes among carriers of 482Ser allele
Kang et al.(52)	T2DM n=198, Korean	Rosiglitazone	PPAR-γ2	Pro12Ala	Ala allele associated with degree of (FPG)level (50.6±27.8mg/dL vs 24.3±41.9 mg/dL(non-carriers); p=0.026) and ↓ in HbA1c level (1.41%±1.47% vs 0.57%±1.16%(non-carriers), p=0.015)
Kang et al.(55)	T2DM n=166, Korean	Rosiglitazone	ACDC	+45T/G +276G/T	GG in both SNP's associated with lower ↓ in FPG and HbA1c ΔFPG PM: 30.5±43.3mg/dl vs ΔFPG GG/GG: 0.5±56.7mg/dl ΔHb A1c PM: 0.85±1.05% vs ΔHb A1c GG/GG: 0.18±1.23%

Table I. continued (3)

Reference	Population	Therapy	Gene	Polymorphism	Allelic interaction
Zacharova et al.(56)	IGT n=356	Acarbose	ACDC	+45T/G +276G/T	TT of SNP+276 associated with ↑ conversion to T2DM than GG OR 2.83 ([95%CI 1.26-6.36] p=0.012) combination G-allele/TT further ↑ risk OR 3.05 ([95%CI 1.34-6.96] p=0.008)
Sesti et al. (58)	T2DM n=525	SU	KCNJ11	E23K	E23K variant associated with secondary failure to SU (relative risk of K allele vs E23E homozygotes; 1.45; p=0.04)
Florez et al. (59)	IGT n=3234	Metformin	KCNJ11	E23K	Preventive against DM in E/E HR: 0.55 (0.54-1.67), E/K HR:0.89 (0.66-1.19) and K/K HR:0.95 (0.54-1.67) vs placebo
Pearson et al. (62)	T2DM n=4469	SU Metformin	TCF7L2	Rs12255372G/T Rs7903146	Carriers of TT ↑ rate of failure vs GG: OR(95%CI):1.95[1.23-3.06] ↔between metformin response and polymorphisms
Sagen et al.(64)	MODY n=7	Tolbutamide	HNF-1α		normal response to tolbutamide
Pearson et al.(65)	MODY n=26	Gliclazide	HNF-1α		improved response of fasting glucose to gliclazide FPG ↓ from baseline (mmol/l) T2DM vs HNF-1α: 1.2[0;2.4] vs 4.7[3.3;6.2]
Pearson et al.(65)	MODY n=26	Metformin	HNF-1α		↔ in response
Wang et al. (66)	T2DM n=113, Chinese	Pioglitazone	LPL	S447X	significant ↓ in FBG in X allele treated with rosiglitazone OR(95%CI): 0.54 (0.30-0.97)

PM: polymorphism; ND: Non Diabetic; T2DM: Type 2 Diabetes Mellitus; HI: Hyperinsulinemic; PCOS: Polycystic Ovary Syndrome; GDM: Gestational Diabetes Mellitus; IGT: Impaired Glucose Tolerance; MODY: Maturity Onset Diabetes of the Young; SU: Sulfonylurea; NC: non clearance

Table 2 Frequency of studied candidate genes.

Gene	Frequency (%)	Population	Reference
CYP2C9*2	10.7 –15.0	Caucasians	75-79
	0	Korean, Chinese	80-82
CYP2C9*3	7.4 – 9.8	Caucasians	75-78
	1.1	Korean	80
	1.7 -4.9	Chinese	81, 82
CYP2C19 PM	12.6	Korean	83
	11.1-17.65	Chinese	84
CYP2C8*3	13	Caucasians	85
SLCO1B1 (-11187 G>A)	14.3-17.7	Finnish	30, 86
OCT (Met408Val)	0.19-0.28	Japanese / T2DM	34
SUR1			
intron16-3t/c	47(t)	Caucasians / T2DM	87
C/T exon 18	3(t)	Caucasians / T2DM	87
combined	4	Caucasians / T2DM	87
IRS-1	9,8 - 11,6	Caucasians / T2DM	88
PPAR-γ2 (Pro12Ala)	10 (Ala)	Caucasians / T2DM	89
PGC-1α (Gly482Ser)	35.6	Danish / Metabolic syndrome	90
ACDC			
+45T/G	31.3	Korean / T2DM	55
	19.4 (G)	Caucasian / IGT	56
+276G/T	28.3	Korean / T2DM	55
	50.8 (T)	Caucasian / IGT	56
Kir6.2			
E23K	61.5(K)	Caucasian / T2DM	91
HNF-1α	5.2	Caucasian / MODY3	92

PM: Polymorphism; T2DM: Type 2 Diabetes Mellitus; IGT: Impaired Glucose Tolerance; MODY: Maturity Onset Diabetes of the Young

Table 3 Gene-drug interactions stratified according to pathway of drug response. Results presented are comparisons between carriers of the variant gene vs. wild type unless stated otherwise.

Drug/outcome	Genes in the PK pathway	Genes in the PD pathway	Genes in the causal pathway
SU-derivatives			
Hypoglycemia	↑ (CYP2C9*3)		
Insulin Response		↔ (SUR1)	
Secondary Failure*		↑ (IRS-1, KIR6.2)	
Early Failure**		↔ (SUR1)	
Tolbutamide			
Clearance	↓ (CYP2C9*2/*3)		
Blood glucose	↔ (CYP2C9*2/*3)		
Insulin Response	↔ (CYP2C9*2/*3)	↔ ↓ (SUR1)	↔ (HNF-1α)
Glyburide			
Clearance	↓ (CYP2C9*3)		
Blood glucose	↔ (CYP2C9*2)		
	↓ (CYP2C9*3)$		
Insulin Secretion	↔ (CYP2C9*2)		
	↑ (CYP2C9*3) $		
Glimepiride			
Clearance	↓ (CYP2C9*3)		
Blood glucose	↔ (CYP2C9*2/*3)		
Chlorpropamide			
Clearance	↓ (CYP2C9*3)		

Table 3 continued (1)

Drug/outcome	Genes in the PK pathway	Genes in the PD pathway	Genes in the causal pathway
Gliclazide			
Fasting Plasma Glucose			↓ (HNF-1α)
Meglitinides			
Nateglinide			
Clearance	↔(CYP2C9*2, CYP2D6*4/*5) ↓ (CYP2C9*3)		
Repaglinide			
Clearance	↓ (CYP2C9*3, CYP2C8*3)		
Blood glucose	↔ (CYP2D6*4/*5, SLCO1B1)		
Biguanides			
Metformin			
Fasting Insulin Level		↓ non carriers (IRS-1)	
Insulin Resistance (HOMA)		↓ non carriers (IRS-1)	
Insulin Response (HOMA)			↔ (HNF-1α)
HbA1c		↔ (OCT1-2)	
TZD			
Pioglitazone			
Blood Glucose		↔ (PPAR-γ2)	
Troglitazone			
Blood Glucose		↔ (PPAR- γ2)	

