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Type 2 Diabetes Mellitus and Metabolic Syndrome

Modulators and Prognosis

THIJS T. W. VAN HERPT

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Thijs van Herpt

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Type 2 Diabetes Mellitus and Metabolic Syndrome: Modulators and Prognosis

Type 2 diabetes mellitus en het metabool syndroom:
modulatoren en prognose

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Promotoren:
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Prof. dr. H.R. Haak

Copromotoren:
Dr. M. van Hoek
Dr. A.G. Lieverse

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PART I

Introduction to the Thesis

Chapter 1

General introduction

Obesity, metabolic syndrome and type 2 diabetes mellitus are multifactorial conditions, that have detrimental effects on both individual's health perspectives and functional outcome as well as governmental health care expenditures. It is therefore important to gain more insight into the magnitude, etiology and complications of these diseases.

1.1 OBESITY

The term overweight refers to a body mass index between 25 and 30, whereas obesity is defined as a body mass index of 30 and over. Due to an overabundance of food and sedentary lifestyles in both developed and developing countries the worldwide incidence and prevalence of obesity is rising (1,2). In the United States for example, the overall prevalence of obesity has increased from 13.4% in 1960 to 30.9% in 1999 and 37.7% for the year 2013 (3). Obesity is associated with insulin resistance (4–6), lipid disorders (7–10), hypertension (7,11,12) and cardiovascular disease (13,14). These conditions are therefore becoming increasingly more common and so are their long-term complications with associated morbidity and mortality (15–20). Global efforts are needed to deflect the widespread epidemic of obesity in order to reduce their burden on quality of life, mortality and health care expenditures.

1.2 METABOLIC SYNDROME

The metabolic syndrome is a constellation of risk factors known for their association with type 2 diabetes mellitus and cardiovascular disease and consists of obesity, hyperglycemia, dyslipidemia and hypertension (21–24). In 1988 the term was first introduced as a pathophysiological entity with insulin resistance as a common denominator (5). However, in time it became mainly used to cluster and predict cardiovascular disease and type 2 diabetes mellitus. As a predictor, a number of issues have arisen. There are a number of definitions with different cut-off values for its components (25–27), which may have led to heterogeneity in the results of prediction and risk association studies. In addition, the added value of metabolic syndrome as a disease entity on top of its individual components in terms of risk association with type 2 diabetes, cardiovascular disease and mortality has been questioned since its introduction (28–31).

1.3 TYPE 2 DIABETES

Type 2 diabetes is a metabolic condition characterized by insulin resistance and insufficient beta-cell capacity resulting in a dysregulation of glucose metabolism (32,33). A short-term complication of type 2 diabetes mellitus is hyperglycemia. However, the main burden of the disease comes from its long-term complications such as retinopathy (34–36), nephropathy (37), neuropathy (38–41) and macrovascular disease (12, 42–44). Because of the increased prevalence of obesity, the prevalence of type 2 diabetes also continues to rise worldwide (19). Currently, an estimated 1 in 11 adults or 415 million individuals worldwide have diabetes (19). A low estimate of health care expenditures on diabetes in 2014 was USD 612 billion, which is 11% of global health expenditures (45). Although there has been a tremendous amount of research devoted to the role of genetic, biochemical and environmental factors in type 2 diabetes and its vascular complications, much is still unknown on exact pathophysiological disease mechanisms. A staggering 55% global increase in prevalence of diabetes by the year 2040 emphasizes the need to improve treatment options and preventive strategies.

1.4 RISK FACTORS FOR TYPE 2 DIABETES AND ITS COMPLICATIONS

Type 2 diabetes is a multifactorial disease with impaired insulin secretion and insulin resistance as its main pathophysiological components (32,33,46). Both genetic and environmental factors influence disease-risk which makes it more difficult to determine the exact cause of type 2 diabetes in each individual patient. Known risk factors for type 2 diabetes are obesity, a family history of diabetes, ethnicity and lifestyle factors, such as exercise, sleep duration and smoking (47–53). Type 2 diabetes morbidity and mortality are mainly due to its macro- and microvascular complications (42,54–58). Glycaemia is associated with the risk of cardiovascular disease and microvascular complications in T2DM (42,59,60). However also other metabolic derangements associated with type 2 diabetes and obesity are important risk factors for the occurrence of complications, such as hypertension and dyslipidemia.

It is well known that treatment of blood pressure and dyslipidemia reduces the risk on vascular complications in both primary and secondary prevention in the general population (61,62). Treatment and prevention strategies in type 2 diabetes focus on aggressive reduction of dyslipidemia and hypertension with successful results in the reduction of vascular disease risk (63–65). By strict glycemic control, the risk of microvascular disease can also be reduced substantially (59,66). However, interventions applying more strict glycemic control were unsuccessful or showed adverse effects (67,68) in the reduction

of macrovascular disease. The relation between glycemic control and prevention of macrovascular events therefore remains incompletely understood. Also, despite optimal treatment efforts for all vascular risk factors, a substantial residual risk of macro- and microvascular complications remains.

Diabetes care in the Netherlands is organized in primary care and outpatient clinics. In the outpatient clinic setting, the more complex patients are situated and care is performed by internal medicine consultants. The primary care is performed by general practitioners and consists of more elderly patients with a relatively large proportion on oral treatment and diets. It is important to gain more insight into characteristics and effect of organizing diabetes care this way.

Apart from environmental factors that play a major role in the development of type 2 diabetes, there is clear evidence for genetic susceptibility (47). The genetic risk component of type 2 diabetes is polygenic, which means many genes with small effects are involved . Up to now, a total of 128 signals at 113 loci have been independently associated with type 2 diabetes (69–71). However, only 5 to 20 % of the attributional predisposition to T2DM can be explained by these genetic variants (70,72).

1.5 PREDIABETES, LIFETIME RISK AND PREVENTION

An important condition for preventive efforts to become successful is the ability to identify individuals at high risk to develop a disease. Individuals with an impaired glucose homeostasis below the threshold of diabetes are at high risk to develop diabetes. This prediabetes state emerges long before a diagnosis of diabetes is made (33), and can therefore be used to identify individuals to apply preventive efforts to. Pharmacological and lifestyle interventions have proven their preventive capacity for diabetes when applied to individuals with prediabetes (73–76). However, two definitions of prediabetes, by the American Diabetes Association (ADA) and World Health Organisation (WHO), exist (77,78). A substantial difference in these definitions is the lower threshold of the glycemic index by the ADA definition. This may change the balance of sensitivity to identify individuals at risk versus overtreatment of false positive identified individuals that will never progress to diabetes. Therefore, it is important to investigate the consequences of this difference in definition for the relation between prediabetes and progression to diabetes. In order to obtain successful individual disease management and preventive effects, clear communication of risks and treatment goals from clinician to patient is key. Non-transparent relative risk estimates have less persuasive power when compared to absolute disease risks (79–82). Better patient disease awareness improves therapy adherence. Lifetime

risks provide a clear message to patients, clinicians and policy makers by providing cumulative risks of developing a disease during an individual's remaining lifespan. There are some reports on life time risks of type 2 diabetes in the US and Australia, however these are based on simulated data. Therefore there is a clear need for real-life prospective population-based cohort follow-up, high-quality data on impaired glucose metabolism, its treatment and complications to gain more insight in to the development and progression of the disease. More importantly, by providing patients and physicians with lifetime risk estimates of diabetes and prediabetes, a clear communication tool in the doctor's office is provided to persuade patients to adhere to their therapy and create self-awareness in disease management.

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Chapter 2

Aims and scope

The aim of this thesis is to investigate the clinical value of the metabolic syndrome, to assess the lifetime risk of diabetes and prediabetes in the Netherlands and to examine the prevalence of vascular complications in the Dutch diabetes patient population. Secondly, we investigated association of biochemical markers and genetic variants with risk of type 2 diabetes.

In Part II (Chapter 3), we study the clinical value of the metabolic syndrome by comparing three commonly applied definitions, study their prevalence in the Dutch population and investigate the associations of the definitions and their added value above their individual components with cardiometabolic diseases.

In part III (chapter 4 and 5) we study the burden of type 2 diabetes in the Netherlands in a unique and large prospective population-based study in Ommoord, Rotterdam with accurate data on glucose and thus prediabetes and diabetes onset. Through calculation of cumulative risks in all person-years of follow-up, we provide lifetime risk estimates which help to elucidate an individual's diabetes risk based on age and body mass index. (chapter 4) Furthermore, by comparing the different definitions of prediabetes in this population with respect to lifetime risk in both women and men (chapter 5), we uncover strengths and weaknesses of both definitions from a WHO and ADA perspective.

In part IV, (chapter 6,7 and 8) we present a diabetes population from The Netherlands in which we evaluated the presence of micro- and macrovascular complications and the prevalence of type 2 diabetes-associated risk factors and genetic risk alleles (chapter 6). Furthermore, in chapter 7, we present a candidate-gene approach study in which we find a genetic variant in SLC6A20, (involved in proline metabolism) to be associated with type 2 diabetes in both a European as well as a Chinese population. In chapter 8, we find ADAMTS13 to be a novel risk marker for type 2 diabetes (chapter 8)

Finally, in part V, in chapter 9, I will summarize the main findings of this thesis, methodological issues, their implication for clinical practice and future research directions.

PART II

Metabolic syndrome in modern society: definitions and predictive ability

Chapter 3

The clinical value of metabolic syndrome and risks of cardiometabolic events and mortality in the elderly: the Rotterdam Study.

Chapter 3

The clinical value of metabolic syndrome and risks of cardiometabolic events and mortality in the elderly: The Rotterdam Study

Thijs T.W. van Herpt
Abbas Dehghan
Mandy van Hoek
M. Arfan Ikram
Albert Hofman
Eric J.G. Sijbrands
Oscar H. Franco

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ABSTRACT

Introduction: To evaluate the clinical value of metabolic syndrome based on different definitions (American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI), International Diabetes Federation (IDF) and European Group for the study of Insulin Resistance (EGIR)) in middle-aged and elderly populations.

Methods: We studied 8,643 participants from the Rotterdam Study (1990-2012; mean age 62.7; 57.6% female), a large prospective population-based study with predominantly elderly participants. We performed cox-proportional hazards models for different definitions, triads within definitions and each separate component for the risk of incident type 2 diabetes mellitus, coronary heart disease, stroke, cardiovascular- and all-cause mortality.

Results: In our population of 8,643 subjects, metabolic syndrome was highly prevalent (prevalence between 19.4% and 42.4%). Metabolic syndrome in general was associated with incident type 2 diabetes mellitus (median follow-up of 6.8 years, hazard ratios 3.13 to 3.78). The associations with coronary heart disease (median follow-up of 7.2 years, hazard ratios 1.08 to 1.32), stroke (median follow-up of 7.7 years, hazard ratios 0.98 to 1.32), cardiovascular mortality (median follow-up of 8.2 years, ratios 0.95 to 1.29) and all-cause mortality (median follow-up of 8.7 years, hazard ratios 1.05 to 1.10) were weaker. AHA/NHLBI- and IDF-definitions showed similar associations with clinical endpoints compared to the EGIR, which was only significantly associated with incident type 2 diabetes mellitus. All significant associations disappeared after correcting metabolic syndrome for its individual components.

Conclusions: Large variability exists between and within definitions of the metabolic syndrome with respect to risk of clinical events and mortality. In a relatively old population the metabolic syndrome did not show an additional predictive value on top of its individual components. So, besides as a manner of easy identification of high-risk patients, the metabolic syndrome does not seem to add any predictive value for clinical practice.

BACKGROUND

The metabolic syndrome (MetS) is a combination of risk factors for type 2 diabetes mellitus and cardiovascular disease (CVD). Although MetS was designed to cluster and predict risk for type 2 diabetes mellitus and CVD, controversy remains on its usefulness in clinical practice. This is due to the fact that it is still not fully clear whether MetS has an added value to the prediction of diabetes, cardiovascular disease and mortality above the effect of its individual components [1-6].

There are a number of different definitions according to which MetS can be defined which may have led to heterogeneity. The currently applied definitions have substantial differences in the predefined components and cut-off values [7-10]. Furthermore, most studies on the association between MetS and cardiovascular disease, mortality and diabetes have been performed in middle-aged populations [11-15], while the associations of MetS with type 2 diabetes mellitus, CVD and mortality and the added value of MetS above its individual components in elderly populations has received less attention and has led to inconsistent results [2-5, 16-19].

Therefore, the aim of our study was to determine the clinical value of MetS in a large prospective Dutch predominantly elderly population comparing three commonly applied definitions. We investigated the associations of the definitions, their exact composition and the added predictive value above their individual components with risk of type 2 diabetes mellitus, coronary heart disease (CHD), stroke, cardiovascular - and all-cause mortality.

METHODS

Study Population

Analyses were performed in the Rotterdam Study, an ongoing prospective population-based cohort study in Rotterdam, The Netherlands. In 1989, all residents aged 55 years or older in a well-defined district of Rotterdam were invited to participate in the original cohort (RS-I). A total of 7,983 (78.1%) agreed to participate in the follow-up study. The study was extended in 2000 with a cohort of individuals who had reached age 55 or moved into the study area after the initial cohort (n=3,011). In 2006, a third cohort of 3,932 participants aged 45 years or older was enrolled, bringing the total study size to 14,926 individuals. There were no eligibility criteria to enter the Rotterdam Study cohorts except the minimum age and residential area. A more detailed description of the methods of the Rotterdam Study can be found elsewhere [20,21].

Participants are being monitored for type 2 diabetes mellitus, CHD, stroke and mortality by continuous linkage to files from general practitioners in the study area, information from medical specialists and discharge reports after hospitalization. All information was obtained through trained research employees and reviewed by two independent medical doctors, supervised by a specialist in each separate medical field.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

Population for analysis

A total of 14,926 participants were included in three subsequent cohorts in the Rotterdam Study. From the first cohort entering the study in 1990 (n=7,983) we used data from their third examination (n=4,797, 1997-1999) because of the availability of fasting blood samples. Furthermore, we used data from participants of the second (n=3,011, 2000-2001) and third cohort (n=3,932, 2006-2008).

From the 11,740 participants mentioned above, 10,599 went to the research center for blood sampling and anthropometric measurements. Only fasting participants were included in the study (n=9,819) Subsequently, we excluded 1,176 prevalent cases of type 2 diabetes mellitus resulting in a population for analysis of n=8,643. For each given endpoint, prevalent cases of that endpoint where excluded. An average of 1.7% had missing data on MetS-components. These were imputed by using the multiple imputation method described by Sterne et al. [22].

Definitions of MetS

MetS was defined according to 3 definitions (supplemental table S1): 1) as stated by the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) [8], which was later used without adjustments in the consensus definition of IDF and AHA/NHLBI in 2009 [10], 2) according to the International Diabetes Federation (IDF) [9]and 3) according to the European Group for the study of Insulin Resistance (EGIR) [7]. A diagnosis of MetS according to AHA/NHLBI-criteria consists of at least 3 of the following components: (1) waist circumference > 102 cm for males or > 88 cm for females; (2) HDL-cholesterol <1.03 mmol/l for males or HDL-cholesterol <1.29 mmol/l for females, (3) triglycerides ≥ 1.7 mmol/l, (4) systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or antihypertensive treatment; and (5) fasting glucose of ≥ 5.6 mmol/l or drug treatment for elevated glucose.

According to IDF-criteria, a diagnosis of MetS includes the component of central obesity (COB) as defined by waist circumference ≥ 94 cm for males or waist circumference ≥80 cm for females. If BMI is > 30 kg/m^2, central obesity is assumed and waist circumference does not need to be measured. Central obesity is the central component in the definition of MetS according to IDF. In addition to central obesity, two of the following four components should be present: (1) raised triglycerides ≥ 1.7 mmol/l, (2) HDL-cholesterol < 1.03 mmol/l for males or HDL-cholesterol < 1.29 mmol/l for females, (3) systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or treatment of hypertension, (4) raised fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed type 2 diabetes mellitus. According to EGIR-criteria, the upper quartile of fasting insulin in a non-diabetes population is required together with two of the following components: (1) hyperglycemia ≥ 6.1 mmol/l but not having diabetes, (2) systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or treatment of hypertension, (3) dyslipidemia as defined by triglycerides >2.0 mmol/l or HDL-C <1.0 mmol/l, (4) central obesity as defined by a waist circumference ≥94 cm for males or waist circumference ≥80 cm for females.

Components and triads

According to the AHA/NHLBI-, IDF- and EGIR-criteria we defined MetS at baseline. Triads where defined as the simultaneous combination within a participant of any 3 different components of the MetS that would guarantee a diagnosis of MetS (a participant could have >1 triad at the same time).

Definition of type 2 diabetes mellitus

Incident type 2 diabetes mellitus was defined in accordance with the guidelines of the American Diabetes Association [23,24] and World Health Organization (WHO) [25] as a (1) fasting glucose level ≥ 7.0 mmol/L or (2) a non-fasting glucose level ≥ 11.1 mmol/L or (3) treatment with oral glucose-lowering medication or insulin, and (4) diagnosis of diabetes as registered by a general practitioner or medical specialist. Prevalent cases of diabetes were diagnosed at baseline by a (1) non-fasting or post-load glucose level (after oral glucose tolerance test) ≥ 11.1 mmol/L or (2) treatment with oral glucose-lowering medication or insulin, and (3) diagnosis as registered by a general practitioner.

Definition of CHD

Incident CHD was defined as (1) myocardial revascularization (as a proxy for significant coronary artery disease), (2) Myocardial Infarction (MI, fatal and nonfatal) and (3) fatal CHD. Specific details on definitions in each categories in the Rotterdam Study can be found elsewhere [26].

Definition of stroke

Stroke was defined according to WHO-criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin [27]. History of stroke at baseline was assessed during the baseline interview and verified by review of medical records. A more profound description on methods of data collection for stroke can be found elsewhere [28].

Definition of cardiovascular mortality and all-cause mortality

Cardiovascular mortality was classified as mortality as a consequence of (1) CHD, (2) cerebrovascular disease, (3) atherosclerotic disease other than CHD or cerebrovascular disease (including ruptured abdominal aortic aneurysm, peripheral vascular disease, and visceral vascular disease) and (4) other cardiovascular disease. Specific details on definitions in each categories and methods of data collection of cardiac outcomes in the Rotterdam Study can be found elsewhere [26]. With respect to all-cause mortality, information was obtained on a weekly basis from the central registry of the municipality in Rotterdam and through general practitioners working in the study area.

Statistical Analysis

Normally distributed continuous variables were expressed as mean ± standard deviation (SD). Continuous variables that were not normally distributed were log-transformed for the analysis and are expressed as a median with interquartile range. Age- and sex-adjusted logistic regression and chi-square tests were used to compare baseline characteristics of MetS and non-MetS participants. Cox proportional hazards models corrected for age, sex and ethnicity served to analyze the associated hazard ratio of MetS and incident type 2 diabetes mellitus, CHD, stroke, cardiovascular- and all-cause mortality. All models were initially adjusted for age, sex and ethnicity. Ethnicity did not have a significant effect in any of the models and was therefore left out. To investigate whether the metabolic syndrome as a syndrome captures more of the risk for clinical endpoints than the individual components, we subsequently corrected the hazard ratios of MetS for each individual component. We imputed missing values by using the multiple imputation method, which has been proven to be a reliable method [22]. Participants with prevalent or unknown disease status were excluded from analyses on type 2 diabetes mellitus, CHD and stroke. Participants with prevalent or unknown stroke and/or CHD status were excluded from the analyses on cardiovascular mortality. For the analysis on incident diabetes we performed sensitivity analyses in which we excluded participants with impaired fasting glucose levels (fasting glucose ≥ 5.6 mmol/l). All analyses were adjusted for age, sex and ethnicity. All analyses were performed with SPSS version 21.0 (SPSS, Chicago, IL, USA) and a 2-sided α smaller than 0.05 was used to claim statistical significance.

RESULTS

Baseline characteristics

Baseline characteristics are shown in table 1. The overall mean age at baseline was 62.7 years. Participants were more often female (57.6% vs. 42.4%). Between definitions, the mean age of the participants having MetS ranged from 64.2 years (AHA/NHLBI) to 62.1 years (EGIR). From our study population, 97.8% were of Caucasian descent. Other baseline characteristics are being displayed in table 1.

Table 1: Baseline characteristics of population diagnosed with MetS according to different definitions.

Characteristic	AHA/NHLBI	IDF	EGIR	Total population
Participants having MetS (n,%)	3055 (35.3)	3646 (42.2)	1680 (19.4)	8643
Age, y	64.2 (58.8-72.5)*	64.0 (58.8-72.6)*	62.1 (57.1-70.8)	62.7 (57.6-71.2)
Female sex (n, %)	1790 (58.6)	2090 (57.3)	919 (54.7)*	4983 (57.7)
Body Mass Index (kg/m^2)	29.3 ± 4.1*	28.9 ± 3.9*	30.3 ± 4.3*	27.0 ± 4.1
Waist-circumference, cm	100.0 ± 10.8*	98.9 ± 10.5*	102.0 ± 11.2*	92.8 ± 11.8
Systolic Blood pressure, mmHg	145.6 ± 19.0*	145.3 ± 19.1*	144.1 ± 18.9*	138.3 ± 20.8
Diastolic Blood pressure, mmHg	81.4 ± 11.4*	81.3 ± 11.4*	82.6 ± 11.7*	78.8 ± 11.4
Total cholesterol, mmol/L	5.8 ± 1.1*	5.8 ± 1.1*	5.7 ± 1.1	5.8 ± 1.0
HDL cholesterol, mmol/L	1.2 (1.0-1.4)*	1.2 (1.0-1.4)*	1.2 (1.0-1.4)*	1.4 (1.1-1.7)
Triglycerides, mmol/L	1.8 (1.4-2.3)*	1.7 (1.3-2.2)*	1.8 (1.3-2.4)*	1.3 (1.0-1.8)
Insulin, pmol/L	92 (67-129)*	88 (64-123)*	131 (111-166)*	69 (49-97)
Glucose, mg/L	5.8 (5.4-6.1)*	5.7 (4.5-6.1)*	5.8 (5.4-6.2)*	5.4 (1.1-1.7)
CRP, mg/mL	2.2 (1.0-4.4)*	2.0 (0.9-4.1)*	2.2 (1.0-4.6)*	1.5 (0.6-3.3)
Hypertension treatment (n, %)	1045 (34.2)*	1197 (32.8)*	92 (41.2)*	1873 (21.7()
Antidiabetic treatment (n, %)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lipid treatment (n, %)	563 (18.4)*	630 (17.3)*	353 (21.0)*	1255 (14.5)
Current smoking (n, %)	287 (9.4)	329 (9.0)*	114 (6.8)*	813 (9.4)
Caucasian descent (n, %)	2732 (98.2)	3255 (98.1)	1482 (96.9)	7655 (97.8)
Prevalent type 2 diabetes (n, %)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0%)
Prevalent CHD (n, %)	218 (7.1)*	249 (6.8)*	121 (7.2)*	518 (6.0)
Prevalent Stroke (n, %)	95 (3.1)*	107 (3.0)*	54 (3.2)*	196 (2.3)

Continuous data are mean ± SD when normally distributed. Otherwise median with interquartile range. AHA/NHLBI, American heart association / national heart, lung, and blood institute; IDF, International Diabetes Federation; EGIR, European Group for the study of Insulin Resistance; MetS, metabolic syndrome; HDL, High-Density Lipoprotein; CRP, C-reactive Protein; CHD, coronary heart disease. *, Significant difference between MetS and non-MetS after correction for age and sex (P<0.05).

Prevalence of MetS

At baseline, a total of 4,118 participants (47.6%) were diagnosed with MetS according to either definition. The concordance of diagnoses using AHA/NHLBI, IDF and EGIR-definitions is displayed in Figure 1. Thirty-five percent had a diagnosis according to AHA/NHLBI, 42.2% according to IDF, and 19.4% according to EGIR (table 2).

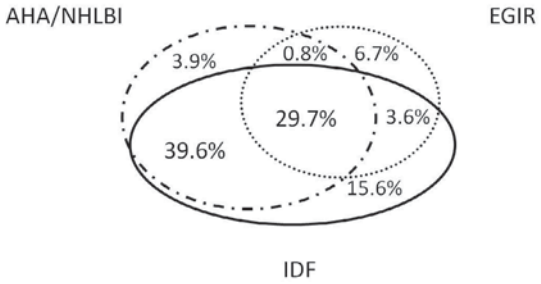

Figure 1. Concordance and disparity in diagnosis of metabolic syndrome according to different definitions. AHA/NHLBI, American heart association / national heart, lung, and blood institute; IDF, International Diabetes Federation; EGIR, European Group for the study of Insulin Resistance

Prevalence of components and triads of MetS

Table 2 shows the prevalence of components and triads in each definition of MetS.

A combination of hyperglycemia, high blood pressure and central obesity was the most frequent triad within a diagnosis of MetS according to AHA/NHLBI and IDF. In the EGIR-definition, high blood pressure and central obesity together with hyperinsulinemia were most frequently prevalent in MetS-diagnosed participants.

Risk of incident type 2 diabetes mellitus

During a median follow-up of 6.8 years 768 individuals developed type 2 diabetes mellitus. MetS was significantly associated with the risk of type 2 diabetes mellitus regardless of the definition chosen (table 3) in Cox proportional hazards models. Ethnicity did not have a significant effect and was therefore left out of the model. The cox proportional hazard ratio (HR) was 3.78 (95%CI 3.24-4.41) for AHA/NHLBI-definition, 3.53 (95%CI 3.01-4.14) for IDF definition, and 3.13 (95%CI 2.69-3.64) for EGIR-definition. The risk of type 2 diabetes mellitus was highly variable dependent on the composition of diagnosis (supplemental table S2). In MetS according to AHA/NHLBI, a combination of GLYC-HDL-WC (HR 6.75; 95%CI 5.53-8.25) was associated with the highest risk of type 2 diabetes mellitus. In MetS according to IDF, a combination of COB-HDL-GLYC (HR 6.07; 95%CI 5.01-7.35) was associated with the highest risk of type 2 diabetes mellitus. For EGIR-diagnosis, the highest risk of type 2 diabetes mellitus was associated with a combination of INS-DYSL-GLYC (HR 7.35; 95%CI 5.92-9.13). After correction for sex, age and individual components none of the

Table 2: Prevalence of components / triads at RS-I-3 within diagnosis of MetS

AHA/NHLBI	IDF	EGIR
Metabolic Syndrome	Metabolic Syndrome	Metabolic Syndrome
3055 (35.3%)	3646 (42.2%)	1680 (19.4%)
Components within diagnosis	**Components within diagnosis**	**Components within diagnosis**
BP 2810 (92.0%)	COB 3646 (100%)	INS 1680 (100%)
WC 2319 (75.9%)	BP 3302 (90.6%)	WC 1627 (96.8%)
GLYC 2140 (70.0%)	GLYC 2489 (68.3%)	BP 1347 (80.2%)
HDL 1688 (55.3%)	HDL 1750 (48.0%)	DYSL 864 (51.4%)
TRIG 1849 (60.5%)	TRIG 1896 (52.0%)	GLYC 577 (34.3%)
Triads within diagnosis	**Triads within diagnosis**	**Triads within diagnosis**
GLYC-BP-WC 1469 (48.1%)	COB-GLYC-BP 2265 (62.1%)	INS-WC-BP 1302 (77.5%)
BP-TRIG-WC 1075 (35.2%)	COB-HDL-BP 1474 (40.4%)	INS-WC-DYSL 821 (48.9%)
GLYC-BP-TRIG 1004 (32.9%)	COB-TRIG-BP 1620 (44.4%)	INS-BP-DYSL 611 (36.4%)
BP-HDL-WC 984 (32.2%)	COB-TRIG-HDL 1074 (29.5%)	INS-WC-GLYC 549 (32.7%)
BP-TRIG-HDL 970 (31.8%)	COB-TRIG-GLYC 1067 (29.3%)	INS-BP-GLYC 431 (25.7%)
GLYC-BP-HDL 838 (27.4%)	COB-HDL-GLYC 926 (25.4%)	INS-DYSL-GLYC 278 (16.5%)
GLYC-TRIG-WC 728 (23.8%)		
TRIG-HDL-WC 710 (23.2%)		
GLYC-HDL-WC 627 (20.5%)		
GLYC-TRIG-HDL 633 (20.7%)		

AHA/NHLBI, American heart association / national heart, lung, and blood institute; IDF, International Diabetes Federation; EGIR,
European Group for the study of Insulin Resistance. GLYC, hyperglycemia; BP, hypertension; TRIG, hypertriglyceridemia; HDL, low HDL-cholesterol;
WC, increased waist circumference; COB, central obesity; DYSL dyslipidemia; INS, highest quartile of fasting Insulin not having type 2 diabetes;

Table 3: Metabolic syndrome and hazard ratios for incident clinical endpoints.

Outcome	Events in population	AHA/NHLBI	IDF	EGIR
Type 2 diabetes mellitus	765/8567	3.78 (3.24-4.41)*	3.53 (3.01-4.14)*	3.13 (2.69-3.64)*
Coronary heart disease	544/7864	1.32 (1.11-1.56)*	1.38 (1.16-1.63)*	1.08 (0.87-1.35)
Stroke	458/8304	1.29 (1.07-1.56)*	1.32 (1.10-1.59)*	0.98 (0.77-1.25)
Cardiovascular mortality	418/7724	1.21 (0.99-1.47)	1.29 (1.05-1.57)*	0.95 (0.73-1.23)
All-cause mortality	2244/8586	1.10 (1.01-1.20)*	1.09 (1.01-1.19)*	1.05 (0.94-1.17)

Data are presented as hazard ratios with 95% confidence intervals. All analysis corrected for age and sex. * = statistically significant. AHA/NHLBI, American heart association / national heart, lung, and blood institute; IDF, International Diabetes Federation; EGIR, European group for the study of Insulin Resistance.

MetS-definitions itself was significantly associated with the risk of type 2 diabetes mellitus (table 4). The results were similar in a sensitivity analysis in which all participants with impaired fasting glucose levels (fasting glucose ≥ 5.6 mmol/l) were excluded from the cox regression modelling (supplementary table S7).

Risk of incident CHD

During a median follow-up of 7.2 years in which 544 individuals developed CHD, MetS as defined by AHA/NHLBI (HR 1.32; 95%CI 1.11-1.56) and IDF (HR 1.38; 95%CI 1.16-1.63 P<0.001) were significantly associated with the risk of CHD (table 3). In our population, the EGIR definition was not associated with the risk of incident CHD. In MetS according to AHA/NHLBI, a combination of BP-TRIG-WC (HR 1.77; 95%CI 1.41-2.23) was associated with the highest risk of CHD (supplemental table S3). In MetS according to IDF, a combination of COB-TRIG-BP (HR 1.76; 95%CI 1.44-2.15) was associated with the highest risk of CHD. For

Table 4: MetS according to different diagnosis and hazard ratios for incident clinical endpoints corrected for individual components.

	AHA/NHLBI	IDF	EGIR
Type 2 diabetes mellitus	MetS 1.19 (0.90-1.58) GLYC 4.01 (3.30-4.87)* HDL 1.48 (1.24-1.76)* WC 1.48 (1.23-1.78)* BP 1.28 (1.04-1.58)* TRIG 1.24 (1.04-1.49)*	MetS 1.11 (0.82-1.49) GLYC 4.20 (3.45-5.12)* HDL 1.52 (1.29-1.80)* BP 1.34 (1.08-1.65)* COB 1.33 (0.99-1.78) TRIG 1.32 (1.12-1.55)*	MetS 0.91 (0.56-1.49) GLYC 5.12 (4.38-5.98)* DYSL 1.64 (1.40-1.92)* WC 1.33 (1.08-1.64)* INSUL 1.54 (0.98-2.44) BP 1.39 (1.18-1.64)*
Coronary Heart Disease	MetS 0.73 (0.53-1.01) BP 1.67 (1.31-2.14)* TRIG 1.48 (1.18-1.85)* WC 1.38 (1.11-1.71)* HDL 1.34 (1.07-1.68)* GLYC 0.94 (0.77-1.15)	MetS 1.18 (0.86-1.58) BP 1.53 (1.20-1.95)* TRIG 1.29 (1.04-1.59)* HDL 1.17 (0.95-1.44) COB 0.99 (0.76-1.28) GLYC 0.82 (0.67-1.01)	MetS 1.00 (0.55-1.81) BP 1.50 (1.23-1.82)* DYSL 1.36 (1.12-1.67)* WC 1.10 (0.90-1.35) GLYC 0.93 (0.74-1.18) INSUL 0.87 (0.50-1.52)
Stroke	MetS 1.09 (0.76-1.58) BP 1.44 (1.10-1.89)* HDL 1.36 (1.06-1.75)* WC 1.06 (0.83-1.34) GLYC 0.98 (0.78-1.22) TRIG 0.84 (0.65-1.10)	MetS 1.12 (0.80-1.56) BP 1.42 (1.08-1.86)* HDL 1.35 (1.07-1.71)* COB 1.11 (0.83-1.48) GLYC 0.96 (0.76-1.21) TRIG 0.84 (0.66-1.08)	MetS 0.91 (0.46-1.77) BP 1.44 (1.16-1.79)* GLYC 1.22 (0.96-1.56) WC 1.20 (0.95-1.52) DYSL 1.11 (0.88-1.41) INSUL 0.87 (0.47-1.61)
Cardiovascular mortality	MetS 0.86 (0.58-1.27) BP 1.47 (1.10-1.97)* TRIG 1.24 (0.94-1.62) WC 1.21 (0.94-1.55) HDL 1.13 (0.86-1.48) GLYC 1.00 (0.79-1.26)	MetS 1.06 (0.74-1.54) BP 1.39 (1.04-1.86)* COB 1.21 (0.89-1.63) TRIG 1.14 (0.89-1.47) HDL 1.05 (0.81-1.35) GLYC 0.92 (0.71-1.18)	MetS 0.79 (0.40-1.57) BP 1.35 (1.07-1.69)* WC 1.30 (1.01-1.67)* GLYC 1.14 (0.88-1.47) DYSL 1 .07 (0.84-1.37) INS 1.00 (0.54-1.86)
All-cause mortality	MetS 0.97 (0.82-1.14) HDL 1.25 (1.11-1.41)* BP 1.09 (0.97-1.22) WC 1.02 (0.92-1.13) GLYC 1.00 (0.90-1.10) TRIG 0.97 (0.86-1.10)	MetS 0.98 (0.85-1.14) HDL 1.24 (1.11-1.39)* BP 1.08 (0.97-1.22) COB 1.05 (0.93-1.18) GLYC 0.99 (0.89-1.10) TRIG 0.96 (0.86-1.08)	MetS 0.81 (0.62-1.05) INSUL 1.18 (0.93-1.50) DYSL 1.15 (1.03-1.27)* GLYC 1.11 (0.99-1.24) BP 1.10 (1.00-1.21)* WC 1.04 (0.94-1.15)

Data are presented as hazard ratios with 95% confidence intervals. All analysis corrected for age and sex. * = statistically significant. AHA/NHLBI, American heart association / national heart, lung, and blood institute; IDF, International Diabetes Federation; EGIR, European group for the study of Insulin Resistance; GLYC, hyperglycemia; BP, hypertension; TRIG, hypertriglyceridemia; HDL, low HDL-cholesterol; WC, increased waist circumference; COB, central obesity; DYSL dyslipidemia; INS, highest quartile of fasting Insulin not having type 2 diabetes.

EGIR-diagnosis, the highest risk of CHD was associated with a combination of INS-BP-DYSL (HR 1.26; 95%CI 0.29-1.72). After correction for age, sex and individual components none of the MetS-definitions were significantly associated with CHD (table 4).

Risk of incident stroke

During a median follow-up of 7.7 years in which 458 participants suffered from incident stroke, MetS according to AHA/NHLBI (HR 1.29; 95%CI 1.07-1.56) and IDF (HR 1.32; 95%CI 1.10-1.59) showed a significantly increased risk of stroke (table 3a). No association of the EGIR definition and incident stroke was found. In MetS according to AHA/NHLBI, a combination of GLYC-HDL-WC (HR 1.75; 95%CI 1.31-2.34) was associated with the highest risk of stroke (supplemental table S4). In MetS according to IDF, a combination of COB-HDL-GLYC (HR 1.62; 95%CI 1.26-2.10) was associated with the highest risk of stroke. For EGIR-diagnosis, the highest risk of stroke was associated with a combination of INS-BP-DYSL (HR 1.02; 95%CI 0.70-1.49). After correction for age, sex and individual components none of the MetS-definitions were significantly associated with stroke (table 4).

Risk of cardiovascular mortality

During a median follow-up of 8.2 years in which 418 cardiovascular mortalities occurred, only the IDF-diagnosis was associated with significantly increased risk of cardiovascular mortality (HR 1.29; 95%CI 1.05-1.57; P=0.01) (table 3). Within each definition, a large variability in hazard ratios for cardiovascular mortality was found (supplemental table S5). In MetS according to AHA/NHLBI, a combination of BP-TRIG-WC (HR 1.48 (95%CI 1.13-1.94)) was associated with the highest risk of cardiovascular mortality. In MetS according to IDF, a combination of COB-TRIG-BP (HR 1.45 (95%CI 1.13-1.85)) was associated with the highest risk of cardiovascular mortality. Neither the EGIR diagnosis nor its triads were significantly associated with cardiovascular mortality. After adjustments for age, sex and individual components none of the MetS definitions were significantly associated with cardiovascular mortality (table 4).