Table 3 continued (2)

Drug/outcome	Genes in the PK pathway	Genes in the PD pathway	Genes in the causal pathway
Rosiglitazone			
Fasting Plasma Glucose		↓↓ (PPAR- γ2)	
		Less Reduction (ACDC)	
HbA1c		↓↓ (PPAR- γ 2)	
		Less Reduction (ACDC)	
α -glucosidase			
Acarbose			
Conversion Diabetes Mellitus		↑ (PPAR- γ 2)	
		↓ (ACDC)	

PK: pharmacokinetic; PD: pharmacodynamic

*secondary failure is defined as requiring insulin due to uncontrolled hyperglycemia after adding metformin in patients whose plasma glucose rose to >300mg/dl after SU treatment,

**early failure is defined as receiving insulin treatment in the first 5 years after diabetes diagnosis

$ in Chinese

↓: decreased

↑: increased

↔: no effect

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CHAPTER FIVE

General discussion

Introduction

Diabetic patients have a higher risk of developing microvascular and macrovascular complications and lower extremity amputations. The main goal in the treatment of diabetes is improving glycaemic control which clearly reduces the risk of microvascular disease and has shown to lower the risk of atherosclerosis and macrovascular disease. Treatment for individuals with diabetes mainly focused on control of glycaemia, however, during the last decades, evidence has grown that controlling blood pressure and cholesterol levels plays an important role in managing diabetes or even in the prevention of it[1-5]. Controlling blood pressure in diabetic patients has even been reported to be a cost-effective intervention in order to improve cardiovascular health outcomes[6].

The first reports on a possible effect of different classes of antihypertensive drugs on incident diabetes mellitus date from the late 1950s[7-10]. Since then, increasing numbers of epidemiologic and experimental studies have confirmed that there are differences in the effect of antihypertensive drugs on glucose tolerance and incident diabetes[11]. The current national clinical practice guidelines in the Netherlands[12] support the use of thiazide diuretics as an initial therapeutic agent for the treatment of hypertension in patients with type 2 diabetes without albuminuria.

However, the use of thiazide diuretics, in order to aggressively treat high blood pressure, has been argued for many years as the glucose tolerance-impairing character of thiazide diuretics has been known for more than 40 years[7, 8, 13].

Besides the variety in response to the different classes of antihypertensive drugs, there is also considerable variability in antihypertensive drug response among individuals receiving similar doses of the same drug.

Thus, when prescribing a treatment regimen to a patient, one should keep in mind that not every patient responds similarly to a given drug or treatment with regard to adverse drug events and effectiveness. This interindividual variability can be caused by environmental factors, comorbidity, compliance, use of co-medication and the genetic profile of the patient[14].

The first report on genes playing a role in the response to a substance appeared in the early 1930s. In this study, a group of patients was not able to taste the chemical compound phenylthiocarbamide because they carried two recessive alleles which disabled the production of an enzyme required to taste the phenylthiocarbamide chemical[15]. The discovery of differently reacting chemicals due to genetic predisposition was followed by studies in the

1940s and 1950s and during the First World War. All these studies showed that inherited traits could cause adverse drug reactions but also determine the effectiveness of the drugs[16]. These discoveries gave birth to a new discipline called pharmacogenetics. Pharmacogenetics is defined as the study of hereditary variations in drug response and this term first appeared in literature in 1959[17]. In the beginning, the focus of pharmacogenetics was mainly on drug-metabolizing genes influencing the pharmacokinetics. Polymorphisms in the cytochrome P450 system are among the most extensively studied. Over the last decade however, polymorphisms of genes, encoding drug transporters and drug targets have been identified. It is in this context that polymorphisms which affect the pharmacodynamics of drugs, which at least partially defines the effectiveness of the drugs, are receiving more and more attention.

Several polymorphisms have been identified in systems related to interindividual variation in response to drug treatment. A key system to target when prevention of diabetes is an issue in hypertensive subjects is the blockade of the Renin-Angiotensin System (RAS). **Figure 1** shows the site of manipulation by angiotensin converting enzyme inhibitors (ACE inhibitors-ACEi), angiotensin II type 1 (AT_1) receptor antagonists also called angiotensin receptor blockers[18](ARB) and renin blockers[19].

Polymorphisms in the genes encoding ACE, angiotensinogen, and AT_1R have been associated with RAS activity[20-23]. The response and the risk of diabetes of RAS blockers may be influenced by these polymorphisms[24].

In this thesis, we investigated the effect of single nucleotide polymorphisms (SNP) in the RAS and salt sensitivity genes on the risk of diabetes associated with the use of antihypertensive drug treatment (thiazide diuretics, Angiotensin Converting Enzyme Inhibitor and Angiotensin Receptor Blocker), was examined. We also provided a systematic review of candidate genes possibly modifying the response to glucose-lowering drugs.

The main findings of this thesis will be presented here together with the overall limitations and strengths. We will conclude this chapter with the clinical implications of these studies and provide some recommendations for the future.

Main findings: we confirmed that thiazide diuretics increased the risk of diabetes in a dose dependent way. However, the addition of potassium sparing drugs or potassium supplements did not alter this effect. We found no major differences in the risk of diabetes mellitus associated with different diuretic drugs. The combination of thiazides with other antihypertensive drugs such as ACEi's or ARBs was less diabetogenic than thiazide monotherapy.

Several polymorphisms in two salt sensitivity genes and three RAS genes modified the association between antihypertensive drugs and the incidence of new onset diabetes.
The AGTR1 1166CC genotype carriers did not show an increased risk of becoming diabetic while on thiazide diuretics, even for doses higher than 1 DDD/day. ACE 4656GG and GNB3 825CC polymorphism carriers had a higher risk of diabetes, while on thiazide diuretics, compared to the C allele carriers and the T allele carriers respectively.
The use of ARB significantly increased the risk of becoming diabetic in carriers of the AGTR1 1166CC genotype, while high dose ACEi use only increased the risk of diabetes in ACE 4656 GG genotype carriers. Though we were not able to show an effect between the AGT M235T polymorphism and the use of RAAS inhibitors, the AGT T allele in combination with an ACE Inhibitor was shown to increase the risk of T2DM[25]. Recently a study performed by Becker et al. showed that the single nucleotide polymorphism rs10494366 in the NOS1AP (Nitric Oxide Synthase 1 adaptor protein) gene is associated with the incidence of diabetes mellitus in patients using CCBs[26].

Our review of pharmacogenetics of glucose lowering drugs identified several important interactions between genetic variants related to the pharmacokinetics and dynamics of these drugs.
In conclusion, our pharmacogenetic studies demonstrate that genetic variation can explain variation in response to RAS inhibitors and thiazide diuretics with regard to the risk of diabetes mellitus.