Risk of all-cause mortality

During a median follow-up of 8.7 years in which 2,244 participants deceased, MetS according to AHA/NHLBI (HR 1.10 (1.01-1.20) P =0.03) and IDF (HR 1.09 (95%CI 1.01-1.19) P = 0.03) were associated with all-cause mortality. There was variability within definition as displayed by their triads (supplemental table S6). In MetS according to AHA/NHLBI, a combination of TRIG-HDL-WC (HR 1.24 (95%CI 1.07-1.45)) was associated with the highest risk of all-cause mortality. In MetS according to IDF, a combination of COB-HDL-GLYC (HR 1.18 (95%CI 1.04-1.34)) was associated with the highest risk of all-cause mortality. After adjustments for age, sex and individual components none of the diagnoses showed a significantly increased risk of all-cause mortality (table 4).

DISCUSSION

In our large predominantly elderly prospective population-based study, we show there is large variability between and within the definitions of MetS with respect to prevalence- and risk estimates for important cardiovascular and metabolic clinical endpoints. In addition, we confirm that MetS does not have an additional value in the risk estimation of type 2 diabetes mellitus, CHD, stroke and mortality on top of its individual components.

MetS is a highly prevalent condition in our Dutch population. This is in line with previous reports on MetS in middle-aged and elderly populations in the United States and Europe that reported equal or higher prevalence estimates [29-31]. We diagnosed the MetS according to the definitions of AHA/NHLBI, IDF and EGIR. The IDF-definition diagnosed the largest proportion of our population with MetS, followed by AHA/NHLBI and EGIR respectively, which is similar to previous studies [5, 32, 33]. This can be explained by the lower IDF cut-off points for waist circumference and BMI, resulting in more individuals that meet the central obesity-criterium. The EGIR-diagnosis selects an upper quartile of fasting insulin and excludes prevalent diabetes, resulting in a lower prevalence compared to the other definitions.

In our population, MetS is a strong risk factor for type 2 diabetes mellitus regardless of the definition chosen. This has already been found by several study groups in predominantly middle-aged populations of various ethnicities [12, 15, 34, 35]. Sattar et al. also confirmed this association in elderly, predominantly male subjects and subjects at risk for cardiovascular disease [19]. However, these studies were partly based on self-reported data and the associations were mostly the result of the hyperglycemic component rather than the diagnosis of MetS itself. Our findings are in line with this study, since the association of MetS with type 2 diabetes mellitus disappears after correcting for its components of which the hyperglycemic component constitutes the largest hazard. Our study, being population-based and with larger and meticulous follow-up, therefore adds to the evidence provided by previous studies that MetS does not confer additional risk of type 2 diabetes mellitus above the sum of its components, especially fasting glucose [15,19].

MetS is a known risk factor for CVD in middle aged and elderly populations [13, 14, 19, 36]. We found a relatively weak association of MetS with CVD in concordance with previous associations reported in literature [19]. Our study adds to previous studies including a large meta-analysis [14] that show that MetS does not show additive value to the risk associated with the sum of its individual components [1, 2, 4, 5, 36]. Previous studies did find an independent associative role of MetS [37] and higher hazard ratios for MetS and incident cardiovascular events [38]. However, these studies were done in small numbers

of patients at younger age having essential hypertension [37] or being suspected of having coronary artery disease [38]. Therefore, those results may not be similar to our study, which is a population-based study with predominantly elderly participants. For stroke in particular, Kotani et al. found MetS to have a positive association with stroke in women in a retrospective cohort [39]. We found MetS to be associated with stroke in the general population, but the association disappeared after correcting for the individual MetS components.

Although earlier studies on middle-aged younger individuals suggested otherwise [11, 13, 14, 40], we did not find any significant associations of MetS with all-cause mortality after correction for its individual components in any of the definitions. This could very well be an effect of the relatively higher age of our population making study subjects equally prone to decease due to causes other than cardiometabolic disease, thereby reducing the relative effect of MetS. Our findings on all-cause mortality are in line with results obtained from patients after coronary artery bypass grafting (CABG) in which survival of MetS patients without diabetes resembled their matched background population [41].

Remarkably dyslipidemia and blood pressure were the main contributing factors for cardiovascular disease and cardiovascular- and all-cause mortality effects of MetS. Although these are known as important independent risk factors for coronary heart disease and atherosclerosis [42-47], this finding adds to the evidence that these individual components important predictors in CVD [19].

The strengths of this study are the large sample size, population-based design and the long-term follow-up. Furthermore, data extraction has been done in a systematic way.

Despite the fact that we have executed this study with great care, we have to address some limitations of our study. Participants included in the Rotterdam Study were mainly European Caucasians (97.8%). Therefore our results may not apply to other ethnic groups. Considering the dynamic changes in European demographic, our results should be interpreted accordingly. Unfortunately a small proportion (1.7%) of our population had missing data for the definition of MetS. We addressed this by applying a reliable multiple imputation method.

In this study, we approach the MetS as a predictive tool to identify patients at high risk for cardiometabolic endpoints. However as Tenenbaum and Fisman emphasized [48], MetS is still an interesting biological feature of coexistence of components. Research directed at the underlying mechanisms of their coexistence could lead to important biological in-

sights in underlying cardiometabolic disease pathophysiology. These studies are beyond the scope of our current epidemiological approach for prediction purposes.

In conclusion, MetS shows high variability in its association with clinical endpoints both within and between diagnoses according to different definitions. Also, in a relatively old population MetS did not have additional predictive value on top of its components for any of the cardiometabolic endpoints. Besides as a manner of easy identification of risk patients, MetS does not seem to add any predictive value for clinical practice.

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PART III

Type 2 diabetes mellitus

Lifetime risk and disease course

Chapter 4

Lifetime risk of developing impaired glucose metabolism and eventual progression from prediabetes to type 2 diabetes: a prospective cohort study.

Chapter 5

Lifetime risk to progress from prediabetes to type 2 diabetes among women and men: a comparison between American Diabetes Association and World Health Organization diagnostic criteria

Chapter 4

Lifetime risk of progression from prediabetes to type 2 diabetes: a prospective cohort study

Thijs T.W. van Herpt
Symen Ligthart
Maarten J.G. Leening
Maryam Kavousi
Albert Hofman
Bruno H.Ch. Stricker
Mandy van Hoek
Eric J.G. Sijbrands
Oscar H. Franco
Abbas Dehghan

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ABSTRACT

Introduction: Data on lifetime risk of the full spectrum of impaired glucose metabolism including prediabetes and the risk to eventually progress to diabetes or start insulin therapy are scarce.

Methods: We used data from 10050 participants from the prospective population-based Rotterdam Study. Events were diagnosed by use of general practitioners records, hospital discharge letters, pharmacy dispensing data and serum fasting glucose measurements at the study center visits. Normoglycemia, prediabetes and diabetes were defined according to the WHO criteria for fasting glucose (normoglycemia: ≤6.0 mmol/L; prediabetes: >6.0mmol/L and <7.0mmol/L; diabetes ≥7.0 mmol/L or use of glucose lowering therapy). Lifetime risks were calculated using a modified version of survival analysis adjusted for the competing risk of death. In addition, we estimated the lifetime risk of progression from prediabetes to overt diabetes and from diabetes free of insulin therapy to insulin use. Further, we calculated years lived with healthy glucose metabolism.

Results: During a follow-up of up to 14.7 years, 1148 participants developed prediabetes, 828 diabetes and 237 started insulin therapy. At the age of 45, the remaining lifetime risk (95%CI) was 48.7% (46.2%-51.3%) for prediabetes, 31.3% (29.3%-33.3%) for diabetes and 9.1% (7.8%-10.3%) for insulin use. The lifetime risk to progress from prediabetes at the age of 45 to diabetes was 74.0% (67.6%-80.5%), and 49.1% (38.2%-60.0%) of the individuals with overt diabetes at the age of 45 started insulin therapy. The lifetime risks attenuated with advancing age but increased with increasing body mass index and waist circumference. On average, individuals with severe obesity lived 10 fewer years without glucose impairment compared to normal-weight individuals.

Conclusion: Our results highlight the public health burden posed by glycemic disturbances and demand further investigation into earlier and more effective prevention strategies.

INTRODUCTION

People with elevated blood glucose levels below the threshold of diabetes, a state referred to as prediabetes, have an excess risk of diabetes.[1-3] Today, more than 382 million people live with diabetes worldwide and due to the increasing prevalence of prediabetes and the rapid conversion of prediabetes to type 2 diabetes, the number is predicted to exceed half a billion by 2035.[4] Moreover, many diabetes patients are unable to achieve glycemic control goals through diet or oral medications only and ultimately require insulin treatment.[5-7] Estimates on the progression from prediabetes to diabetes and ultimately insulin therapy are scarce and have been limited to merely annual incidences and absolute risks within a restricted time period.[2]

Lifetime risks provide estimation of the cumulative risk of developing a disease during an individual's remaining lifespan and comprise thus a clear message to patients, clinicians and policy makers.[8-10] A few reports have simulated the lifetime risk of type 2 diabetes in the US and Australia.[10-12] However, estimates using accurate and careful documentation of elevated blood glucose levels, diabetes diagnosis and diabetes drug use are lacking. Prospective population-based cohort studies with long-term follow-up and detailed data on the full spectrum of impaired glucose metabolism including prediabetes, diabetes and the eventual need for insulin therapy, permit estimation of the burden of elevated blood glucose levels in the context of overall survival.

Hence, we used mortality rates and incidences of the disease during every year of life taking into account the competing risk of death to assess the lifetime risks of prediabetes, diabetes and insulin use in a large prospective population-based cohort study of individuals aged 45 years and older. Additionally, we estimated the lifetime risk of individuals with prediabetes to eventually develop diabetes and for diabetes patients to ultimately use insulin.

METHODS

Study design and population

This study is embedded within the framework of the Rotterdam Study, a prospective cohort study among the community-dwelling population aged 45 years and older in the city of Rotterdam, the Netherlands. The study design of the Rotterdam Study has been described in detail previously.[13] Briefly, in 1990 all inhabitants of a well-defined district of Rotterdam were invited, of whom 7983 agreed to participate (78.1%). The study was extended in 2000 with a second cohort of individuals who had reached the age of 55 or moved into the study area after 1990 (n=3011). In 2006, a third cohort was enrolled includ-

ing inhabitants aged 45 years and older (n=3932), bringing the total study size to 14926 individuals. There were no eligibility criteria to enter the Rotterdam study cohorts except the minimum age and residential area based on ZIP codes. We used the third center visit (1997–1999, n=4216) of the first cohort and the first visit of the second and third cohorts as baseline (2000–2001 and 2006–2008, respectively) for the current analysis. To ascertain the absence of prediabetes or diabetes by means of serum glucose measurement and use of blood glucose lowering medication, we excluded 1369 individuals without a valid baseline glucose measurement. Next, for the calculation of the lifetime risk of prediabetes, we only included individuals that were normoglycemic at study baseline (n=7462). To calculate the lifetime risk of diabetes, we only included individuals that were free of diabetes at study baseline (n=8844). Further, to calculate the lifetime risk of insulin use, we only included individuals that were free of insulin use at study baseline (n=9887). Selection of the individuals for the analyses can be found in Figure 1. The individuals with prediabetes (n=1382) were used to study progression from prediabetes to diabetes and individuals with diabetes without insulin treatment (n=1043) were used to study the progression from diabetes to insulin use.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physicians.

Ascertainment of prediabetes and type 2 diabetes

The participants were followed from the date of baseline center visit onwards. At baseline and during follow-up, cases of prediabetes and type 2 diabetes were ascertained through active follow-up using general practitioners' records (including laboratory glucose measurements), hospital discharge letters and serum glucose measurements from Rotterdam Study visits which take place approximately every four years.[14] Diabetes, prediabetes and normoglycemia were defined according to the recent WHO guidelines.[15] Normoglycemia was defined as a fasting blood glucose level ≤6.0 mmol/L; prediabetes was defined as a fasting blood glucose >6.0 mmol/L and <7.0 mmol/L or a non-fasting blood glucose >7.7 mmol/L and <11.1 mmol/L (when fasting samples were unavailable); type 2 diabetes was defined as a fasting blood glucose ≥7.0 mmol/L, a non-fasting blood glucose ≥11.1 mmol/L (when fasting samples were unavailable), or the use of blood glucose lowering medication. Information regarding the use of blood glucose lowering medication was derived from both structured home interviews and linkage to pharmacy dispensing records.[14] At baseline, more than 95% of the Rotterdam Study population was covered by the pharmacies in the study area. All potential events of prediabetes and type 2 diabetes were

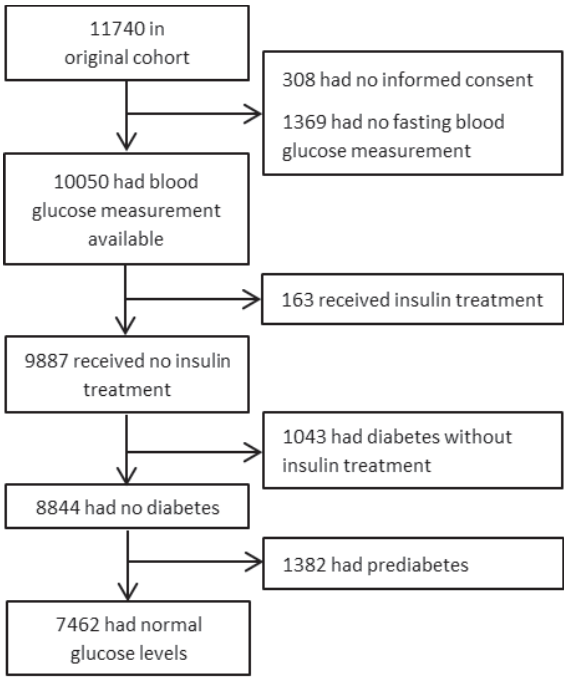

Figure 1. Participants Selection.
Participants without insulin treatment were used for the lifetime risk of insulin dependency, participants without diabetes for the lifetime risk of diabetes and participants with normal glucose levels for the lifetime risk of prediabetes. Progression from prediabetes to diabetes was assessed in the individuals with prediabetes and the progression from diabetes to insulin dependency in the individuals with diabetes without use of insulin treatment.

independently adjudicated by two study physicians. In case of disagreement, consensus was sought with an endocrinologist. Follow-up data was complete until January 1st 2012.

Statistical analysis

Baseline characteristics were compared between normoglycemic individuals, individuals with prediabetes and individuals with type 2 diabetes using linear regression models, Kruskal-Wallis tests for continuous data, and χ^2 tests for categorical data.

Remaining lifetime risks at different ages were calculated for prediabetes, diabetes and insulin use. We used a modified version of survival analysis to take the competing event of death into account for the calculation of the absolute lifetime risk (see appendix page 3 for statistical details). Lifetime risk estimates were calculated at index ages 45, 55, 65, 75, and 85 years for men and women combined and separately. The lifetime risk estimates reflect the remaining risk at the index age to the age of last observation (107 years in our study). In addition, to compare lifetime risks at the index ages with absolute risks in a shorter time period, we also calculated 10-year risks for prediabetes, diabetes and insulin use at all index ages.

Next, we calculated the lifetime risk of diabetes only in individuals with prediabetes in order to obtain an estimate of the lifetime risk to progress from prediabetes to diabetes.

Similarly, we calculated the lifetime risk of insulin use in individuals with diabetes free of insulin therapy to study what percentage of individuals with diabetes will eventually start insulin therapy.

In order to analyze the effect of anthropometric measures on the lifetime risk of prediabetes, diabetes and insulin use, we computed lifetime risks at the age of 45 stratified by BMI and waist circumference. The individuals were stratified into four categories of BMI (<25 kg/m^2, 25-30 kg/m^2, 30-35 kg/m^2, and >35 kg/m^2) and three categories of waist circumference based on the WHO classification scheme (for men: <94 cm, 94-101 cm and ≥102 cm; for women: <80 cm, 80-87 cm and ≥88 cm).[16]

To study the delay in onset of prediabetes, diabetes and insulin use we examined the difference in mean disease-free survival among BMI and waist circumference strata. As censoring precludes estimation of the mean survival time, we used Irwin's restricted mean survival to calculate the mean disease-free survival and overall mean survival.[17] Irwin's restricted mean survival is the mean of the survival time up to a point in time and mathematically is the area under the survival curve up to the selected point in time. As data from individuals aged older than 100 was limited, we set the restriction time point to 100 years of age.

All data were analyzed using the IBM SPSS Statistics version 21.0.0.1 (IBM Corp, Somers, NY, USA) and R version 2.1 with the 'etm' and 'survival' libraries.[18,19]

RESULTS

Baseline population characteristics

The mean (SD) age of the population was 65.2 (9.8) and women made up the majority of the study population (56.5%). Of the 10050 participants at baseline, 7462 (74.2%) had normoglycemia, 1382 (13.8%) had prediabetes, and 1206 (12.0%) had diabetes (Table 1). Prevalences of prediabetes and type 2 diabetes increased with advancing age in both men and women and were higher in men compared to women (appendix page 7). Individuals with prediabetes and diabetes had higher BMI and unfavorable lipid profile compared to normal glycemic individuals. Furthermore, people with diabetes had a higher prevalence of stroke, coronary heart disease and were more often smokers compared to normoglycemic individuals.

Lifetime risk of prediabetes, diabetes and insulin use

During 56230 person-years of follow-up in normoglycemic individuals, 1148 individuals developed prediabetes and 1343 died (incidence rate per 1000 person-years (IR): 20.4 (95%CI 19.3 to 21.6); mortality rate per 1000 person-years (MR): 23.9, 95%CI 22.7 to 25.2). We observed 828 cases of diabetes during 69639 person-years of follow-up in non-diabetic individuals and 1709 deaths (IR: 11.9, 95%CI 11.1 to 12.7; MR: 24.5, 95%CI 23.4 to 25.7). Among non-insulin users, 237 incident cases of insulin use were observed during 80832 person-years of follow-up (IR: 3.4, 95%CI 3.0 to 3.9) and 2183 individuals died (MR: 27.0, 95%CI 25.9 to 28.1). The remaining lifetime risk for a 45-year old individual to develop prediabetes was 48.7% (95%CI 46.2 to 51.3), whereas the lifetime risks of diabetes and insulin use were 31.3% (95%CI 29.3 to 33.3) and 9.1% (95%CI 7.8 to 10.3), respectively (Table 2). The cumulative incidences function subsequent to 45 years of age, are depicted in Figure

Table 1: Baseline characteristics of participants by prevalent glycemic state.

Characteristics	Normal glucose (n = 7462)	Prediabetes (n = 1382)	Diabetes (n = 1206)	P-value
Women (n, %)	4411 (59.1)	692 (51.0)	582 (47.7)	<0.001
Age (y)	64.4 ± 9.8	66.6 ± 9.4	67.5 ± 9.6	<0.001
Waist circumference (cm)	90 ± 11	96 ± 12	101 ± 12	<0.001
Body mass index (kg/m^2)	26.3 ± 3.8	27.9 ± 4.2	29.4 ± 4.8	<0.001
Total cholesterol (mmol/L)	5.7 ± 1.0	5.8 ± 1.0	5.4 ± 1.1	<0.001
HDL cholesterol (mmol/L)	1.4 (1.2-1.7)	1.3 (1.1-1.5)	1.2 (1.0-1.4)	<0.001
Triglycerides (mmol/L)†	1.3 (1.0-1.7)	1.5 (1.1-2.1)	1.7 (1.2-2.3)	<0.001
non HDL cholesterol (mmol/L)	4.3 ± 1.0	4.4 ± 1.0	4.2 ± 1.1	<0.001
LDL cholesterol (mmol/L)	3.7 ± 0.9	3.6 ± 0.9	3.4 ± 0.9	<0.001
Insulin (pmol/L)†	66 (47-93)	93 (64-133)	110 (73-177)	<0.001
Glucose (mmol/L)†	5.3 (5.0-5.6)	6.3 (6.1-6.5)	7.7 (7.0-9.5)	<0.001
eGFR (mL/min/1.73 m^2)	81 ± 17	80 ± 18	83 ± 22	<0.001
C-reactive protein (mg/L)	1.4 (0.6-3.1)	2.0 (0.9-4.2)	2.5 (1.1-4.9)	<0.001
Systolic blood pressure (mmHg)	137 ± 21	145 ± 21	147 ± 22	<0.001
Diastolic blood pressure (mmHg)	78 ± 11	81 ± 12	79 ± 12	<0.001
Hypertension (n, %)	3448 (46.7)	873 (64.0)	875 (73.3)	<0.001
History of stroke (n, %)	178 (2.4)	35 (2.5)	77 (6.4)	<0.001
History of CHD (n, %)	422 (5.8)	110 (8.1)	164 (13.8)	<0.001
Use of blood pressure lowering drugs (n, %)	1408 (19.6)	437 (32.8)	474 (40.5)	<0.001
Use of lipid lowering agents (n, %)	1043 (14.4)	239 (17.8)	1178 (27.4)	<0.001
Current smoking (n, %)	707 (9.5)	144 (10.4)	147 (12.3)	0.001
Former smoking (n, %)	2769 (37.4)	553 (40.2)	480 (40.1)	0.001

Values are mean ± standard deviation or median (interquartile range) for characteristics with skewed distributions. eGFR denotes estimated glomerular filtration rate, HDL high-density-lipoprotein, and CHD coronary heart disease. †Only fasting samples.

Table 2: Remaining lifetime and 10-year risks of prediabetes, diabetes and insulin use.

Age, years		N	Lifetime risk prediabetes (95%CI)	N	Lifetime risk diabetes (95%CI)	N	Lifetime risk insulin use (95%CI)
45	Lifetime	7462	48.7% (46.2-51.3)	8844	31.3% (29.3-33.3)	9887	9.1% (7.8-10.3)
	10-year	1233	8.4% (5.4-11.4)	1344	3.4% (2.1-4.8)	1400	0.5% (0.0-1.0)
55	Lifetime	6939	44.5% (42.5-46.6)	8291	29.2% (2..4-31.0)	9329	8.8% (7.6-10.0)
	10-year	3788	13.2% (11.4-15.0)	4456	7.0% (5.8-8.3)	4914	2.2% (1.4-3.0)
65	Lifetime	5109	37.6% (35.6-39.5)	6257	24.8% (2..1-26.5)	7179	7.0% (6.0-7.9)
	10-year	3901	19.3% (17.7-20.9)	4754	11.2% (10.0-12.3)	5414	3.0% (2.4-3.6)
75	Lifetime	3073	25.8% (23.7-28.0)	3850	17.4% (15.7-19.1)	4547	4.7% (3.8-5.6)
	10-year	2885	19.1% (17.3-20.9)	3618	12.2% (10.9-13.6)	4273	2.6% (2.0-3.2)
85	Lifetime	1072	13.1% (10.4-15.7)	1405	8.9% (6.9-10.9)	1725	3.3% (2.2-4.4)
	10-year	1060	11.9% (9.5-14.4)	1390	8.2% (6.4-10.0)	1707	3.3% (2.2-4.4)

The lifetime risk of prediabetes is for individuals with normal glucose levels at the index age, lifetime risk of diabetes for individuals without diabetes at the index age and the lifetime risk of insulin use for individuals free of insulin use at the index age.

1. Lifetime risks of prediabetes, diabetes and insulin use subsequent to increasing ages attenuated. Compared to the lifetime risks, the 10-year risks of prediabetes, diabetes and insulin use were lower at all index ages. The remaining lifetime risks did not differ by gender irrespective of age (appendix page 8-9). With adjustment for the competing risk of death, the lifetime risks were lower as compared to the unadjusted risk derived from Kaplan-Meier estimates (appendix page 4).

Progression to diabetes and insulin use

In 1382 individuals with prediabetes we observed 425 incident cases of diabetes, whilst 257 died without diabetes (IR: 43.0, 95%CI 39.2 to 47.2; MR: 26.0, 95%CI 23.0 to 29.3). The lifetime risk for individuals that experience prediabetes at 45 years of age to progress to diabetes was 74.0% (95%CI 67.6 to 80.5). Further, among 1043 individuals with diabetes, we observed 183 incident cases of insulin use, whilst 302 died without ever using insulin treatment (IR: 24.0, 95%CI 20.8 to 27.7; MR: 39.7, 95%CI 35.5 to 44.3). The lifetime risk for individuals with diabetes at the age of 45 to start insulin therapy was 49.1% (95%CI 38.2 to 60.0).

Stratification by BMI and waist circumference

Stratification by BMI revealed that people with normal weight at the age of 45 have a significantly lower prediabetes lifetime risk compared to overweight and obese individuals (Table 3). Stratification by waist circumference revealed similar effects on the lifetime risks of prediabetes. In accordance with the lifetime risks for prediabetes, lifetime risks for diabetes and insulin use were higher with increasing BMI and waist circumference. The cumulative incidences by BMI strata as a function of age, for 45 year olds, are depicted in Figure 2. When we stratified individuals within the BMI strata by waist circumference

Table 3: Lifetime risk at the age of 45 for prediabetes, diabetes and insulin use by body mass index and waist circumference strata.

BMI (kg/m²)	N	Lifetime risk prediabetes (95%CI)	P-value	N	Lifetime risk diabetes (95%CI)	P-value	N	Lifetime risk Insulin use (95%CI)	P-value
< 25	2686	36.9% (33.1-40.6)		2955	18.8% (15.8-21.7)		3124	4.6% (3.0-6.2)	
25-30	3452	52.1% (48.0-56.2)	<0.001	4132	33.1% (30.2-36.0)	<0.001	4598	9.6% (7.7-11.5)	<0.001
30-35	1013	60.2% (54.1-66.2)	0.02	1324	43.9% (38.6-49.2)	<0.001	1613	14.2% (10.5-17.9)	0.01
> 35	240	71.3% (60.5-82.0)	0.04	352	56.6% (46.7-66.6)	0.01	462	17.1% (9.9-24.2)	0.24
Waist circumference									
Small	2127	37.5% (33.2-41.7)		2329	19.5% (16.0-23.1)		2449	4.5% (2.8-6.3)	
Medium	2220	46.6% (41.1-52.2)	0.005	2589	25.1% (21.6-28.6)	0.01	2815	6.5% (4.4-8.5)	0.08
Large	2801	57.8% (54.2-61.5)	<0.001	3553	41.8% (38.5-45.0)	<0.001	4205	12.6% (10.5-14.8)	<0.001

*Waist circumference categories small, medium and large represent the WHO classification scheme (for men: <94 cm, 94-102 cm and ≥102; for women: <80 cm, 80-88 cm and ≥88). BMI denotes body mass index. P-values are for the comparison with the lower BMI or waist category.
The lifetime risks are subsequent to the age of 45.

Table 4: Lifetime Risk to Develop Diabetes in Individuals with Prediabetes at the Age of 45 and the Lifetime Risk to Use Insulin in Individuals aged 45 with Diabetes but Free of Insulin Use, Stratified by Body Mass Index and Waist Circumference.

BMI (kg/m²)	N	Prediabetes to diabetes (95%CI)	P-value	N	Non-insulin dependent diabetes to insulin use (95%CI)	P-value
< 25.0	269	35.9% (18.4-53.4)		169	58.6% (34.2-83.1)	
25 – 30	680	76.3% (68.9-83.7)	<0.0001	466	57.5% (45.2-69.9)	0.47
30 – 35	311	87.7% (79.4-96.0)	0.02	289	25.0% (0.0-60.1)	0.04
> 35	112	80.9% (65.9-95.9)	0.22	110	46.4% (28.6-64.1)	0.14
Waist circumference						
Small	202	49.8% (28.6-70.9)		120	43.0% (24.4-61.7)	
Medium	369	70.0% (56.4-83.7)	0.06	226	55.9% (38.6-73.2)	0.16
Large	752	78.8% (70.4-87.1)	0.14	652	45.0% (29.0-61.0)	0.18

*Waist circumference categories small, medium and large represent the WHO classification scheme (for men: <94 cm, 94-102 cm and ≥102; for women: <80 cm, 80-88 cm and ≥88). P-values are for the comparison with the lower BMI or waist category.

categories, we observed an increasing risk of diabetes with increasing waist, except in the lowest BMI category (appendix page 10).

The lifetime risk to progress from prediabetes to diabetes was also substantially lower for individuals with a normal weight compared to overweight and obese individuals. However, the risk to start insulin therapy in individuals with diabetes did not differ substantially between strata of BMI (Table 4).

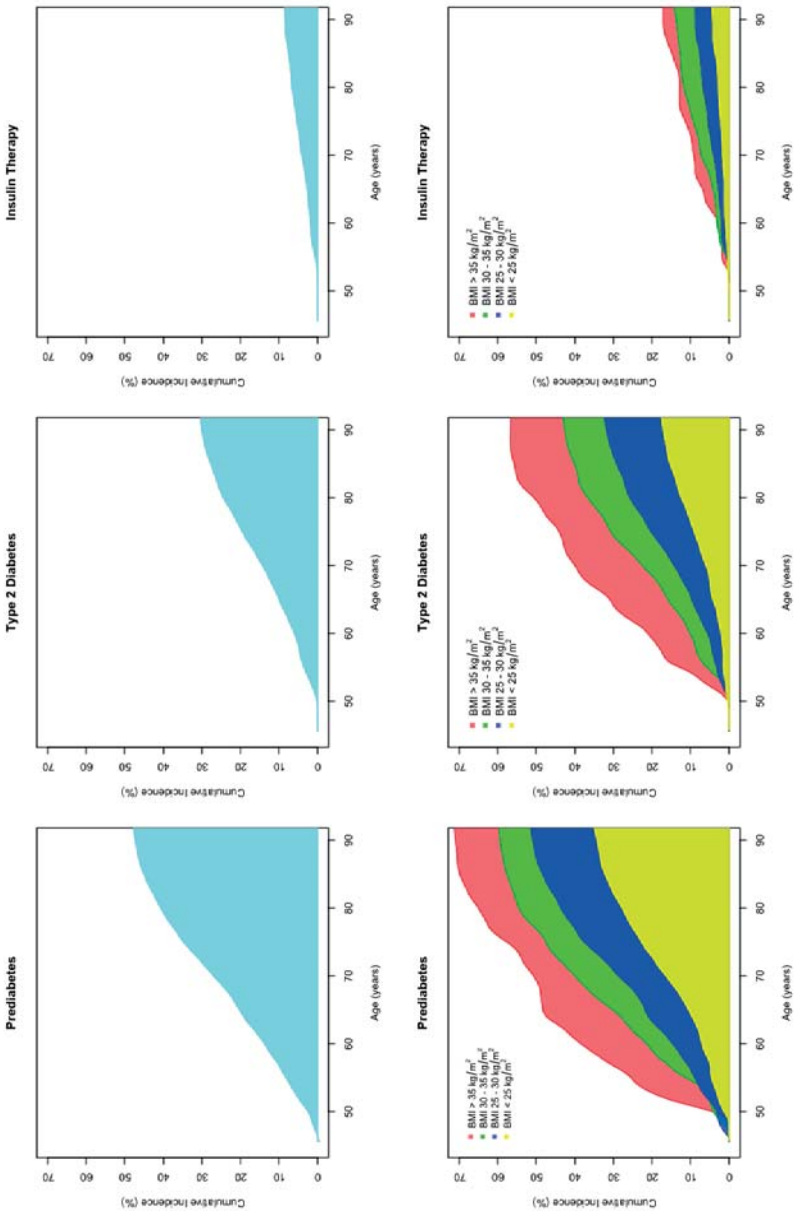

Figure 2. Lifetime risk of prediabetes, type 2 diabetes and insulin therapy among individuals at 45 years of age, adjusted for the competing risk of death. The upper panel depicts the cumulative incidence of prediabetes (fasting glucose >6.0 mmol/L), type 2 diabetes (fasting glucose ≥7.0 mmol/L or use of glucose lowering medication) and insulin use among all individuals at 45 years of age, adjusted for the competing risk of death. The lower panel depicts the cumulative incidences of prediabetes, type 2 diabetes and insulin therapy among individuals at 45 years of age, according to body mass index and adjusted for the competing risk of death.

Diabetes-free survival

Remaining life years free of prediabetes, diabetes and insulin use from the age of 45 by sex, BMI and waist circumference strata are depicted in appendix page 5-6. Overall, years lived with normal glucose metabolism diminished with increasing levels of obesity. Also, individuals with higher BMI experienced more years lived with diabetes. For example, the average age of onset of prediabetes in men with normal weight was more than 10 years later compared to men with a BMI >35 kg/m^2. On average, insulin therapy is only used in a short time period at the end of life.

DISCUSSION

The lifetime risk of prediabetes for an individual aged 45 is one in two, and one in three individuals aged 45 will develop diabetes. The vast majority of individuals that have prediabetes at age 45 will eventually progress to diabetes and one in two diabetes patients aged 45 will start insulin therapy. Furthermore, obesity substantially affects the risk to progress from prediabetes to diabetes and compresses the years lived with normal glucose metabolism.

Individuals with prediabetes have an increased risk of diabetes, cardiovascular disease, cancer and mortality.[20-22] Despite the high prevalence of prediabetes, estimates of what proportion of the population will eventually present with prediabetes have not been previously published. Evidence regarding the preventive effects of both lifestyle and pharmacological interventions on the progression of prediabetes to diabetes increases.[23-25] Lifetime risk estimates may indicate the proportion of individuals for whom early intervention would be applicable. We observed that half of our population will sooner or later present with prediabetes and may qualify for potential interventions during their lifespan.

In contrast to diabetes, prediabetes is a more fluctuating health state. The lifetime risk estimates of prediabetes in the current study should be interpreted as ever experiencing a serum glucose in the prediabetes range. However, individuals diagnosed with prediabetes could return to normoglycaemia. In the Diabetes Prevention Program (DPP), 19% of the placebo group returned to normoglycemia within 10 year.[26] In the pioglitazone for diabetes prevention study, 28% returned to normoglycemia in the placebo arm during a median follow-up of 2.4 years. These estimates are based on a limited time period and longer follow-up may result in different estimates. Our lifetime estimates show that 3 in 4 individuals with a glucose level in the prediabetes range at the age of 45 progress to diabetes. These estimates provide a better long-term perspective of individuals who ever meet prediabetes criteria, irrespective whether an individual remains prediabetes or

returns to normoglycemia in the following years. This is in agreement with the American Diabetes Association expert panel suggesting that 70% of the individuals with prediabetes progress to diabetes.[2] The higher prevalence of obesity in the US raises the concern of even higher progression rates compared to the estimates from our European population.

A previous report simulated the lifetime risk of diabetes in a US population based on questionnaire data for the adjudication of diabetes which does not comprise undiagnosed diabetes.[11] As the prevalence of undiagnosed diabetes is more than 25%,[27] the risk estimates in the US study are likely to be underestimated. Furthermore, an Australian study estimated the lifetime risk of diabetes using two cross-sectional examinations (diabetes defined based on fasting plasma glucose (≥7.0 mmol/L) and 2hr plasma glucose (≥11.1 mmol/L)) with a short time interval (5 years) in a population with a large number of dropouts (39%) and without active follow.[12] Instead, we used active follow-up data and fasting glucose measurements as an objective and comprehensive assessment of diabetes diagnosis enabling us to provide accurate estimates of the entire spectrum of impaired glucose metabolism.

We observed a substantial impact of obesity on the remaining lifetime risk of prediabetes, diabetes and insulin use, which is in line with a previous report.[28] Also, obesity increased the risk to progress from prediabetes to diabetes. This is in agreement with the observation in the placebo group of the DPP in which obese individuals had a higher risk to progress to diabetes (9 vs 14 cases/100 person-years).[23] Furthermore, obesity compressed the survival with normal glucose metabolism and influenced the time lived within each glycemic state underscoring the importance of weight management.

We estimate that one in two patients with diabetes eventually start insulin treatment. Together, the lifetime risk of diabetes and insulin use show the burden of pharmacological treatment of diabetes in our Western population. Despite the consensus statements from the ADA and the European Association for the Study of Diabetes in 2006[29] and 2009,[30] physicians consider a variety of non-standardized factors to initiate glucose lowering treatment.[31] Therefore, our lifetime risks of insulin use may not reflect country and population-specific prescription behaviors. Furthermore, recent developments in diabetes care include the initiation of insulin therapy for beta-cell preservation, which has not been common practice in the calendar time period of our study. The lifetime risk of 9.1% in non-insulin users at the age of 45 may therefore be an underestimation of the risk of insulin use in current diabetes clinical care.

The strength of our study is the comprehensive assessment of incident diabetes diagnosis through use of blood glucose lowering treatment using medical records from hospitals

and general practitioners, standardized blood glucose measurements at the repeated study center visits, and electronic linkage with the pharmacy dispensing records in the study area. Also, we used data from a prospective population-based cohort study with long-term follow-up and adjusted the lifetime risks for the competing risk of death to avoid overestimation. We need to address some limitations. First, we calculated remaining lifetime risks at the age of 45 because we did not have data for individuals younger than 45. Nevertheless, the cumulative incidence of type 2 diabetes before the age of 45 is low.[10,11] Also, for estimating the lifetime risk in BMI and waist circumference strata, we used anthropometric data at older ages than 45 as not all individuals entered the study at age 45. This could have led to the misclassification of individuals across the different categories as BMI and waist circumference could have changed with age. Third, we used data from an completely unselected sample of the general Dutch population with high participation rates (72.0%).[13] However, all studies requiring active participation are to some extent subject to the "healthy volunteer effect" and this generally leads to slight underestimations of absolute risk estimates at short term follow-up. However, this underestimation attenuates at long-term follow-up.[32] Last, the vast majority of the Rotterdam Study participants are white (97%) and we therefore present lifetime risks for individuals from European ancestry.

Half of the general population will sooner or later develop prediabetes defined as fasting glucose >6.0 mmol/L. Up to three in four of those with prediabetes aged 45 will progress to diabetes and one in two diabetics aged 45 eventually starts insulin therapy. These lifetime risks demonstrate the burden of impaired glucose metabolism on our society and demand earlier and more effective prevention strategies.