Potential mechanisms

A possible mechanism for the increased risk of diabetes mellitus due to thiazide use is that thiazide diuretic-induced hypokalemia causes an indirect reduction in insulin secretion leading to elevated serum glucose concentrations[27].
Although the precise mechanisms have not yet been fully elucidated, it is suggested that thiazides block the renal sodium chloride channel (NCC) and thereby increase sodium and chloride excretion. In the distal convoluted tubule, thiazides deliver a high sodium load with a resulting increased potassium excretion. In addition, thiazides activate the renin angiotensin aldosterone system (RAS) through volume depletion which leads to increased aldosterone secretion, further enhancing potassium excretion. One of the hypotheses is that this diuretic-induced hypokalemia leads to a higher secretion ratio of proinsulin to insulin which is

biologically less active compared to insulin[28], which in turn results in decreased insulin secretion[29].

But there is still some controversy surrounding the association between thiazide diuretics and the development of diabetes and this, therefore, needs further investigation, especially when the use of potassium sparing drugs or potassium supplements are considered in the prevention of thiazide-induced glucose tolerance and insulin hyposecretion[30]. This hypothesis is further confirmed by a randomized clinical trial performed by Shafiq et al. where patients treated with a thiazide showed a 45% higher adjusted diabetes risk for each 0.5mEq/L decrease in serum potassium compared to placebo use. After one year, chlorthalidone use was not associated with increased diabetes risk which suggests a thiazide-induced diabetes early after initiation treatment mediated by changes in serum potassium[31].

Another important finding was that thiazide diurectic monotherapy was significantly associated with diabetes compared to other antihypertensive monotherapy. However, when thiazides were used in combination with other antihypertensive drugs such as ARB and ACEi, the risk of developing diabetes was not statistically different from calcium antagonist monotherapy, which was used as a reference. Burke et al.[32] studied the effect of antihypertensive drugs and drug combination on the incidence of new-onset type-2 diabetes mellitus (NOD). They showed that most double and triple combinations of antihypertensives were associated with a significantly increased risk of diabetes mellitus compared to ACEi-monotherapy except ACEi plus thiazide. Also, any combination therapy without ACEi showed an increase of 27% of NOD compared to other combinations.

Both the AGTR1 CC genotype and the ACE GG genotype are related to increased activity of the RAS. When subjects carrying the ACE GG genotype are treated with thiazides (also increasing the RAS activity) this might further increase the angiotensin II signalling, leading to diminished insulin signalling and glucose transport, leading to an increased risk of diabetes. The AGTR1 CC genotype carriers have a greater sensitivity for angiotensin II due to a greater number of receptor binding sites and affinity for angiotensin-II. This means that the response to thiazides would be smaller due to increased counter-regulation.

The ACE GG (deletion) genotype is associated with higher circulating ACE plasma levels. Since the use of ACE inhibitors may improve insulin-stimulated glucose uptake of the whole organism, carriers of the ACE GG genotype might need higher doses of ACE inhibitors to achieve a similar effect as seen in C allele carriers (insertion)

Although we did not find a significant interaction between ACE inhibitors and the AGT M235T polymorphism, carriers of the T allele of the AGT gene using ACE inhibitors tended

to have a 2-fold higher incidence of diabetes in comparison to MM carriers (chapter 3.1). An analysis in the Rotterdam study confirmed that the AGT T allele in combination with an ACE Inhibitor increased the risk of T2DM[25].

Limitation and strengths

We used data from two observational, population-based studies; The Monitoring Project on Cardiovascular Risk Factors (Doetinchem) and a pharmacogenetic study of antihypertensive drugs. Both studies showed overlap with the PHARMO-RLS population, and these studies were used to collect data on genotypes and potential risk factors for diabetes mellitus. None of the studies used diabetes as the primary outcome; for the Doetinchem study the primary outcomes of interest were cardiovascular disease risk factors while in the pharmacogenetic study of antihypertensive drugs this was non-fatal myocardial infarction.

A limitation of these studies was the diagnosis of diabetes and hypertension. Since diabetes was not the primary outcome of interest the only available data on this outcome was the self-reported data found in the questionnaires and the pharmacotherapy assessed by PHARMO-RLS. In a small validation study among 83 subjects for whom general practitioner records were available (24 cases and 59 controls), we found that 91.7% (22) of the cases could be confirmed according to WHO criteria for a diagnosis of type 2 diabetes mellitus (either a fasting plasma glucose>7.0 mmol/l and/or random (non-fasting) blood glucose>11.1 mmol/l, and/or use of oral antidiabetic medication and/or use of insulin and/or treatment by diet and registered by a general practitioner as having type 2 diabetes mellitus), whereas 100.0% (59) of the controls had no diagnosis of type 2 diabetes mellitus.

The advantage of prescription based identification of diabetes is the unique use of glucose lowering drugs. While these are prescribed for diabetes specific conditions only, the use of antihypertensives are not *per se* used in hypertensive conditions only. However, specific effects of antihypertensive drugs beyond lowering blood pressure are likely to be similar for subjects with and without hypertension. Therefore, we do not expect this to be a major limitation of our studies. Another limitation of our studies was that we used an observational case-control approach. Therefore, our findings might suffer from residual confounding due to unmeasured or inaccurately measured confounding factors. In our studies we were able to correct for the most important confounding factors such as physical activity, obesity, use of alcohol, high cholesterol and smoking. Although these risk factors were assessed based on self-reported questionnaire information and therefore potentially causing recall bias, we

confirmed most risk factors for diabetes such as obesity, use of alcohol, and reduced physical activity.

Two major approaches to identifying the genetic variants associated with disease or treatment response include a family-based approach and a population-based approach. Compared to the population-base approach, a family-based approach has the advantage that fewer markers are needed to fully cover the genetic variation present. A disadvantage of a family-based approach is that, in order to observe the outcome, all family members would have to be users of a specific drug or drug class. For pharmacogenetic studies, a population-based approach would therefore be more feasible.

With regard to the identification of genetic variants, either genome-wide scanning or a candidate gene approach can be applied. Genome-wide scanning does not use a priori knowledge to select genetic variants, but uses a fixed set of markers spread across the genome. Genome-wide scanning is a powerful way to rapidly and systematically uncover new associations[33]. Over the last decade, genome-wide association studies have received increasing attention. This strategy has been very successful in identifying and confirming several common genetic variants associated with the risk of type 2 diabetes mellitus. The candidate gene approach uses a priori information to identify polymorphisms that may be biologically relevant to the disease or treatment response of interest. The selection of candidate genes could, of course, be expanded to several thousands of genes[34].