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SUPPLEMENTAL MATERIAL

Statistical analysis lifetime risk

We used data from individuals at each age during follow-up that they attained free of the disease (i.e. prediabetes, diabetes or insulin therapy).[1,2] Individuals who reached to age j free of the disease at some point during follow-up constituted the population at risk for any age j (risk set, Rj). If an individual developed the disease, died or was censored at age j, he or she was removed from the risk set for age j+1 and older. If an individual entered the study at age j+1, he or she was added to the risk set for age j+1.[3] For the lifetime risk at 45, for instance, hazards (hj), age-specific incidences (fj), cumulative incidences (Fj), and survival probabilities (Sj) were calculated according to the standard Kaplan-Meier methods for each age j (assuming F44 = 0 and S44 = 1):

hj = ej / Rj (ej = # of events at age j)
f j = hj × Sj – 1
Fj = ji = 45fi
Sj = 1 – Fj

It should be noted that Fj is the cumulative incidence of the disease (prediabetes, diabetes or insulin therapy) which applies to individuals who survive through age j-1. This cumulative incidence does not take into account the competing risk of death from another cause. This means that individuals that decease count as withdrawals and are assumed to have the same risk of the disease compared to the individuals that are alive at censoring. However, individuals who die before age j have a zero future risk of the disease. This competing risk of death will result in overestimation of the lifetime risk.[4] Therefore, we used a separate survival function (Uj) with death included as an event alongside prediabetes, diabetes or insulin therapy to adjust for the competing risk of death. The adjusted incidence and true lifetime risk was calculated as follows:

fj* = hj × Uj – 1
Fj* = ji = 45fi*
Sj* = 1– Fj*

We used similar methods to calculate lifetime risks at the starting age 55, 65, 75 and 85. We set the FT-1 and UT-1 to 0 for every index age T and used the original hazard (hj) to calculate Uj for j T. The analysis methods for prediabetes, diabetes and insulin dependency were similar. Furthermore, we calculated the lifetime risks stratified by body mass index and waist circumference. Finally, we calculated the lifetime risk of diabetes conditional on the presence of prediabetes to study the progression from prediabetes to overt diabetes.

Similarly, we studied the lifetime risk of insulin therapy conditional on the presence of diabetes (without insulin therapy) to study the progression from insulin-free diabetes to diabetes with insulin therapy.

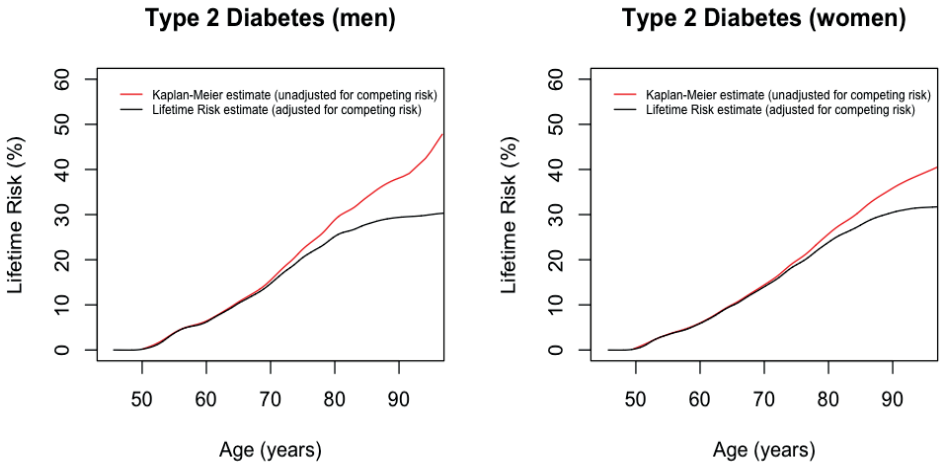

Figure S1. Kaplan-Meier Estimate Compared to Competing-Risk Estimate of Lifetime Risk of Type 2 Diabetes.

Comparison of the cumulative incidence of type 2 diabetes adjusted for the competing risk of death (Cumulative Incidence estimates) with the Kaplan-Meier estimate of the lifetime risk of type 2 diabetes (unadjusted for the competing risk of death) in men (n = 3741) and women (n = 5103) aged 45.

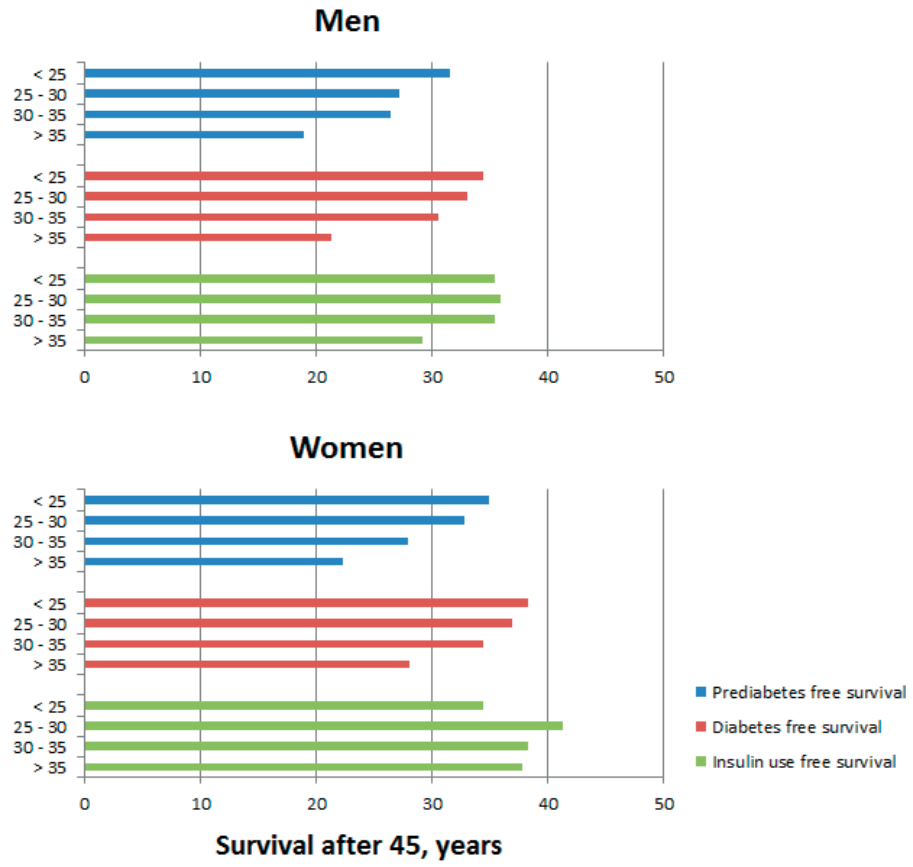

Figure S2. Survival free of prediabetes, diabetes and insulin use after the age of 45 by body mass index strata.

The blue bars represent the prediabetes-free survival in men and women with normal glucose levels at the age of 45. The red bars represents the diabetes-free survival in men and women without diabetes at the age of 45. Finally, the green bars represent the insulin-free survival in men and women that do not use insulin at the age of 45. All analyses are stratified by body mass index and adjusted for the competing risk of death.

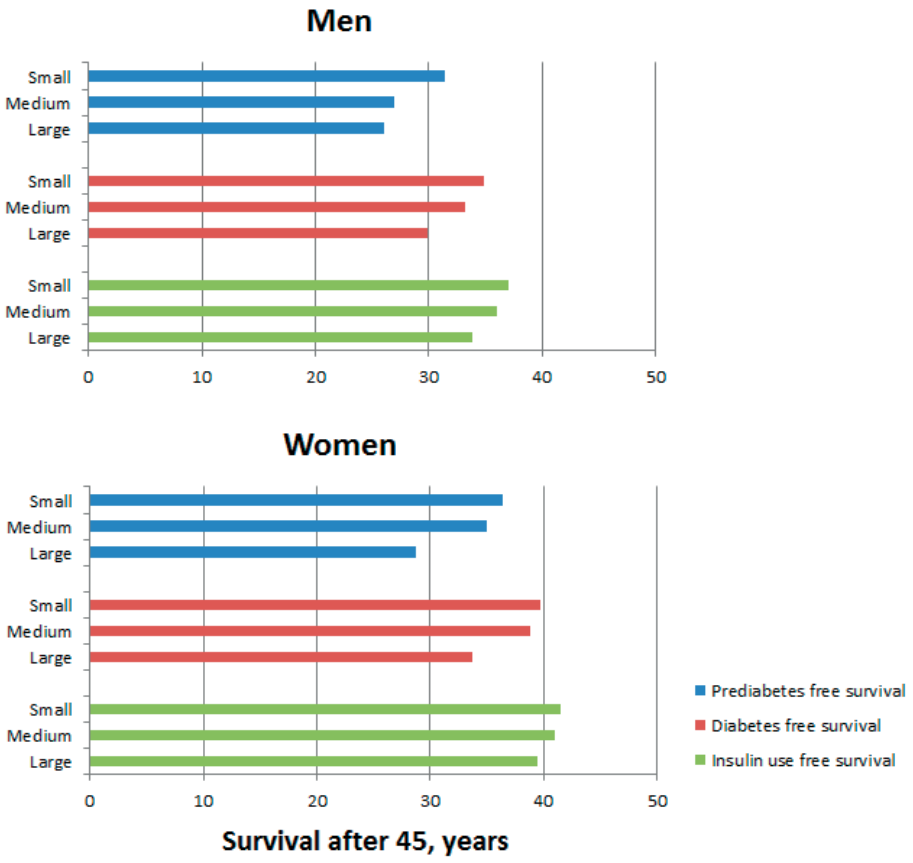

Figure S3. Survival free of prediabetes, diabetes and insulin use after the age of 45 by waist circumference strata.

The blue bars represent the prediabetes-free survival in men and women with normal glucose levels at the age of 45. The red bars represents the diabetes-free survival in men and women without diabetes at the age of 45. Finally, the green bars represent the insulin-free survival in men and women that do not use insulin at the age of 45. All analyses are stratified by waist circumference and adjusted for the competing risk of death.

Table S1: Prevalence of Prediabetes and Diabetes at Baseline by Sex and Age (n = 10050).

Age (years)	Men (n = 4368)		Women (n = 5682)	
	Total N (cases)	Prevalence (95%CI)	Total N (cases)	Prevalence (95%CI)
Prediabetes				
45-55	622 (58)	0.09 (0.07-0.12)	795 (43)	0.05 (0.04-0.07)
55-65	1824 (318)	0.17 (0.16-0.19)	2311 (279)	0.12 (0.11-0.13)
65-75	1243 (198)	0.16 (0.14-0.18)	1453 (191)	0.13 (0.11-0.15)
75-85	596 (105)	0.18 (0.15-0.21)	928 (146)	0.16 (0.13-0.18)
>85	83 (11)	0.13 (0.07-0.23)	195 (33)	0.17 (0.12-0.23)
Diabetes				
45-55	622 (43)	0.07 (0.05-0.10)	795 (40)	0.05 (0.04-0.07)
55-65	1824 (254)	0.14 (0.13-0.16)	2311 (206)	0.09 (0.08-0.10)
65-75	1243 (195)	0.16 (0.14-0.18)	1453 (169)	0.12 (0.10-0.13)
75-85	596 (118)	0.20 (0.17-0.23)	928 (135)	0.15 (0.12-0.17)
>85	83 (17)	0.20 (0.13-0.31)	195 (29)	0.15 (0.11-0.21)

CI denotes confidence interval.

Table S2: Remaining Lifetime Risk of Prediabetes, Type 2 Diabetes and Insulin Dependent Type 2 Diabetes in Men by Categories of Age, Body Mass Index, and Waist Circumference.

Age (years)	N	Lifetime risk prediabetes (95%CI)	N	Lifetime risk diabetes (95%CI)	N	Lifetime risk insulin use (95%CI)
45	3051	48.4% (44.1-52.6)	3741	30.5% (27.5-33.5)	4282	10.7% (8.7-12.7)
55	2835	42.6% (39.5-45.8)	3505	28.1% (25.4-30.7)	4045	10.3% (8.4-12.2)
65	2070	35.1% (32.2-38.1)	2619	24.1% (21.6-26.6)	3085	7.8% (6.3-9.3)
75	1228	24.5% (21.2-27.7)	1557	16.0% (13.4-18.6)	1887	5.3% (3.9-6.8)
85	346	12.9% (8.2-17.5)	463	8.3% (4.9-11.8)	584	4.0% (2.0-6.0)
BMI (kg/m^2)						
< 25	1047	37.5% (31.6-43.3)	1174	19.6% (15.3-23.9)	1273	6.9% (3.5-10.3)
25-30	1596	51.2% (43.6-58.8)	1979	31.5% (27.3-35.7)	2254	11.1% (8.3-13.8)
30-35	346	58.4% (49.1-67.8)	492	42.0% (33.7-50.3)	614	16.5% (10.3-22.7)
>35	41	72.4% (48.6-96.2)	69	59.7% (38.4-81.0)	110	16.8% (3.8-29.8)
Waist circumference						
Small	1129	40.2% (34.3-46.1)	1266	21.8% (17.0-26.6)	1357	5.0% (2.6-7.4)
Medium	1005	52.1% (41.6-62.6)	1237	29.0% (23.5-34.6)	1392	9.6% (6.2-12.9)
Large	797	53.4% (46.7-60.1)	1087	39.4% (33.8-44.9)	1359	15.3% (11.2-19.3)

*Waist circumference categories small, medium and large represent the WHO classification scheme (for men: <94 cm, 94-102 cm and ≥102 cm). BMI denotes body mass index. The lifetime risk of prediabetes is for individuals with normal glucose levels at the index age, lifetime risk of diabetes for individuals without diabetes at the index age and the lifetime risk of insulin use for individuals free of insulin use at the index age. For BMI and waist circumference, the lifetime risks are subsequent-the age of 45.

Table S3: Remaining Lifetime Risk of Prediabetes, Type 2 Diabetes and Insulin Dependent Type 2 Diabetes in Women by Categories of Age, Body Mass Index, and Waist Circumference.

Age (years)	N	Lifetime risk prediabetes (95%CI)	N	Lifetime risk diabetes (95%CI)	N	Lifetime risk insulin use (95%CI)
45	4411	49.1% (46.1-52.0)	5103	31.9% (29.3-34.6)	5605	7.8% (6.2-9.3)
55	4104	46.1% (43.4-48.8)	4786	30.1% (27.7-32.5)	5284	7.5% (6.1-9.0)
65	3039	39.4% (36.8-42.1)	3638	25.3% (23.1-27.6)	4094	6.3% (5.0-7.6)
75	1845	26.8% (24.0-29.6)	2293	18.5% (16.2-20.7)	2660	4.2% (3.0-5.3)
85	726	13.2% (10.0-16.4)	942	9.2% (6.8-11.6)	1141	2.9% (1.6-4.2)
BMI (kg/m^2)						
< 25	1639	36.6% (31.6-41.5)	1781	18.0% (14.0-22.0)	1851	3.2% (1.6-4.8)
25-30	1856	53.5% (49.3-57.7)	2153	34.8% (30.7-38.8)	2344	8.0% (5.4-10.7)
30-35	667	61.3% (42.8-69.8)	832	43.4% (36.7-50.1)	999	13.0% (8.3-17.6)
>35	199	71.0% (58.4-83.6)	283	55.9% (44.6-67.3)	352	16.1% (7.8-24.4)
Waist circumference						
Small	998	33.9% (27.8-39.9)	1063	16.0% (10.8-21.1)	1092	4.1% (1.3-7.0)
Medium	1215	42.4% (36.8-48.0)	1352	21.6% (17.1-26.1)	1423	2.9% (0.8-5.0)
Large	2004	59.7% (55.4-64.1)	2466	42.8% (38.8-46.8)	2846	11.4% (8.8-13.9)

*Waist circumference categories small, medium and large represent the WHO classification scheme (for women: <80 cm, 80-88 cm and ≥88 cm). BMI denotes body mass index. The lifetime risk of prediabetes is for individuals with normal glucose levels at the index age, lifetime risk of diabetes for individuals without diabetes at the index age and the lifetime risk of insulin use for individuals free of insulin use at the index age. For BMI and waist circumference, the lifetime risks are subsequent-the age of 45.

Table S4: Remaining Lifetime Risk of Diabetes at Age 45 by Body Mass Index and Waist Circumference.

Waist circumference	Body mass index (kg/m^2)			
	<25	25-30	30-35	>35
Small	18.4% (14.3-22.5)	22.8% (15.9-29.8)	N/A	N/A
Medium	18.5% (13.5-23.5)	28.5% (23.9-33.2)	27.6% (10.2-44.9)	N/A
Large	17.7% (10.5-24.9)	39.0% (34.6-43.4)	44.6% (39.0-50.1)	57.1% (46.8-67.3)

* Waist circumference categories small, medium and large represent the WHO classification scheme (for men: <94 cm, 94-102 cm and ≥102 cm; for women: <80 cm, 80-88 cm and ≥88 cm).

PART IV

Type 2 diabetes mellitus

Risk factors and complications

Chapter 6

Introduction of the DiaGene Study: clinical characteristics, pathophysiology and determinants of vascular complications of type 2 diabetes

Thijs T.W. van Herpt
Roosmarijn F.H. Lemmers
Mandy van Hoek
Janneke G. Langendonk
Ronald J. Erdtsieck
Bert Bravenboer
Annelies Lucas
Monique T. Mulder
Harm R Haak
Aloysius G. Lieverse
Eric J.G. Sijbrands

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ABSTRACT

Introduction: Type 2 diabetes is a major healthcare problem. Glucose-, lipid-, and blood pressure-lowering strategies decrease the risk of micro- and macrovascular complications. However, a substantial residual risk remains. To unravel the etiology of type 2 diabetes and its complications, large-scale, well-phenotyped studies with prospective follow-up are needed. This is the goal of the DiaGene study. In this manuscript, we describe the design and baseline characteristics of the study.

Methods: The DiaGene study is a multi-centre, prospective, extensively phenotyped type 2 diabetes cohort study with concurrent inclusion of diabetes-free individuals at baseline as controls in the city of Eindhoven, The Netherlands. We collected anthropometry, laboratory measurements, DNA material, and detailed information on medication usage, family history, lifestyle and past medical history. Furthermore, we assessed the prevalence and incidence of retinopathy, nephropathy, neuropathy, and diabetic feet in cases. Using logistic regression models, we analyzed the association of 11 well known genetic risk variants with type 2 diabetes in our study.

Results: In total, 1886 patients with type 2 diabetes and 854 controls were included. Cases had worse anthropometric and metabolic profiles than controls. Patients in outpatient clinics had higher prevalence of macrovascular (41.9% vs. 34.8%; P=0.002) and microvascular disease (63.8% vs. 20.7%) compared to patients from primary care. With the exception of the genetic variant in KCNJ11, all type 2 diabetes susceptibility variants had higher allele frequencies in subjects with type 2 diabetes than in controls.

Conclusions: In our study population, considerable rates of macrovascular and microvascular complications are present despite treatment. These prevalence rates are comparable to other type 2 diabetes populations. While planning genomics, we describe that 11 well-known type 2 diabetes genetic risk variants (in TCF7L2, PPARG-P12A, KCNJ11, FTO, IGF2BP2, DUSP9, CENTD2, THADA, HHEX, CDKAL1, KCNQ1) showed similar associations compared to literature. This study is well-suited for multiple omics analyses to further elucidate disease pathophysiology. Our overall goal is to increase the understanding of the underlying mechanisms of type 2 diabetes and its complications for developing new prediction, prevention, and treatment strategies.

BACKGROUND

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease characterized by overweight, insulin resistance and beta-cell dysfunction [1-3]. Because of ageing and the rising prevalence of obesity, the incidence and prevalence of T2DM are increasing [4-7]. T2DM accounts for a large proportion of present and future health care expenditure in Western societies [5, 7, 8]. People affected by T2DM have an increased risk of cardiovascular events [9-13], and a poor prognosis after these events [14, 15]. In addition, T2DM gives rise to microvascular complications such as retinopathy, nephropathy and neuropathy [16-19]. We have collected a new large cohort of individuals with and without T2DM with prospective follow-up in the Netherlands: the DiaGene study.

The care for T2DM in the Netherlands is organized in primary care by general practitioners and at hospital-based outpatient clinics by medical specialists. This systematic care is based on local and international treatment guidelines aiming to reduce morbidity and mortality through optimal treatment of hyperglycemia and associated metabolic complications, such as dyslipidemia, vascular dysfunction and high blood pressure [20, 21]. Treatment of these components has proven to reduce the risk of cardiovascular morbidity and mortality in T2DM [22-30]. However, a substantial residual risk remains. Improving knowledge on genetic, biochemical and environmental (lifestyle and anthropometric) determinants of T2DM and its micro– and macrovascular complications can have large implications for prevention, treatment and prognosis of T2DM [22, 23, 31]. Through high throughput sequencing, about 80 common genetic variants associated with T2DM have been discovered [31, 32]. These common variants only explain 5-10% of the overall predisposition of T2DM [33]. There clearly is a need to expand these analyses to additional populations.

In this paper, we present the DiaGene Study, a new, multicenter T2DM cohort study collected in the Netherlands in both primary and secondary care. The main purpose of the DiaGene Study is to study the analyses of genetic, biochemical and environmental determinants of T2DM and its complications. Here we describe the characteristics of our population, the prevalence of complications and future perspectives.

METHODS

Study design

The DiaGene-study is a multicenter cohort study that was coordinated by the vascular section of internal medicine of the Erasmus Medical Center and the Diabetes subunit

of the Máxima Medical Center, and collected in the city of Eindhoven, The Netherlands. Eindhoven is a medium-sized city with 170,668 adult (> 21 years) inhabitants in 2011. Both hospitals in Eindhoven participated in the DiaGene study: Catherina Hospital and Máxima Medical Center. In addition, the local Primary Care Diagnostic Centre participated. Hence, virtually all diabetes patients in Eindhoven were approached for inclusion through this population-based approach. Between 2006 and 2011, physicians at all three centers included a total of 2,065 patients with T2DM. Of these, 179 patients were excluded from analysis. Reasons for exclusion where: no diabetes (n=1), Type 1 diabetes (n=30), Maturity-Onset Diabetes of the Young (n=4), Latent auto-immune diabetes in adults (n=3), double inclusion (n=77), post-pancreatitis diabetes (n=3), refusal during study period (n=2) and missing written informed consent (n=59); resulting in a total of 1,886 patients in the study population.

The control group consisted of two groups: 1. subjects recruited via advertisement in local newspapers, and 2. subjects that where included through invitation of friends and self-reported unrelated family members of participating patients. Inclusion criteria for controls was age 55 years or older. Exclusion criteria were the presence of any kind of diabetes, use of metformin or Cushing's disease. Subjects who were approached had at least 7 days of decision-time to fully reflect on research goals and methods using physician-provided information, before giving their written informed consent. Eventually, 904 diabetes-free subjects participated as controls. Of these, 50 were excluded from all analyses based on missing written informed consent (n=14), double inclusion (n=17), and suspected or confirmed diagnosis of diabetes (n=19), resulting in a total of 854 controls included in the final population. This study was approved by the Medical Ethics Committees of the Erasmus MC, Catherina Hospital and Máxima Medical Center. Written informed consent was obtained from all participants.

Definition of T2DM

Information on the diagnosis of T2DM was retrieved from the patient's medical records. In accordance with American Diabetes Association – and World Health Organization – guidelines [34, 35], diabetes was defined as a fasting plasma glucose ≥ 7.0 mmol/L and/or a non-fasting plasma glucose level ≥ 11.1 mmol/l measured at least at 2 separate time points, treatment with oral glucose-lowering medication or insulin, and/or the diagnosis of T2DM as registered by a medical specialist. Persons with the diagnosis of type 1 diabetes (as derived from medical records and patient-questionnaires) or other types of diabetes mellitus were excluded from the study. Control subjects with fasting glucose ≥ 7.0 mmol/L or glycated hemoglobin (HbA1c) ≥ 47.5mmol/mol were excluded. Information on T2DM status was checked by two investigators. If they did not reach consensus, the participant's treating physician was consulted.

Medical and family history

Each participant filled out an extensive questionnaire on their medical history (history of diabetes, metabolic disease, vascular disease, medication use and intoxications) and ethnicity of their parents (Supplement 1). We classified a participant to be Caucasian if both parents were reported to be Caucasian. Furthermore, the participant's family history regarding diabetes and cardiovascular disease and medication usage was recorded through the questionnaire.

Sample collection

A 20cc Ethylene diamine tetra acetic (EDTA) fasting blood sample was taken from all participants. Samples were centrifuged (3000rpm; 1800G for 15 minutes at 4°C). Directly after centrifugation, the plasma and the buffy coat were separated and stored (at -80°C) for DNA analysis and future measurements.

Diabetes and complications of diabetes

Data on body mass index (BMI) (kg/m^2) and blood pressure (mmHg) were extracted from medical records at inclusion. Similarly, laboratory results were extracted around time of inclusion and contained fasting glucose, glycated hemoglobin (HbA1c), total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein-cholesterol (HDL-cholesterol), triglycerides, creatinin and urinary albumin/creatinine-ratio. The majority of measurements were collected within 6 months prior to or after the actual date of inclusion. To estimate kidney function, the estimated glomerular filtration rate was calculated with the Modification of Diet in Renal Disease-formula. Information on the presence of cardiovascular disease in the patients treated in the hospital-based outpatient clinics was retrieved from their medical records. Cardiovascular disease comprised myocardial infarction, percutaneous coronary intervention / coronary arterial bypass graft (PCI/CABG), cerebrovascular accident, transient ischemic attack and peripheral arterial disease. PCI/CABG was defined as any invasive intervention to treat coronary arterial disease (PCI, CABG). Peripheral arterial disease was defined as an ankle-brachial index below 0.80 or below 0.90 with typical complaints, any intervention to treat peripheral arterial disease (supervised exercise training, stenting, bypass and percutaneous transluminal angioplasty, or the self-reported presence of intermittent claudication. Information on cardiovascular disease in patients from primary care and diabetes-free controls was based on self-reporting.

Microvascular complications were subdivided into retinopathy, nephropathy and neuropathy. Diabetic foot was additionally assessed. Retinopathy was scored according to the report of an ophthalmologist as absent or present and classified as non-proliferative, proliferative, or retinopathy treated with photo coagulation or intra-vitreal injections.

Neuropathy was defined by a podotherapist, neurologist or the patients' treating physician. Nephropathy was defined present when micro-albuminuria (Albumin/creatinin-ratio (ACR)≥2.5 for men or ≥3.5 for women) was present at two of three consecutive measurements, or when high micro-albuminuria or macro-albuminuria was present at one measurement (ACR≥12.5 for men or ≥17.5 for women). Diabetic foot was established by a podotherapist or physician according to the SIMM's classification [36]. All information on laboratory data, macrovascular, and microvascular events in case and control subjects at baseline that was retrieved from medical records was separately checked by two investigators. When they did not reach consensus, the participant's physician was consulted.

Genotyping

DNA was isolated using the Invisorb® Blood Universal Kit from Stratec Molecular (Berlin, Germany). Eleven well-known T2DM genetic risk variants were genotyped: TCF7L2(rs7903146), PPARG-P12A(rs1801282) , DUSP9(rs594532), CENTD2(rs1552224), THADA(rs7578597), HHEX(rs1111875), CDKAL1(rs7754840) and KCNQ1(rs231362)which had previously been genotyped for replication in DIAGRAM [37], and KCNJ11(rs5219), IGF2BP2(rs4402960) and FTO(rs8050136). These risk variants were chosen because of their relatively large effect sizes on T2DM risk in previous studies [32, 37-42]. Genotyping was performed with TaqMan allelic discrimination assays, designed and optimized by Applied Biosystems (Foster City, CA, USA). Reactions were performed on the Taqman Prism 7900 HT platform.

Follow-up data

Currently, we are finalizing the first collection of prospective follow-up in our study population. This encompasses all anthropometric and laboratory measurements and data on metabolic, microvascular and macrovascular complications of T2DM and enables us to perform prospective analyses.

Statistical analysis

Continuous variables are expressed as median with interquartile range unless otherwise specified. Comparisons between groups were performed with Mann-Whitney U tests for continuous and X^2-tests for categorical data. Deviation from the Hardy-Weinberg equilibrium was assessed by X^2-testing. Associations of the genotypes with T2DM were tested using logistic regression models. We have calculated interaction effects of odds ratios for T2D to compare our results with previous genetic studies according to the method of Altman et al [43]. All models were adjusted for age and sex. Additionally, models were adjusted for center of inclusion as a categorical covariate. Cases and controls of non-Caucasian ethnicity were excluded from the genetic analyses. P-values smaller than 0.05 were considered to be statistically significant. Statistical analysis was performed with SPSS-software version 22.0 (SPSS, Chicago, IL, USA).

RESULTS

General characteristics

The most relevant general characteristics of the cohort are displayed in Table 1. A total of 1886 patients with T2DM and 854 diabetes-free controls were included. Of all anthropometric measurements, 90.6% and 96.1% were performed within 6 and 12 months of inclusion, respectively. Of laboratory data, 81.8% and 93.2% were measured within 6 and 12 months of inclusion, respectively. The cases and controls were of similar age. When compared to controls, cases had higher BMI (29.5 (Interquartile range (IQR) 26.4-32.7) vs. 25.5 (IQR 23.3-27.7) kg/m^2 ; P<0.001), higher HbA1c (50.8 (IQR 43.7-57.9) vs. 37.7 (IQR 36.1-39.3) mmol/mol; P<0.001), higher creatinin (78 (IQR 66-91) vs. 72 (IQR 63-81) umol/L; P<0.001), higher triglycerides (1.4 (IQR 0.9-1.9) vs 1.2 (IQR 0.9-1.5) mmol/L; P<0.001), lower HDL-cholesterol (1.1 (IQR 0.9-1.3) vs 1.4 (IQR 1.2-1.6) mmol/L; P<0.001) and lower LDL-cholesterol (2.4 (0.8) vs. 3.6 (0.9) mmol/L; P<0.001). A larger proportion of cases had reduced estimated glomerular filtration rate (19.7% vs. 4.7%, P<0.001) and prevalent macrovascular disease (38.0% vs 8.3%, P<0.001) compared to diabetes-free controls. More cases had a first-degree relative with T2DM compared to controls (64.4% vs 33.3%, P<0.001). More baseline characteristics can be found in Table 1.

Primary care versus hospital-based outpatient clinic

Table 2 shows baseline characteristics of patients with T2DM in primary care and hospital-based outpatient clinic. Patients with T2DM from the outpatient clinic had longer median duration of diabetes compared to primary care (12.5 (IQR 7.2-17.8) vs. 4.6 (IQR 1.2-7.9) years; P<0.001) while they were diagnosed at a younger age (50.8 (10.8) vs. 58.4 (11.3) years, P<0.001). At the outpatient clinic, participants had higher BMI (30.2 (IQR 26.8-33.7) vs. 29.0 (IQR 26.0-32.0) kg/m^2; P<0.001), HDL-cholesterol (1.2 (IQR 1.0-1.4) vs.1.1 (IQR 0.9-1.3); P<0.002), HbA1c (56.3 (IQR 48.1-64.5) vs. 48.6 (43.7-53.6) mmol/mol; P<0.001) and higher creatinin (81 (67-95) vs. 76 (64-88) umol/L; P<0.001). Total cholesterol (4.3 (0.9) vs 4.2 (0.9); P=0.04) and LDL-cholesterol (2.6 (0.8) vs. 2.3 (0.8); P<0.001) was higher in primary care patients. A larger proportion of patients with T2DM at the outpatient clinic had reduced estimated glomerular filtration rate (25.5% vs. 15.2%, P<0.001), macrovascular disease (41.9% vs. 34.8%; P=0.002) and microvascular disease (63.8% vs. 20.7%) compared to patients with T2DM from primary care. We could not retrieve reliable data on neuropathy nor diabetic foot in primary care population. More patients from the outpatient clinic had a first-degree relative with T2DM compared to controls (64.4% vs. 33.3%, P<0.001).

Genetics

Table 3 shows the associations of 11 well-established genetic T2DM variants in our study population. Hardy-Weinberg's equilibrium was met for all variants. With the exception

Table 1: General baseline characteristics of participants with and without T2DM

	Cases	Controls	p-value
Number of participants	1886	854	
Female sex, n (%)	874 (46.4)	511 (59.8)	<0.001
Age, yr, median (IQR)	65.7 (58.5-72.9)	64.9 (60.4-69.4)	0.72
Age of onset diabetes, yr, median (IQR)	55 (47-63)	N/A	N/A
Duration of diabetes, yr, median (IQR)	8.1 (2.8-13.5)	N/A	N/A
BMI, kg/m^2, median (IQR)	29.5 (26.4-32.7)	25.5 (23.3-27.7)	<0.001
HbA1c, mmol/mol, median (IQR)	50.8 (43.7-57.9)	37.7 (36.1-39.3)	<0.001
Diabetes treatment, % (n / n-available / n-missing)			
No glucose-lowering medication	19.2 (340 / 1772 / 114)	N/A	N/A
Oral glucose-lowering agent	64.3 (1140 /1773 / 113)	N/A	N/A
Insulin	32.3 (572 / 1772 / 114)	N/A	N/A
Systolic blood pressure, mmHg, median (IQR)	140 (129-151)	137 (124-150)	<0.001
Diastolic blood pressure, mmHg, median (IQR)	78 (71-85)	82 (76-89)	<0.001
Total Cholesterol, mmol/L, median (IQR)	4.2 (3.6-4.8)	5.6 (4.9-6.2)	<0.001
Triglycerides, mmol/L, median (IQR)	1.4 (0.9-2.0)	1.2 (0.9-1.5)	<0.001
HDL-cholesterol, mmol/L, median (IQR)	1.1 (0.9-1.3)	1.4 (1.2-1.6)	<0.001
LDL-cholesterol, mmol/L, median (IQR)	2.3 (1.8-2.8)	3.5 (2.9-4.1)	<0.001
Creatinin, µmol/L, median (IQR)	78 (66-91)	72 (63-81)	<0.001
eGFR < 60ml/min, % (n / n-available / n-missing)	21.2 (372 / 1756 / 130)	5.0 (40 / 795 / 59)	<0.001
Cardiovascular disease, % (n / n-available / n-missing)			
Any macrovascular disease	38.0 (660 / 1738 / 148)	8.3 (68 / 824 / 30)	<0.001
Ischemic heart disease	28.0 (497 / 1778 / 108)	4.9 (41 / 842 / 12)	<0.001
Ischemic brain disease	12.0 (211 / 1757 / 129)	1.4 (12 / 840 / 14)	<0.001
Peripheral arterial disease	10.8 (193 / 1783 / 103)	2.2 (18 / 823 / 31)	<0.001
Microvascular diabetes complications, % (n / n-available / n-missing)			
Any microvascular disease	34.3 (561 / 1637 / 249)	N/A	N/A
Diabetic retinopathy	17.3 (308 / 1778 / 108)	N/A	N/A
Diabetic nephropathy	23.0 (387 / 1684 / 202)	N/A	N/A
Family history, % (n / n-available / n-missing)			
First-degree relative with T2DM	64.4 (1104 / 1714 / 172)	33.3 (269 / 809 / 45)	<0.001
First-degree relative with CVD	68.3 (1086 / 1590 / 296)	68.7 (519 / 755 / 99)	0.87
Any relative with early-onset CVD	45.0 (780 / 1732 / 154)	41.5 (342 / 825 29)	0.09
Descent:			
Caucasian descent, % (n / n-available / n-missing)	91.9 (1613 / 1755)	96.1 (810 / 843 / 11)	<0.001
Age of death father, yr, median (IQR)	73 (65-82)	75 (67-84)	<0.001
Age of death mother, yr, median (IQR)	78 (70-86)	81 (73-89)	<0.001

Table 1 shows baseline characteristics of participants from the DiaGene Study. BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate calculated with the Modification of Diet in Renal Disease-formula; IQR, interquartile range; n-total, total number of participants for whom information was available; T2DM, type 2 diabetes mellitus; Yr, year.

Table 2: general baseline characteristics of participants in primary care and hospital-based outpatient clinic at inclusion.