Multiple testing is particularly an issue when large numbers of SNP's are studied simultaneously. This implies that in order to find an association, more than one statistical comparison will be conducted on the same patient sample, which will increase false-positive results[35]. In our study we usually studied one SNP based on a specific a priori hypothesis, and therefore we do not expect our results to suffer from multiple testing.

A major strength of our study was the large sample size. The power of a genetic association study depends on the size of the allelic effect, the recombination fraction and the number of SNP's genotyped. When the recombination fraction is low and the mutation has a large effect on the phenotype, a relatively small number of SNP's can already yield a high power[36]. In general, studies of interactions require even larger sample sizes than studies of main associations. Increasing the sample size will greatly improve the power to detect associations and interactions. When many genes for complex diseases, each with a small effect, are involved, one can better increase the sample size rather than search additional SNP markers in order to detect the association or interaction. Most of the pharmacogenetic studies suffer from small sample size partially explaining the non-replication of the weak associations found.

The use of varying outcome definitions, different environmental exposures of the subjects, the interaction with other genes and differences in genetic background of populations can all lead to the non-replication of genetic association studies. In one of our studies we defined new onset diabetes based on medication only, whereas in the other studies we used a definition based on both self-reported information and medication refill data. In comparison, the Rotterdam study defined incident diabetes by the diagnosis of diabetes as registered by a general practitioner or treatment with antidiabetic medication (oral medication or insulin) during follow-up. The replication of these types of study is very important for a pharmacogenetic interaction. However, this is not so easy to implement since there are major limitations which cannot fully be compensated by using the same case definitions or by adjusting for environmental factors specific for each population. A possible way to deal with non-replication is to increase the sample size of the study so that the power to detect the interaction will increase further. Another way to deal with non-replication in other studies is to perform a replication in similar populations. Both methods were used in this thesis. While our relatively large sample size compensates for the most common limitations seen in genetic association studies, the replication of the association found between the use of ACE inhibitors and the insertion allele of the ACE gene in a combined analysis of two population based studies (The Monitoring Project on Cardiovascular Risk Factors and the pharmacogenetic study of antihypertensive drugs) and the whole set of the antihypertensive drugs study, showed the consistency of our result. One should also note that the second study was not an exact replica of the first as the populations only showed some overlap.

Clinical implications and recommendations for the future

There has long been an argument against the use of thiazides as first line treatment of hypertension as thiazides have been shown to increase the risk of becoming diabetic. This fear of prescribing thiazides is mainly based on findings from trials where diabetes was not the main outcome of interest but more a complication or side effect of the drug. Due to its long-term efficacy with regard to important clinical outcomes such as myocardial infarction and stroke and the fact that it is the least expensive antihypertensive drug available, thiazides should be considered as first line treatment in hypertensive patients. Even in patients with modest increase in risk factors for the metabolic syndrome, the benefits of an aggressive blood pressure reduction on the overall cardiovascular risk might outweigh the side effects such as K+ depletion, leading to an increased risk of diabetes.

Perhaps patients who are suspected of being at high risk of developing diabetes should also be put on thiazides but administered at lower doses and in combination with other antihypertensive drugs such as ACE inhibitors. While the addition of K+ sparing drugs and K+ supplementation has no clear proven benefit in reducing the risk of diabetes due to thiazide use, these agents reduce the risk of sudden cardiac death[37]. In this light, we support the recommendation of national clinical practice guidelines in the Netherlands where the use of thiazides as first line treatment is recommended even in patients with higher risk of becoming diabetic or who already are diagnosed with diabetes[38].

Though the findings of this thesis need to be confirmed in larger studies, one could foresee that in the future, when prescribing antihypertensive drugs to patients with increased risk of diabetes, polymorphisms in the RAS and salt sensitivity genes should be taken into account. The benefit-risk balance for treatment of hypertension with low-dose thiazide diuretics could then be individualised based on the genetic characteristics of the patient. Without taking genetic factors into account, the 5 year Number-Needed-to-Treat (NNT) for a hypertensive patient with a 5-year risk of CVD of 10% (threshold for treatment with antihypertensive agents in the Netherlands[39]) and a relative reduction of the risk of a CVD event by 24%[40] would be 42. The 5 year Number-Needed-to-Harm (NNH) for a hypertensive patient with a 5-year risk of type 2 diabetes mellitus of 6.8% (risk in placebo groups of hypertension treatment trials) and a relative increase of the risk of type 2 diabetes mellitus of 1.34[11] would be 43. However, for hypertensives with the GNB3 825T allele, the RR of type 2 diabetes associated with thiazide therapy is 1.36 which would correspond to a 5-year NNH of 41, whereas for hypertensives without the GNB3 825T allele with an RR of 2.01, the NNH would be 15. Therefore, the benefit-risk balance of thiazide treatment for subjects with the GNB3 825 T allele is more positive than for subjects without this allele. More formal weighing of benefit-risk such as proposed by van Staa et al could be applied to quantify the overall benefit-risk balance and help health care providers and patients take individualised decisions[41].

Based on our study findings, we constructed a decision table (**Table1**) which, under the assumption that the results will be replicated and confirmed in larger studies, could facilitate the process of prescribing an appropriate treatment regimen for hypertensives with increased risk of diabetes, taking into account polymorphisms in relevant genes. It should be noted that this is merely a conceptual model and that actual decision making is currently not possible due to the lack of conclusive data. The table presents the ORs of antihypertensive drugs and the risk of type 2 diabetes mellitus that were assessed in the studies described in this thesis.

Before pharmacogenetic tests are widely available, several limitations should be overcome. Challenges of the future for pharmacogenetic testing include the correct interpretation of these tests, test availability, cost and reimbursement.

As the evaluation of the clinical value of pharmacogenetic tests requires a detailed knowledge of pharmacology and, taking into account the limited availability of scientific evidence, the need for guidelines that link the result of pharmacogenetic test to therapeutic recommendations is clear. An important proposal for pharmacogenetic-based guidance was for the therapeutic use of antidepressants which included CYP2D6-related dose recommendations coming from pharmacokinetic studies[42]. This is, however, not the entire solution to the problem as the use of recommendations in routine clinical practice remains difficult due to non-accessibility during the decision-making process. To overcome this, the Royal Dutch Association for the Advancement of Pharmacy (KNMP) established the Pharmacogenetics Working Group (PWG) which is responsible for the development of pharmacogenetic-based therapeutic recommendations which are then integrated into the Dutch drug information database, the G-Standaard[43].

The recommendations, including dosage adjustments, advice for an alternative drug, alerts for increased risk of adverse drug events or diminished effectiveness are made per genotype or phenotype and appear on the screen of the healthcare professional while prescribing or dispensing a drug that interacts with a specific gene. Until now, pharmacogenetic based recommendations have been described for 40 drugs mainly based on genetic variation in the pharmacokinetic pathway.