	Primary Care	Outpatient clinic	p-value
Number of participants	1056	830	
Female sex, n (%)	494 (46.8)	380 (45.9)	0.71
Age, yr, median (IQR)	65.5 (58.1-72.9)	65.9 (58.9-72.9)	0.66
Age of onset diabetes, yr, median (IQR)	59 (51-67)	51 (44-59)	<0.001
Duration of diabetes, yr, median (IQR)	4.5 (1.2-7.9)	12.5 (7.2-17.8)	<0.001
BMI, kg/m^2, median (IQR)	29.0 (26.0-32.0)	30.2 (26.8-33.7)	<0.001
HbA1c, mmol/mol, median (IQR)	48.6 (43.7-53.6)	56.3 (48.1-64.5)	<0.001
Diabetes treatment, % (n / n-available / n-missing)			
No medication	24.7 (248 / 1004 / 52)	12.0 (92 / 768 / 62)	<0.001
Oral glucose-lowering agents	72.9 (732 / 1004 / 52)	53.1 (408 / 768 / 62)	<0.001
Insulin	8.6 (86 / 1004 / 52)	63.3 (486 / 768 / 62)	<0.001
Systolic blood pressure, mmHg, median (IQR)	146 (133-159)	134 (126-143)	<0.001
Diastolic blood pressure, mmHg, median (IQR)	79 (72-86)	75 (70-80)	<0.001
Total Cholesterol, mmol/L, median (IQR)	4.2 (3.6-4.9)	4.1 (3.6-4.6)	0.047
Triglycerides, mmol/L, median (IQR)	1.4 (0.9-1.9)	1.5 (1.0-2.0)	0.104
HDL-cholesterol, mmol/L, median (IQR)	1.1 (0.9-1.3)	1.2 (1.0-1.4)	0.002
LDL-cholesterol, mmol/L, median (IQR)	2.5 (2.0-3.1)	2.1 (1.7-2.6)	<0.001
Creatinin, µmol/L, median (IQR)	76 (64-88)	81 (67-95)	<0.001
eGFR < 60ml/min % (n / n available/ n-missing)	16.4 (160/978/78)	27.2 (212/778/52)	<0.001
Cardiovascular disease, % (n / n-available/ n-missing)			
Any macrovascular disease	34.8 (335 / 963 / 93)	41.9 (325 / 775 / 55)	0.002
Ischemic heart disease	25.2 (252 / 1000 / 56)	31.5 (245/ 778 / 52)	0.004
Ischemic brain disease	12.7 (124 / 973 / 83)	11.1 (87 / 784 / 46)	0.302
Peripheral arterial disease	9.2 (88 / 958 / 98)	12.7 (105 / 825 / 5)	0.018
Microvascular diabetes complications, % (n / n-available / n-missing)			
Any microvascular disease	20.7 (172 / 830 / 226)	48.2 (389 / 807 / 23)	<0.001
Diabetic retinopathy	6.1 (59 / 962 / 94)	30.5 (249 / 816 / 15)	<0.001
Diabetic nephropathy	15.4 (134 / 868 / 188)	31.0 (253 / 816 / 15)	<0.001
Neuropathy	Unknown	31.2 (238 / 762 / 68)	N/A
Family history, % (n / n-available / n-missing)			
First-degree relative with T2DM	61.4 (586 / 955 / 101)	68.2 (518 / 759 / 71)	0.003
First-degree relative with CVD	67.6 (608 / 899 / 157)	69.2 (478 / 691 / 139)	0.712
Any relative with early-onset CVD	45.0 (436 / 968 / 88)	45.0 (344 / 764 / 66)	1.0
Descent			
Caucasian descent, % (n / n-available / n-missing)	90.3 (892 / 988 / 132)	94.0 (721 / 767 / 63)	0.005
Age of death father, yr, median (IQR)	73 (65-82)	73 (65-82)	0.728
Age of death mother, yr, median (IQR)	79 (72-87)	77 (69-85)	0.055

Table 2 shows baseline characteristics of participants from the DiaGene Study in both primary care and hospital-based outpatient clinic. BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate calculated with the Modification of Diet in Renal Disease-formula; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; n-total, total number of participants for whom information was available; T2DM, type 2 diabetes mellitus; Yr, year.

Table 3: Allele frequencies, odds ratios and 95% confidence intervals of genetic variants and risk of T2D in DiaGene, original discovery studies and most recent meta-analysis of genome-wide-association studies.

		DiaGene		Original discovery study results			Morris et al. 2012 [52]
Locus- Marker	Risk allele /other	Risk allele frequency Case / control / total	OR (95%CI)	Ceu-Hap Map	OR (95%CI)	Reference original study	OR (95%CI)
CDKAL1 - rs7754840	C/G	0.36 / 0.31 / 0.35	**1.23 (1.06-1.44) ; p = 0.006**	0.31	1.12 (1.08-1.16)	[38,39,41,42]	1.15 (1.11-1.19)
CENTD2 - rs1552224	A/C	0.86 / 0.86 / 0.86	0.95 (0.77-1.16) ; p = 0.60	0.88	1.14 (1.11-1.17)	[37]	1.13 (1.08-1.19)
DUSP9 - rs5945326	G/A	0.22 / 0.22 / 0.22	1.02 (0.88-1.18); p = 0.77	0.12	1.27 (1.18-1.37)	[37]	N/A (on X-chromosome)
FTO - rs8050136	A/C	0.40 / 0.38 / 0.39	1.06 (0.91-1.22); p = 0.45	0.45	1.15 (1.09-1.22)	[41]	1.11 (1.07-1.15)
HHEX - rs1111875	C/T	0.64 / 0.63 / 0.63	1.01 (0.87-1.16) ; p =0.95	0.56	1.13 (1.08-1.17)	[38]	1.15 (1.11-1.18)
IGFBP2 - rs4402960	T/G	0.33 / 0.32 / 0.33	1.01 (0.87-1.18) ; p = 0.89	0.29	1.17 (1.10-1.25)	[41]	1.13 (1.09-1.17)
KCNJ11 - rs5219	T/C	0.37 / 0.39 / 0.37	0.92 (0.80-1.07) ; p = 0.29	0.50	1.15 (1.09-1.21)	[38]	1.08 (1.05-1.12)
KCNQ1 - rs231362	G/A	0.53 / 0.49 / 0.52	**1.16 (1.00-1.34) ; p = 0.04***	0.52	1.08 (1.06-1.10)	[40]	1.11 (1.07-1.16)*
PPARG-P12A - rs1801282	C/G	0.89 / 0.88 / 0.89	1.13 (0.91-1.42) ; p = 0.27	0.92	1.14 (1.08-1.20)	[38,39,42]	1.16 (1.11-1.22)
TCF7L2 rs7903146	T/C	0.36 / 0.28 / 0.34	**1.37 (1.17-1.60) ; p <0.001**	0.25	1.37 (1.28-1.47)	[41]	1.40 (1.35-1.46)
THADA rs7578597	T/C	0.91 / 0.88 / 0.90	**1.36 (1.08-1.71) ; P =0.01**	0.92	1.15 (1.10-1.20)	[41]	1.14 (1.08-1.22)

Table shows odds ratios of association with type 2 diabetes for different known risk alleles tested in our study population. Logistic regression analysis is age, sex and center of inclusion-adjusted. CEU, Caucasian; OR, odds-ratio; CI, confidence interval. * statistically significant difference of odds ratio in association of genetic variant with T2DM when compared to Morris et al. 2012 [52].

of the variant in KCNJ11, all T2DM susceptibility variants had higher allele frequencies in cases with T2DM than in controls. TCF7L2 showed the highest odds ratio for prevalent T2DM (OR 1.37 (95%CI 1.17, 1.60; P<0.001). These results were unaffected by additional correction for center of inclusion. After calculation of interaction effects**,** the associations of all genetic variants except for KCNJ11 did not significantly differ from the large scale meta-analyses of Morris et al. [44].

DISCUSSION

In this manuscript, we present the baseline characteristics and future perspectives of the DiaGene study, a new multi-centre cohort study with prospective follow-up on biochemical and genetic determinants of T2DM and its complications. We show that the population is representing both primary and secondary care and that despite treatment, considerable rates of macrovascular and microvascular complications are present. To further elucidate determinants of T2DM and its complications, multi-layer omics and prospective analyses will be of great value. Our study offers excellent opportunities to perform these analyses.

In the Netherlands, primary care practices are led by general practitioners, who are easily accessible and offer essential family medicine. Outpatient clinics of hospitals provide specialized care and require referral by the general practitioner for reimbursement by insurance companies. Therefore, complex and more severely affected patients will be referred to the hospital-based outpatient clinics. This is reflected in the higher prevalence of micro- and macrovascular complications at the outpatient clinics in our population.

The risk of microvascular disease can be reduced substantially by glycemic control and general measures to prevent cardiovascular disease such as lifestyle, blood pressure and lipid optimization [22, 23, 25]. Rates of microvascular disease in our study at baseline were 17.3%, 23.0% and 31% for retinopathy, nephropathy and neuropathy, respectively. This incidence of retinopathy in T2DM is comparable to a report from the Dutch National Institute for Public Health and the Environment [45] and in line with a worldwide meta-analyses for diabetes with a duration of less than 10 years [46], but higher than in a screening study for T2DM from the Netherlands [47]. In the latter study, the duration of T2DM was short and this probably explains the difference. For nephropathy, our rate is slightly lower than in the United Kingdom Prospective Diabetes Study (25%), also probably because of shorter follow-up [16]. Our primary care population appeared to have lower rates of nephropathy compared to studies on prevalent diabetes and newly diagnosed diabetes in patients of general practitioners in the Netherlands [47, 48]. Although the single urinary measurement-based prevalence rates in the latter could be an explanation for this dis-

crepancy. The percentage of patients with T2DM and neuropathy in our population (31%) is lower compared to a prospective study (50%) with 25 years of follow-up from diagnosis [19] and comparable to a cross-sectional study on peripheral neuropathy in the United Kingdom [49].

The risk of macrovascular disease in T2DM can be successfully reduced by applying lifestyle interventions, lipid lowering therapies and antihypertensive treatment. The relationship with glycemic control is more complex. Even though glycemic control epidemiologically is strongly related to cardiovascular disease in T2DM, interventions applying strict glycemic control were unsuccessful [22, 50] or even showed adverse effects [51]. Macrovascular disease rates in our population with T2DM is comparable to previous reports in the Netherlands [45, 52], but lower than in an interview-based study in diabetes patients in the USA [53]. Our population is on average 5 years older than the patients in this American study, and also contains a significant proportion of patients from outpatient clinics having further progressed disease.

T2DM and its complications are multifactorial in their pathophysiology's. Genetics, epigenetics, biological mechanisms and environmental factors are probably interacting at multiple levels. Therefore a pathway-based approach in well-defined cohorts is needed, supported by full use of information technology. High throughput research has been mainly focused on genome wide genetic associations. This has elucidated interesting associations. Yet the results only explain disease susceptibility to a small extent [31, 33]. We are planning to perform genome-wide association analysis in the near future. The quality control of this future genomic work will include analyses of the genetic-based ethnic background to definitively determine population sub-structures. Here, we restricted our analyses of well-known genetic T2DM risk variants to the sub-group of self-reported Caucasians. These DNA polymorphisms showed similar associations in our mainly Caucasian population as in previous extensive meta-analyses: most genetic variants had similar direction of their associations as earlier reported and for TCF7L2, THADA, KCNQ1 and CDKAL1 this was significant [32, 44, 54]. KCNJ11 and CENTD2 showed a slight but not statistically significant opposite association to what has previously described, with estimates close to 1 and confidence intervals embracing the estimates from literature [32]. Except for KCNJ11, all genetic variants had non-significant interaction effects for odds ratios of T2DM-risk variants compared to the latest meta-analysis [44]. The significant difference for KCNJ11 can be an effect of population-specific variance, differences in environmental factors, age or interactions of these factors with the genetic variant [44].

To study the aetiology of T2DM and its complications we need well phenotyped cohorts with prospective follow-up. Our population has these characteristics. We therefore plan to

analyse several omics layers for their associations with T2DM and its complications. We are currently measuring total N-glycomics with matrix-assisted laser desorption-ionization-time of flight (MALDI-TOF), -Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR) [55] and IgG-glycomics with ultra-performance liquid chromatography [56]. In the near future, we aim to include lipidomics, with a focus on lipoprotein(a), metabolomics, and proteomics. Also we plan to perform genomics using the Illumina chip, for mendelian randomization and multilayer interaction analyses. The overall goal being to elucidate new pathophysiological pathways for prediction, prevention and treatment of T2DM.

Although we have performed our study with precision, we need to consider a number of limitations. A large majority of our population is of self-reported Caucasian ethnicity, which limits extending conclusions from our analyses to non-Caucasian populations. However, it also makes our analyses less vulnerable to genetic population stratification bias. Self-reported Caucasian mono-ethnicity in two generations results in a very limited risk of misclassifying genetic admixtures [57, 58]. In addition, a small proportion of diabetes-free subjects where recruited by asking T2DM subjects to invite unrelated family members and friends. Hence, absence of family ties was self-reported, with a small possibility of hidden relatedness. In the near future, we will perform genome-wide association analysis, which will allow us to perform formal quality control and accurately account for hidden relatedness and genetic population stratification bias [59]. Another limitation of this study was our inability to retrieve information on neuropathy in primary care setting. Conclusions on neuropathy are therefore restricted to the secondary care setting. We have made extensive efforts to optimise the reliability of our data by having two independent investigators collect the data and reach consensus. This means we did have to rely on common clinical practice and adequate record keeping in primary and secondary care. For macrovascular events in primary care we had to rely on self-reported data. For validation, we have therefore checked self-reported myocardial infarction data from hospital-based participants and found that in only 6.0% of participants with self-reported myocardial infarction this diagnosis was not confirmed in hospital data. These events have therefore been scored as missing. Underestimation of the incidence of myocardial infarction based on hospital discharge data has however been described before [60]. And although the questionnaire on lifestyle, medication, clinical events and family history was straightforward and easy to use, it is not an externally validated questionnaire. At last, our preliminary genetic results had approximately 10% missing values. We are currently collecting additional samples from the participants whose DNA was not available at the time of the current genetic analysis to improve our genetic analysis. Further strengths of our study are the meticulous hands-on medical file review for each patient by two separate physicians, which produced high-quality data that enable us to research both T2DM itself as well as

its complications in great detail. Currently, we are finalizing the first collection prospective follow up on all T2DM complications. The prospective cohort setting with concurrent inclusion of diabetes-free individuals at baseline, will allow us to perform cross-sectional and prospective end-point analyses to study aetiology and progression of type 2 diabetes and its complications.

CONCLUSION

In conclusion, this manuscript describes the design and baseline characteristics of the DiaGene Study, a large multi-centre prospective follow-up cohort study on environmental, biochemical and genetic risk factors of T2DM and related vascular complications. By studying both clinical and complex biochemical parameters with a current focus on glycomics, genomics and lipidomics, the DiaGene Study aims to contribute to the pathophysiological understanding of T2DM and all its vascular complications in a prospective case-control setting.

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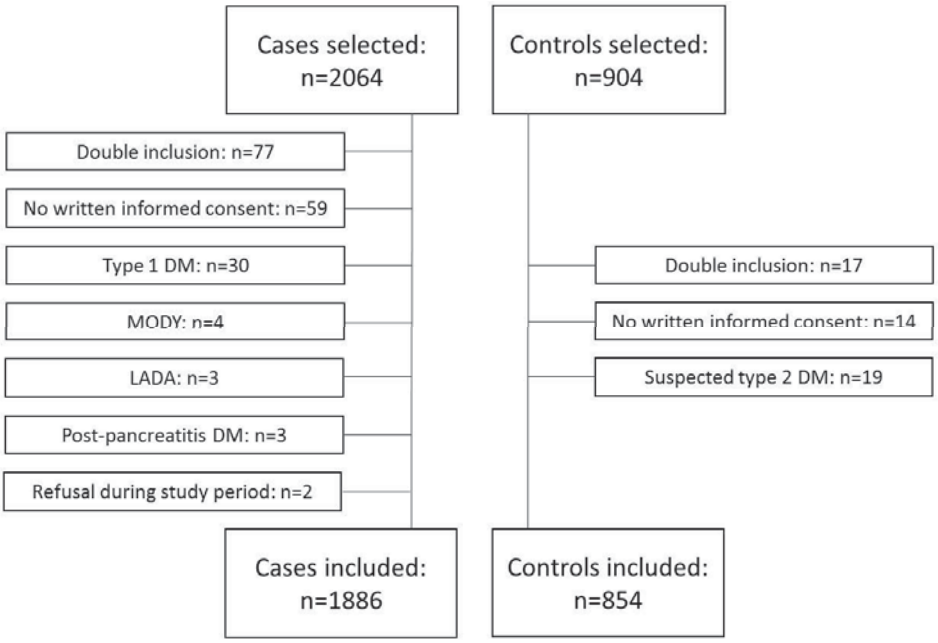

Supplementary material: Flow chart of the inclusion of cases and controls
DM**,** diabetes mellitus; MODY, Maturity-Onset Diabetes of the Young; Latent Auto-immune Diabetes of the Adult

Chapter 7

A genetic variant in *SLC6A20* is associated with type 2 diabetes in White European and Chinese populations

Yan Ling
Thijs T.W. van Herpt
Mandy van Hoek
Abbas Dehghan
Albert Hofman
Andre G. Uitterlinden
Sunfang Jiang
Aloysius G. Lieverse
Bert Bravenboer
Daru Lu
Cornelia M. van Duijn
Xin Gao
Eric J.G. Sijbrands

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ABSTRACT

Introduction: SLC6A20 is a proline transporter and is an essential component of proline metabolism. In a recent metabolic study, reduced SLC6A20 activity was found related to type 2 diabetes in primates and mice. However, its role in the pathogenesis of type 2 diabetes in humans is unclear. We investigated whether polymorphisms in *SLC6A20* are associated with susceptibility to type 2 diabetes.

Methods: In the Rotterdam Study, a prospective, population-based cohort (n=5974), 22 tagging polymorphisms with minor allele frequencies>0.05 across *SLC6A20* were studied. Replication studies were performed in an independent Dutch case-control study (DiaGene-Rotterdam Study 2 n=3133), and in a Chinese Han case-control population(n=2279). Finally, meta-analysis was performed.

Results: In the Rotterdam Study, the minor alleles of rs13062383, rs10461016 and rs2286489 increased risk of type 2 diabetes (HR=1.37 (95%CI 1,15-1,63), HR1,30 (95% CI 1,09-1,54) and HR 1,20 (95% CI 1,07-1,35, respectively). In DiaGene/Rotterdam Study 2, the *A* allele of rs13062383 increased risk of type 2 diabetes (OR=1.45, 95% CI 1.19–1.76). In the Chinese Han study, rs13062383 *A* allele also increased risk of type 2 diabetes (OR=1.21, 95% CI 1.03–1.42). Meta-analysis showed a highly significant association of rs13062383 with type 2 diabetes (OR=1.35, 95% CI 1.21–1.47, $p=3.3\times10^{-8}$).

Conclusions: In conclusion, rs13062383 in *SLC6A20* increased the susceptibility to type 2 diabetes in populations with different genetic backgrounds

INTRODUCTION

Solute carrier family 6 (SLC6) is one of the largest membrane transporter families in the human genome and comprises 20 members. [1] Recently, the SLC6A20 kidney transporter was shown have reduced activity in type 2 diabetes in primates and mice.[2] However, the role of SLC6A20in relation to type 2 diabetes in humans is unclear.

SLC6A20 is a proline transporter and is an essential component of proline metabolism. [1] Proline may influence glucose homeostasis through metabolic pathways that involve glutamine and arginine, amino acids involved in gluconeogenesis and energy homeostasis.[3,4] Moreover, it has been shown that the well-replicated type 2 diabetes risk gene HNF1α, is an important regulator of SLC6A20.[5]

In this study, we hypothesized that variants in the *SLC6A20* gene may influence type 2 diabetes risk. Therefore, we investigated whether variants in the *SLC6A20* gene contribute to the risk of type 2 diabetes. Our discovery population is a prospective population-based study in white Europeans. Significant findings were taken forward to a white European and a Chinese replication population.