Considering test availability, cost and reimbursement, it should be stated that while pharmacogenetic tests have improved (tests can genotype multiple loci in a short time, therefore reducing the cost) only a small number of laboratories offer pharmacogenetic tests. This has several clinical implications since the limited test availability can lead to a low turnaround time for the test results, thus leading to a delayed decision-making which, in some cases, is crucial[44] . Due to the lack of evidence supporting clinical utility, several pharmacogenetic tests are considered ‘experimental’ which leads to non-reimbursement by insurance companies which, in turn, leads to a delay in widespread adaptation of pharmacogenetic testing in clinical practice[44]. So, before pharmacogenetic testing in clinical practice can be made, well designed cost-effectiveness studies should be performed in order to evaluate the added value of the genetic test.

The most important factors influencing the effectiveness of pharmacogenetic tests are described in a paper by Flowers et al. [45]. The first involves gene characteristics and, in order to be clinically relevant and cost effective, the test should be made up for a gene variant that is relatively frequent in the population with a high penetration (association between genotype and treatment outcome). The second considers test characteristics such as the accuracy of the test. High sensitivity and specificity of a test leads to increased cost effectiveness. The third factor deals with the disease characteristics because diseases that are infrequent in the population cannot justify the costs required to develop and implement a genomic test. Subsequently, pharmacogenomic strategies can be cost effective if there are significant disease-related outcomes of interest or the outcome that is being avoided is expensive.
The fourth factor takes into account the treatment characteristics when there needs to be an intervention which can be implemented based on test results. This involves the current ability to accurately monitor patients for adverse drug reactions. Pharmacogenomic strategies incorporating drugs with a narrow therapeutic range, few alternative treatment options or that lead to severe adverse drug reactions have a greater likelihood of being cost effective.
It should be repeated that there is a need for large, population based, well designed pharmacogenetic studies to confirm the evidence found so far. This will help with the design of cost-effective, predictive pharmacogenetic tests.
Alongside these practical limitations, which could be overcome in time, several ethical issues have also arisen[44]. One could imagine that the widespread use of genetic testing could lead to human rights abuses (insurance companies) and also that the popularity of certain tests might lead to the differential development of tests for these diseases but not for others, keeping in mind the limitations in terms of interpreting the results. These ethical questions should be followed with caution since the expansion of pharmacogenetics will only raise more questions.

In the following paragraphs we will present some recommendations for future research.

As described earlier in detail in ‘strength and limitations’, there are two main strategies for identifying the relevant genes influencing drug response: the candidate gene approach and the genome-wide scan. The first involves variations in genes that are thought to play a role in the response to drugs and the second uses a fixed set of markers spread across the genome.

Both approaches have advantages and disadvantages but focusing on one gene alone may not be sufficient to fully elucidate the drug-gene association. Often, several SNP's but also environmental factors are involved and one could consider the use of haplotypes or genome-wide scans. Besides several practical and statistical problems which can be overcome in time, few genome-wide association studies were able to establish true associations.

The most limiting factor, when considering the use of the genome-wide scan, is the lack of replication studies since these studies are important in confirming true associations. A meta-analysis of the existing genome-wide studies, according to the recommendations of the Wellcome Trust Case Control Consortium are important for confirming associations.

Another challenge for the future, which also plays an important role when replicability is considered, is related to the design of the study. One can imagine the substantial possibilities in study design and the difficulties involved when comparing study results. The STrengthening the REporting of Genetic Association studies (STREGA) Statement, therefore, proposed a checklist of minimal items needed to maximize transparency and quality when reporting genetic associations.

Besides genes, many proteins and metabolites are part of the disease/treatment cascade. It would be an oversight in our quest to find optimised and individualised treatment regimens, if we do not take into account the increasing amount of biological data available. While pharmacogenomics focuses on genes influencing the response to pharmacological treatment, proteomics deals with individual protein concentrations and protein expression patterns of a cell or tissue, protein-protein, protein-DNA, and protein-RNA interactions. Proteomics can also facilitate the search for genes playing a role in drug response pathways but we would recommend the combination of all available data emerging from genomic, proteomic, physiological, clinical and environmental data.

In conclusion, the findings in this thesis suggest that the risk of developing diabetes in response to antihypertensive drugs is influenced by polymorphism in RAS and salt sensitivity genes.

Figure 1 Site of action of angiotensin II type 1 (AT_1) receptor antagonists and angiotensin-converting enzyme (ACE) inhibitors in the renin-angiotensin-aldosterone system[46].

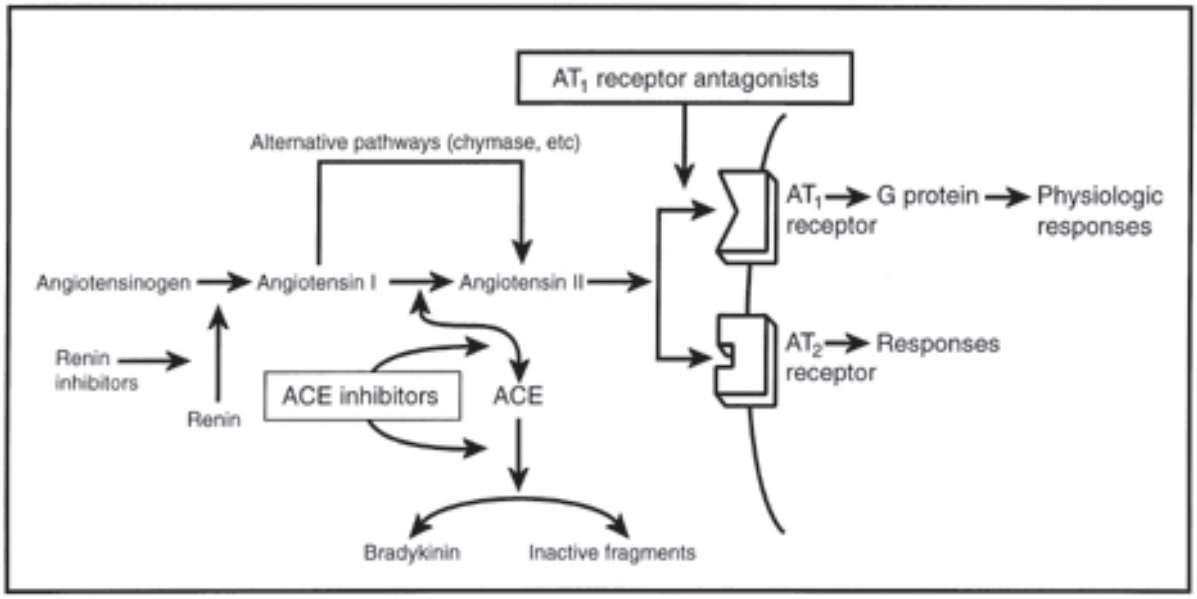

Table 1 Decision table for prescribing an appropriate treatment regimen for hypertensives with increased risk of diabetes, taking into account polymorphisms in relevant genes

Genotype / Treatment	**AGTR1 1166**		**ACE 4656// I/D**		**AGT 235**		**GNB3 825**		**ADD 1460**	
	A allele	*CC*	*GG//DD*	*C//I allele*	*MM*	*T allele*	*CC*	*T allele*	*GG*	*T allele*
Thiazides	1.76	0.97 *(dd)*	3.17 *(dd)*	1.35 *(dd)*	1.66 *(dd)*	1.69	2.01	1.36	1.48	1.81
<1DDD/day	1.39*	0.84	1.61	1.22	1.04	1.46*	1.66*	1.07	1.34	1.16
≥1DDD/day	2.07*	0.57	3.07*	1.49*	2.25*	1.63*	2.22*	1.58*	1.56*	2.26*
ACEi	1.80 *(AA)*	0.65 *(C allele)*	1.55	0.77	0.41	1.28				
<1DDD/day	0.83	0.90	0.70	0.90	0.60	1				
≥1DDD/day	0.24* *(AA)*	1.73* *(C allele)*	0.71	0.59*	0.40	0.67				
	1.05	1.51*	1.99*	0.90	1.20	1.11				
ARB <1DDD/day	1.01	2.72	1.12	1.09	0.71	1.30				
≥1DDD/day	0.67	24.8*	1.97	1.06	0.98	1.31				

* $p<0.05$ =>our studies have found a significant association between antihypertensive treatment and risk of diabetes

(dd): depends on dose

Black box: use of medication should be avoided in order to avoid the increase in risk of diabetes

Light gray box/black letters: use of medication should be promoted in order to reduce the risk of diabetes

Dark gray box/white letters: until no harm has been shown, use of medication is possible

Blank box: no association has been found (neither positive nor negative), some caution needs to be taken when prescribing

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CHAPTER SIX

Summary

Samenvatting

Summary

Being a part of the metabolic syndrome, high blood pressure occurs in a much higher rate among patients with diabetes than in general population. The co-existence of both disorders accelerates microvascular and macrovascular complications and increases cardiovascular risk, risk of stroke and end stage renal disease. Several studies have found that patients with diabetes benefit the most from a more aggressive treatment of hypertension. To achieve optimal blood pressure goals, most diabetic patients with hypertension require combination therapy. Angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers and diuretics are all effective antihypertensive agents in type 2 diabetes mellitus and none of them have shown superiority in either lowering blood pressure or reducing cardiovascular morbidity and mortality in diabetics. The overall aim of this thesis was to gain greater insight into the interaction between antihypertensive drugs and genetic polymorphisms on the development of diabetes.

Despite the superior efficacy in the reduction of the risk of cardiovascular morbidity and mortality, physicians often withhold prescribing thiazide diuretics because of the increased risk of type 2 diabetes. Thiazide diuretics impair carbohydrate metabolism through potassium depletion which decreases the insulin secretory response to glucose. It has been suggested that maintaining normal potassium concentrations may prevent glucose intolerance and/or the development of diabetes[1].
In our first study **(chapter 2.1)** among antihypertensive drug users, the use of potassium supplementation or potassium sparing drugs in combination with thiazides was not shown to be protective against the development of diabetes. The risk of diabetes increased with dose. In the same study we also showed that the combination of thiazides with other antihypertensive drugs such as ACE inhibitors or ARBs is less diabetogenic than thiazide monotherapy. But even if patients were not on combination therapy, low-dose thiazide therapy has shown to be the safest treatment with regard to the risk of diabetes.

Several studies have indeed indicated that not the use of thiazide *per se* leads to a higher risk of diabetes but the used dose[2] should be considered. The precise mechanism through which thiazides increase the risk of diabetes, has not been elucidated yet. A possible mechanism is that thiazide diuretic-induced hypokalemia causes an indirect reduction in insulin secretion leading to elevated serum glucose concentrations[3].

The controversy surrounding the association between thiazide diuretics and the development of diabetes requires further investigation, especially with regard to the use of potassium sparing drugs or potassium supplements.
In our second study **(chapter 2.2)** we examined whether polymorphisms in two salt sensitivity genes and three RAS genes modified the association between thiazide diuretics and the incidence of new onset diabetes. In a study of 5.140 antihypertensive drug users we identified 497 incident cases of diabetes with genotype data available. Thiazide diuretic use was associated with increased risk of diabetes in all genotypes except in AGTR1 CC homozygotes. In these patients even a dose higher than 1DDD/ day did not increase the risk of becoming diabetic. Both subjects with and without the ACE 4656 G variant had a higher risk of incident diabetes due to thiazide use but this risk was more increased in carriers of the ACE 4656GG genotype. The AGT M235T polymorphism did not modify the association. For the salt sensitivity genes only the GNB3 T allele carriers showed less increased risk of diabetes.

While thiazides are known for their diabetogenic effect, at least in higher doses, ACE Inhibitors and ARB have been proposed as possible protective agents against the development of diabetes in hypertensive subjects[4]. However genetic variations in the RAAS can also alter the response of ACE Inhibitors and ARB's on the risk of diabetes[5]. In this thesis two studies were performed to investigate the effect of genetic polymorphisms in RAAS genes on the association between the use of ACE Inhibitors and ARB and the incidence of diabetes **(Chapter 3)**. The first study **(chapter 3.1)** examined the modifying effect of genetic polymorphisms in RAAS genes on the association between the use of ACE Inhibitors and the incidence of diabetes among treated hypertensives. In a combined analysis of two population based studies: The Monitoring Project on Cardiovascular Risk Factors and the pharmacogenetic study of antihypertensive drugs, the AGTR1 1166A/C polymorphism, the ACE I/D polymorphism and the AGT M235T polymorphism (all three genes coding for components of the RAAS) were genotyped in order to assess their effect on the association between ACE inhibitor use and the incidence of treated diabetes. While the AGT M235T polymorphism did not change this association, the AGTR1 AA genotype was strongly linked to a decreased risk of diabetes among ACE inhibitor users compared to users of other antihypertensive drugs. For the AGTR1 C allele carriers, no difference in the risk of diabetes among patients treated with ACE inhibitors and other antihypertensives was observed. Hypertensive subjects carrying the insertion allele of the ACE gene showed a modest reduction of diabetes and no reduction was seen in DD homozygous subjects.

The second study in this chapter **(chapter 3.2)** investigated the modifying effect of the same polymorphisms in the RAAS in a larger sample on the association between diabetes and the response of both ACE Inhibitors and ARB's. The use of ARB and the association with diabetes was not modified by the ACE G4656C polymorphism or the AGT M235T polymorphism but carriers of the AGTR1CC genotype showed a 5 times higher risk of developing diabetes upon the use of ARB's. For the ACE Inhibitor users, the findings of the previous study were confirmed. ACE inhibitor use at doses ≥1DDD/day increased the risk of diabetes among GG (DD of the ACE I/D polymorphism) genotype carriers but not in 4656C allele carriers. However we were not able to confirm our previous findings for the AGTR1 AA genotype and the use of ACE inhibitors.

Although pharmacogenetic studies on glucose-lowering drugs are available, a review of the literature shows that there is still a long way to go before these results can be implemented into clinical practice **(Chapter 4)**. The pharmacogenetic studies summarized in this review, are candidate gene studies which can be classified according to genes affecting pharmacokinetics, pharmacodynamics or genes in the causal pathway. Polymorphisms affecting pharmacokinetics were the most frequent investigated in pharmacogenetic studies of oral antidiabetics. Variations in the Cytochrome P450 [CYP]2 genes and the use of sulfonylureas is one of the most studied associations. For candidate genes affecting the pharmacodynamics of oral antidiabetics, polymorphism in receptor genes were related to reduced response to treatment with several glucose-lowering drugs.
For the pharmacokinetic polymorphisms, the CYP2C9*3 genotype was associated with a decreased clearance of the drugs investigated (sulfonylureas and meglitinides) the association of the other Cytochrome P450[CYP]2 genes with pharmacokinetic parameters was not so clear cut. Among the genes possibly influencing the pharmacodynamics of oral antidiabetics, the IRS1 polymorphism led to secondary failure of sulfonylureas in carriers. While non-carriers who were treated with biguanides showed a decrease in fasting insulin levels and decreased insulin resistance. Polymorphisms in the PPARG gene were linked to decreased fasting plasma glucose and HbA1c in response to rosiglitazone treatment. Carriers of the HNF1A polymorphism, which is a candidate gene in the causal pathway, showed a decrease in fasting plasma glucose in response to gliclazide treatment.

In **Chapter 5** the results of our studies were summarized, placed into a broader perspective of potential clinical implications and further research was discussed.

In conclusion, our pharmacogenetic studies demonstrates that genetic variation can explain variation in response to RAAS inhibitors and thiazide diuretics and other antihypertensives with regard to the risk of diabetes mellitus.

Samenvatting

Hoge bloeddruk, een onderdeel van het metabool syndroom, treedt veel vaker op bij patiënten met diabetes dan onder de algemene bevolking. De co-existentie van beide stoornissen versnelt microvasculaire en macrovasculaire complicaties en verhoogt het cardiovasculair risico, het risico op een beroerte en chronisch nierfalen.
Verschillende studies hebben aangetoond dat patiënten met diabetes het meest profiteren van een strengere behandeling van hypertensie. Om de doelstellingen van optimale bloeddruk te bereiken hebben de meeste diabetes patiënten met hypertensie, combinatietherapie nodig. Angiotensine-converting enzyme-remmers, angiotensine-receptor blokkers en diuretica zijn allen effectieve antihypertensiva bij type 2 diabetes mellitus. Geen enkel van hen hebben een aangetoond superioriteit bij de verlaging van de bloeddruk noch bij de vermindering van cardiovasculaire morbiditeit en mortaliteit bij diabeten. Het algemene doel van dit proefschrift was om meer inzicht te krijgen in de interactie tussen antihypertensiva en genetische polymorfismen op de ontwikkeling van diabetes.

Ondanks de superieure werkzaamheid bij het verminderen van het risico van cardiovasculaire morbiditeit en mortaliteit, zijn artsen vaak terughoudend bij het voorschrijven van thiazide diuretica vanwege het verhoogd risico op type 2 diabetes. Thiazide diuretica tast de glucosestofwisseling aan door een uitputting van kalium dewelke een afname van de glucose geïnduceerde insuline secretie veroorzaakt. Er wordt gesuggereerd dat het behouden van normale kalium concentraties glucose-intolerantie en / of de ontwikkeling van diabetes kan voorkomen[1].

In onze eerste studie **(hoofdstuk 2.1)** onder bloeddrukverlagende medicatie gebruikers is er geen beschermend effect aangetoond van het gebruik van kaliumsupplementen of kaliumsparende geneesmiddelen in combinatie met thiaziden op de ontwikkeling van diabetes. Het risico van diabetes nam toe met de dosis. In dezelfde studie hebben we ook aangetoond dat de combinatie van thiaziden met andere bloeddrukverlagende middelen, zoals ACE-remmers of Angiotensine receptor blokkers(ARB) minder diabetogeen is dan thiazide monotherapie. Maar zelfs bij patiënten die geen combinatietherapie gebruikten bleken lage dosissen thiazide de veiligste behandeling met betrekking tot het risico van diabetes.

Verschillende studies hebben inderdaad aangegeven dat niet het gebruik van thiazide *per se* leidt tot een hoger risico op diabetes, maar dat de gebruikte dosis van belang is[2]. Het precieze mechanisme waardoor thiazide diuretica het risico van diabetes verhogen is nog niet opgehelderd. Een mogelijk mechanisme is dat thiazide diuretica-geïnduceerde hypokaliëmie een indirecte verlaging van de insuline secretie veroorzaakt dewelke leidt tot verhoogde glucoseconcentraties in het serum[3]. De controverse rond de associatie tussen thiazide diuretica en de ontwikkeling van diabetes vereist echter verder onderzoek, vooral met betrekking tot het gebruik van kaliumsparende geneesmiddelen of kaliumsupplementen.

In de tweede studie **(hoofdstuk 2.2)** onderzochten we of polymorfismen in twee zoutgevoeligheidsgenen en drie RAS-genen de associatie tussen thiazide diuretica en de incidentie van nieuwe diabetes modificeerde. In een studie met 5.140 bloeddrukverlagende medicatiegebruikers identificeerden we 497 nieuwe gevallen van diabetes waarvan de genotyperingsdata beschikbaar was. Het gebruik van thiazide diuretica was geassocieerd met een verhoogd risico op diabetes voor alle genotypen, behalve in AGTR1 CC homozygoten. Zelfs bij dosissen van meer dan 1DDD per dag vertoonden deze patiënten geen verhoging van het risico op diabetes. Zowel personen met als zonder de ACE 4656 G-variant toonden een hoger risico op diabetes als gevolg van het gebruik van thiazide, maar dit risico was groter bij dragers van het ACE 4656GG genotype. Het AGT M235T polymorfisme had geen invloed op de associatie. Van de zoutgevoeligheidsgenen vertoonden enkel GNB3 T alleldragers een minder verhoogd risico op diabetes.

Terwijl thiaziden bekend staan om hun diabetogeen effect, althans bij hogere dosissen, worden ACE-remmers en ARB voorgesteld als mogelijke beschermende middelen tegen de ontwikkeling van diabetes bij patiënten met hoge bloeddruk[4]. Maar ook genetische variaties in het RAAS kunnen de reactie van ACE-remmers en ARB's op het risico van diabetes wijzigen[5]. In dit proefschrift werden twee studies uitgevoerd om het effect van genetische polymorfismen in RAAS-genen en de associatie tussen het gebruik van ACE-remmers en ARB en de incidentie van diabetes, te onderzoeken **(hoofdstuk 3)**. De eerste studie **(hoofdstuk 3.1)** onderzocht het effect van genetische polymorfismen in RAAS-genen op de associatie tussen het gebruik van ACE-remmers en de incidentie van diabetes bij hoge bloeddruk behandelde patiënten.

In een gecombineerde analyse van twee populatie gebaseerde onderzoeken: Het Monitoring Project op Cardiovasculaire Risico Factoren en de farmacogenetica studie van antihypertensiva, werden het AGTR1 1166A / C polymorfisme, het ACE I / D polymorfisme en de AGT M235T polymorfisme (alle drie genen die coderen voor onderdelen van het RAAS) gegenotypeerd met het oog op de beoordeling van hun effect op de associatie tussen ACE-remmer gebruik en de incidentie van behandelde diabetes. Terwijl het AGT M235T polymorfisme deze associatie niet veranderde werd het AGTR1 AA genotype sterk gerelateerd aan een verminderd risico op diabetes bij ACE-remmer gebruikers vergeleken met gebruikers van andere antihypertensiva. Bij AGTR1 C alleldragers werd er geen verschil gevonden op het risico van diabetes tussen patiënten behandeld met ACE-remmers en patiënten die andere antihypertensiva gebruikten. Hypertensieve patiënten met het ACE-insertie allel vertoonden een bescheiden reductie van diabetes en er was geen vermindering bij DD homozygoten.

De tweede studie in dit hoofdstuk **(hoofdstuk 3.2)** onderzocht het modificerend effect van dezelfde polymorfismen in het RAAS in een grotere steekproef op de associatie tussen diabetes en de respons op ACE-remmers en ARB. Het gebruik van ARB en de associatie met diabetes werd niet gewijzigd door het ACE G4656C polymorfisme noch het AGT M235T polymorfisme, maar dragers van het AGTR1CC genotype vertoonden een vijfmaal hoger risico om diabetes te ontwikkelen bij gebruik van ARB's. Voor de ACE-remmer gebruikers, werden de bevindingen uit de voorafgaande studie bevestigd. Het gebruik van ACE-remmers bij dosissen ≥1DDD/dag verhoogde het risico op diabetes bij GG (DD van het ACE I / D polymorfisme) genotype dragers, maar niet bij 4656C alleldragers. We waren echter niet in staat om onze eerdere bevindingen over het AGTR1 AA genotype en het gebruik van ACE-remmers te bevestigen.

Hoewel er farmacogenetische studies over bloedsuikerverlagende middelen beschikbaar zijn, toonde een literatuuroverzicht aan dat er nog een lange weg is alvorens deze resultaten kunnen worden toegepast in de klinische praktijk **(hoofdstuk 4)**. De farmacogenetische studies samengevat in deze review, zijn kandidaat-gen studies die kunnen worden ingedeeld op basis van genen die een invloed hebben op de farmacokinetiek, farmacodynamiek en genen in het causale pad.

Polymorfismen die de farmacokinetiek beïnvloedden werden het vaakst onderzocht in studies die de relatie van het genetisch profiel ten opzichte van het gebruik van orale antidiabetica onderzochten. Variaties in het cytochroom P450 [CYP] 2 genen en het gebruik van sulfonylurea was een van de meest bestudeerde associaties. Voor kandidaat-genen die een invloed hebben op de farmacodynamiek van orale antidiabetica werden voornamelijk polymorfismen in de receptor genen bestudeerd en hun effect op een verminderde respons op verschillende glucose-verlagende medicatie.

Voor de farmacokinetische polymorfismen, was het CYP2C9 * 3-genotype geassocieerd met een verminderde klaring van de onderzochte medicatie (sulfonylureas en meglitinides) de associatie van de andere cytochroom P450 [CYP] 2 genen met de farmacokinetische parameters was niet echt duidelijk.
Bij genen die de farmacodynamiek van orale antidiabetica beïnvloeden, vertoonden dragers van het IRS1 polymorfisme secundair falen voor behandeling met sulfonylureum derivaten. Terwijl niet-dragers die behandeld werden met biguaniden een daling van de nuchtere insuline-niveaus en een verminderde insuline resistentie vertoonden. Polymorfismen in het PPARG-gen werden gelinkt aan een vermindering van de nuchtere bloedglucose en HbA1c als reactie op de behandeling met rosiglitazone.
Dragers van het HNF1A polymorfisme, dewelke een kandidaat-gen in het causale pad ligt, toonde een daling van de nuchtere plasma glucose in reactie op de behandeling met gliclazide.

In **hoofdstuk 5** worden de resultaten van onze studies samengevat, in een breder perspectief van potentiële klinische implicaties geplaatst en verder onderzoek wordt besproken. Tot slot toonden onze farmacogenetische studies aan dat genetische variatie, de variatie op de respons op RAAS-remmers en thiazide diuretica en andere antihypertensiva kan verklaren met betrekking tot het risico van diabetes mellitus.

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List of publications

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Dankwoord

Een kuil
Een zaadje
Een handvol aarde
Regelmatige wat water
Geduld
Veel geduld
Plots een steeltje
Dan takken
Veel later bladeren, veel bladeren
Bovengronds onzichtbaar
Maar ook de wortels groeien
Steeds dieper naar de kern van de aarde
Verder vertakkend
Ondersteunend
Verstevigend

De afgelopen jaren, mijn promotieonderzoek, het boekje wat voor u ligt heb ik ervaren als de groei van een boom. Vanaf het prille begin, het graven van de kuil, tot het resultaat wat voor u ligt, de bladeren en de stevige stam, ben ik dank verschuldigd aan zeer veel onder u.
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Özlem Bozkurt was born on 17 august 1981 in Genk, Belgium as the first daughter of a Turkish coalmine worker. She studied Science and Math at the Onze-Lieve-Vrouwlyceum in Genk and in 1999 she started her master Biomedical Sciences at the University of Hasselt and University of Maastricht. For her graduation research she identified the effect of the scorpion toxin BmBKTx1 on isolated metathoracic dorsal unpaired median (DUM) neurons of Locusta migratoria using the whole cell patch clamp technique. The results were published in a peer reviewed journal and this was also her first scientific publication.

After her graduation in 2003 she followed a one year advanced master in Nutrition and Health at the Catholic University of Leuven where she wrote a paper on Cadmium in food.

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