SUBJECTS AND METHODS

Study population

Details of the original Rotterdam Study (RS1) have been described previously.[6] In brief, the Rotterdam Study is an ongoing prospective, population-based, cohort study on 7,983 inhabitants of a suburb in Rotterdam, designed to investigate determinants of chronic diseases in the elderly. Participants were aged 55 years or older. Continuous surveillance on major disease outcomes was conducted between these examinations. Information on vital status was derived from municipal health authorities.

Replication studies

The first replication study was performed in a combined case-control sample of cases of the DiaGene Study, and controls from the second phase of the Rotterdam Study (RS2). The DiaGene study is an ongoing collection of population-based cases with type 2 diabetes from Eindhoven, the Netherlands, and has been described previously.[7] RS2 refers to persons in the Rotterdam Study district that became 55 years since the start of the study or those of 55 years or over that migrated into the study district. This cohort comprises 3,011 participants and started in 1999.[6] Persons without diabetes from the RS2 cohort served as controls in a combined case-control sample with the DiaGene study, as described and validated earlier.[7]

The second replication study included a total of 2279 individuals of Chinese Han ancestry who were residents in Shanghai, China, comprising 1118 persons with type 2 diabetes and 1161 normoglycaemic controls.[8] The cases were inpatients from the department of Endocrinology and Metabolism of Zhongshan hospital, Shanghai, China. Controls were people undergoing health examinations in Zhongshan hospital, Shanghai, China. The controls were older than 40 years and had fasting glucose below 5.6 mmol/l.

Written informed consent was obtained from all participants and all studies were approved by the local ethic committees.

Diabetes

In accordance with the guidelines of the World Health Organization and the American Diabetes Association[9,10], diabetes was diagnosed as fasting plasma glucose levels ≥7.0 mmol/l or a non-fasting plasma glucose levels ≥11.0 mmol/l or treatment with antidiabetic medication (oral medication or insulin) or a diagnosis of diabetes as registered by a general practitioner in the Rotterdam Study (RS1 and RS2), in the DiaGene study and in the Chinese Han population.

In all populations, type 1 diabetes and other known subtypes of diabetes were excluded based on general practitioners and specialists records.

Genotyping

For the first phase of the study, we selected tagging single nucleotide polymorphisms (SNPs) with R^2=1 and minor allele frequency>0.05 across the region of *SLC6A20* (include 20kb upstream and 10kb downstream of the gene) from HapMap Phase II, using the pairwise tagging model in Haploview.[11] Overall, 22 tagging SNPs were chosen and available in Illumina 550K genotyping BeadChip version3 in 5974 subjects. (complete list of SNPs in Supplementary Table 1)

In RS1 and RS2, participants with proper quality DNA samples were considered for genotyping using the Infinium II HumanHap 550K Genotyping BeadChip® version 3 (Illumina, San Diego, USA). After quality control genotyped data was available for 5974 individuals in RS1 and 2157 individuals in RS2.

In the DiaGene Study, DNA material was available for 1472 cases with type 2 diabetes. TaqMan allelic discrimination assays were used for genotyping of significant findings from the first phase. The assays were designed and optimized by Applied Biosystems (Foster City, CA, USA; http://store.appliedbiosystems.com). Reactions were performed on the Taqman Prism 7900HT platform.

In the Chinese Han population, genotyping was performed by primer extension of multiplex products with detection by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy using a MassARRAY platform (MassARRAY Compact Analyzer, Sequenom, San Diego, CA, USA).

Statistical analysis

Analyses were performed with SPSS version 12.0.1. Continuous variables are expressed as means ± SEM. ANOVA was used for comparisons of continuous variable and chi-square tests were used for comparisons of categorical variables between groups. Deviation from Hardy–Weinberg equilibrium was assessed using chi-square tests. SNPs that were not in Hardy-Weinberg equilibrium were excluded from further analyses. Pairwise linkage disequilibrium (LD) including D' and r^2 were estimated by Haploview4.1.[11]

In RS1, we tested the association of the polymorphisms with type 2 diabetes risk in Cox proportional hazards models. Participant with diabetes at baseline were excluded from the analyses. (n=631) We used a Bonferroni correction (0.05/19=0.0026) to adjust for multiple testing.

In both replication studies, we tested the association of the SNPs with type 2 diabetes using logistic regression models. All models were corrected for year of birth and sex. Additional models were corrected for BMI.

We performed a meta-analysis to combine the measure of effects from all three studies. To have comparable measures of effects, we calculated the ORs for RS1 using a logistic regression model, adjusted for the follow-up time. An inverse variance weighting method was used to combine the statistical information from the three independent data sets. Package "meta" , running under "R", was used to perform the meta-analysis.[12] We used the I^2 index to quantify the degree of heterogeneity. The I^2 statistic describes the percentage of variation across studies that is due to heterogeneity rather than chance.[13,14]

RESULTS

General characteristics

In 5974 genotyped participants of RS1, 10.3% developed diabetes during follow-up. A total of 666 individuals were excluded of which 631 had prevalent diabetes at baseline, and 35 had no available data on their diabetes status. (Mean follow-up time20.6years)

The DiaGene study- RS2 replication cohort contained 1472 cases and 1661 controls from RSII. The Chinese Han replication study comprised 2279 participants of which 1118 were type 2 diabetes cases and 1161 were healthy controls. The baseline characteristics of all three populations are presented in Table 1.

Discovery analyses

In RS1, 19 genotyped SNPs were in Hardy–Weinberg equilibrium(χ^2<2.362, df=1, p>0.12) in the total population and in individuals without type 2 diabetes. Three SNPs (rs6770261, rs2531747 and rs12488144) deviated from Hardy–Weinberg equilibrium, and were excluded.

Out of 19 SNPs, 3 were significantly associated with the risk of type 2 diabetes (Table 2). The minor alleles of rs13062383, rs10461016 and rs2286489 increased the risk of type 2 diabetes (HR=1.37, 95% CI 1.15-1.63, p=4.5×10^{-4}; HR=1.30, 95% CI 1.09-1.54, p=0.003; HR=1.20, 95% CI 1.07-1.35, p=0.003, respectively). The association of rs13062383 and rs2286489 with type 2 diabetes remained significant after additional adjustment for BMI (HR=1.34, 95% CI 1.13-1.60, p<9.1×10^{-4}; HR=1.19, 95% CI 1.06-1.35, p=0.004, respectively).

After Bonferroni correction only the association of rs13062383 remained significant and was taken forward for replication.

The replication of rs13062383 in white Europeans

Genotyping success rate was 97.9% for rs13062383 in the DiaGene/RS2 replication study and was in Hardy–Weinberg equilibrium in the total population and in controls (χ^2<2.259, df=1, p=0.13). The A allele of rs13062383 increased the risk of type 2 diabetes (OR=1.45, 95% CI 1.19–1.76, p=0.0001) (Table 2). Additional adjustment for BMI did not change the results.

The replication of 13062383 in the Chinese Han population.

Genotyping success rate was 98.5% for rs13062383 in the Chinese Han replication study. It was in Hardy–Weinberg equilibrium in the total population and in controls (χ^2=0.264, df=1, p=0.61). The A allele of rs13062383 increased the risk of type 2 diabetes adjusted for year of birth and sex (OR=1.21, 95% CI 1.03–1.42, p=0.02). The association became more significant after additional adjustment for BMI (OR=1.26, 95% CI 1.07–1.49, p=0.007) (Table 2).

Table 1 Baseline characteristics of diabetes and non-diabetes in the Rotterdam Study, the DiaGene Study and the Shanghai replication study

Characteristic	Rotterdam Study			DiaGene/RSII Study			Shanghai replication study		
	Incident type 2 diabetes (n=547)	Controls (n=4761)	p value [a]	Cases (n=1472)	Controls (n=1661)	p value [a]	Cases (n=1118)	Controls (n=1161)	P value [a]
Age (years)	68.3±7.78	69.0±9.06	0.06	67.0±10.4	64.6±7.92	3.32×10^{-13}	60.2±13.3	56.5±10.6	2.59×10^{-13}
Men (%)	43.9±0.50	40.5±0.49	0.13	53.7±0.50	44.1±0.50	1.70×10^{-8}	44.6±0.50	42.7±0.50	0.34
Body mass index (kg/m^2)	28.1±3.80	26.0±3.55	4.3×10^{-38}	30.6±5.47	27.0±3.84	$9{,}03 \times 10^{-96}$	24.4±3.75	23.5±2.81	9.88×10^{-11}
Total cholesterol (mmol/l)	6.6±1.14	6.6±1.22	0.70				4.43±1.73	5.10±0.99	2.58×10^{-29}
HDL cholesterol (mmol/l)	1.25±0.34	1.36±0.37	3.49×10^{-11}				1.16±0.44	1.32±0.33	2.02×10^{-22}
Systolic blood pressure (mmHg)	143.3±20.6	137.8±22.0	$2{,}63 \times 10^{-8}$				134.2±16.5	119.4±16.1	7.56×10^{-95}
Diastolic blood pressure (mmHg)	75.4±10.9	73.6±11.4	0.001				80.7±9.37	78.0±9.30	6.57×10^{-12}

Continuous data are expressed as means±SEM

[a] p value for comparison between diabetes and non-diabetes

HDL: high density lipoprotein

Table 2 Risk of type 2 diabetes by *SLC6A20* alleles in the Rotterdam Study, the DiaGene study and the Shanghai replication study

Study	Allele	Non-diabetes (%)	Diabetes (%)	HR/OR1*	95% CI	p value
Rotterdam	rs12490575					
Study	G	85.9	86.6	1	(Ref.)	
	A	14.1	13.4	0.95	0.80-1.13	0.58
	rs17213127					
	G	95.3	95.4	1	(Ref.)	
	A	4.7	4.6	1.00	0.75-1.32	0.98
	rs13062383					
	G	89.8	86.4	1	(Ref.)	
	A	10.2	13.6	1.37	1.15-1.63	4.5×10^{-4}
	rs4327428					
	C	89.7	89.4	1	(Ref.)	
	A	10.3	10.6	1.017	0.84-1.23	0.86
	rs17279465					
	A	56.7	60.2	1	(Ref.)	
	G	43.3	39.8	0.86	0.76-0.97	0.01
	rs10461016					
	A	89.1	85.9	1	(Ref.)	
	G	10.9	14.1	1.30	1.09-1.54	0.003
	rs2286489					
	A	62.7	57.8	1	(Ref.)	
	G	37.3	42.2	1.20	1.07-1.35	0.003
	rs13067466					
	A	82.7	81.2	1	(Ref.)	
	C	17.3	18.8	1.09	0.93-1.26	0.29
	rs2191028					
	G	61.8	66.1	1	(Ref.)	
	A	38.2	33.9	0.84	0.74-0.95	0.01
	rs2531742					
	G	61.5	59.9	1	(Ref.)	
	A	38.5	40.1	1.08	0.95-1.21	0.24
	rs2191027					
	G	69.8	73.3	1	(Ref.)	
	A	30.2	26.7	0.85	0.74-0.97	0.02
	rs2531748					
	A	66.5	66.1	1	(Ref.)	
	G	33.5	33.9	1.02	0.90-1.16	0.72
	rs720625					

Table 2 Risk of type 2 diabetes by *SLC6A20* alleles in the Rotterdam Study, the DiaGene study and the Shanghai replication study (continued)

Study	Allele	Non-diabetes (%)	Diabetes (%)	HR/OR1*	95% CI	p value
	A	62.3	63.0	1	(Ref.)	
	G	37.7	37.0	0.99	0.88-1.12	0.88
	rs9843503					
	A	92.8	93.1	1	(Ref.)	
	C	7.2	6.9	0.96	0.76-1.22	0.75
	rs17078339					
	A	71.3	71.6	1	(Ref.)	
	G	28.7	28.4	1.01	0.88-1.15	0.94
	rs4299518					
	A	52.7	56.3	1	(Ref.)	
	G	47.3	43.7	0.86	0.76-0.97	0.02
	rs2531757					
	C	67.8	66.8	1	(Ref.)	
	A	32.2	33.2	1.05	0.93-1.20	0.41
	rs9848415					
	G	75.0	76.1	1	(Ref.)	
	A	25.0	23.9	0.97	0.84-1.11	0.64
	rs2159272					
	A	56.9	57,8	1	(Ref.)	
	G	43.1	42.2	0.99	0.87-1.11	0.81
DiaGene	Rs13062383					
Study	G	93.6	90.8	1	(Ref.)	
	A	6.4	9.2	1.45	1.19-1.76	0.0001
Shanghai	rs13062383					
Replication	G	86.2	83.9	1	(Ref.)	
Study	A	13.8	16.1	1.21	1.03-1.42	0.02
All studies	rs13062383					
	G			1	(Ref.)	
	A			1.35	1.21 - 1.50	3.3×10^{-8}

* Adjusted for year of birth and sex

HR: hazard ratio OR: odds ratio CI: confidence interval

HR for the Rotterdam Study; OR for the replication studies

Meta-analysis

A heterogeneity test for rs13062383 ORs was performed on the three cohorts, and was found to be homogenous (I^2 = 7.4%). Meta-analysis was performed using the fixed effects model. The combined OR was 1.35 (95% CI 1.21–1.50, p=3.3×10^{-8}).

DISCUSSION

We showed in a large cohort study of white Europeans that individual genetic variants in *SLC6A20* are associated with risk of type 2 diabetes. One of the variants, rs13062383, replicated in an independent Dutch white European population and a Chinese Han population. In the combined analysis of this SNP, the minor allele associated with 35% increased risk of type 2 diabetes and this finding was highly significant.

There are a number of pathways through which SLC6A20 may influence type 2 diabetes risk. SLC6A20 is an essential component in the metabolism of proline.[1] First, proline is synthesized from dietary glutamate and can interconvert with glutamate via the mitochondrial pathway involving pyrroline-5-carboxylate.[15,16] Glutamate and glutamine are major gluconeogenic precursors in the kidney[4,17] and the small intestine[18], contributing to at least 50% of the endogenous glucose production in insulinopenic states, such as fasting[19] and diabetes[20]. Changes in the metabolism of proline, may therefore affect gluconeogenesis in kidney and small intestine. Second, proline is also an important substrate for the synthesis of arginine, the exclusive substrate for the synthesis of nitric oxide (NO). Emerging evidence shows that the arginine-NO pathway is involved in the regulation of the metabolism of glucose, fatty acids and amino acids in mammals.[3,21] Third, a recent metabolomic study revealed that *SLC6A20* kidney expression was reduced in primate and mouse models of type 2 diabetes without the presence of damage to the proximale tubular architecture secondary to type 2 diabetes.[2] In this study, increased levels of glycine betaine, proline, pipecolic acid and glucose were found in urine. Moreover, *HNF1*α, a genome-wide proven risk locus for type 2 diabetes and the causative gene for MODY3, has been shown to be a regulator of SLC6A20.[5] Taken together, we think *SLC6A20* is a promising candidate gene for type 2 diabetes.

Rs13062383 is located in intron 4 of *SLC6A20*. In a genome-wide association study on urine metabolic traits an association between a nonsynonimus functional variant (rs 17279437) in SLC6A20 and N-N-dimethylglycine was found in healthy subjects.[22] This variant is tagged by rs4327428, which was not associated with T2D in our study. However, it is difficult to speculate on the exact mechanisms by which variation in the *SLC6A20* gene influences T2D risk and how this relates to the transport of specific imino acids. Rs13062383

may be linked to one or more functional variants within the *SLC6A20* gene or its regulatory regions. The haploblock in which rs13062383 resides contains the 3'UTR of the gene. There is evidence showing that 3'UTRs are particularly rich in regulatory elements.[23]

We have studied populations with different genetic backgrounds and found similar effect sizes for the associated polymorphism, with no signs of heterogeneity across studies. Haploblock plots of *SLC6A20* show a similar genetic architecture of the gene in Asians and Caucasians. Despite the fact that rs13062383 had a highly significant p-value in our meta-analysis, the variant has not been related to diabetes risk in large-scale genome-wide meta-analyses such as DIAGRAM+, in which RS1 has been a discovery population.[24] A regional plot from DIAGRAM+ results does show a modest, non-significant peak in the area of the *SLC6A20* gene (Supplementary Figure 1. Derived from http://diagram-consortium.org/downloads.html). Additional age-stratified analyses in our discovery population showed a stronger association in the older half of the population (HR 1.42, 95%CI 1.12-1.79; p=0.004) compared to the younger half (HR 1.20, 95% CI 0.94-1.55, p=0.15) (stratified into two equally sized groups with cut off age of 67.7) . Age-stratified analyses in both replication cohorts generated similar results.(data not shown) Since the DIAGRAM+ meta-analyses contain studies with different age-ranges this might explain why this particular SNP did not reach significance in DIAGRAM+. This means we must bear in mind that our finding may not be generalizable to younger populations. Although association studies cannot fully exclude false positive findings, the small p-value reduces the risk of such an erroneous observation.

Strengths of our study include a prospective population-based design in the discovery analyses with up to 10 years of follow-up, and large sample sizes in the following replication studies. Moreover, we were able to study the genetic variant in independent populations with different lifestyles and genetic backgrounds.

Unfortunately, we did not have patient material available to investigate proline and other related metabolites in urine in relation to the polymorphism, to study the underlying mechanism. This may be an interesting next step for future studies on the subject.

In conclusion, this is the first report describing a highly significant relationship between a *SLC6A20* polymorphism and type 2 diabetes in white European, as well as Chinese individuals. Our finding highlights the proline pathway in glucose homeostasis and susceptibility to type 2 diabetes.

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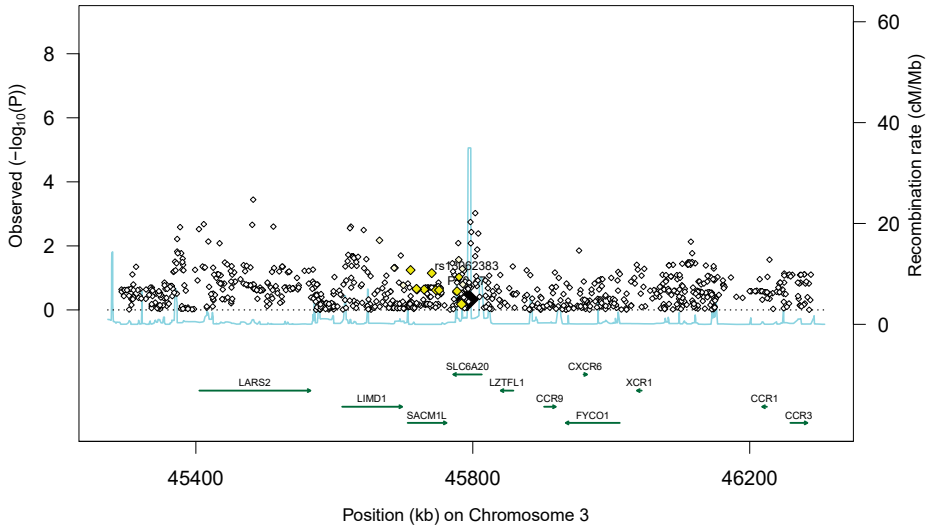

Supplementary Figure 1. Regional plot from the SLC6A20 region derived from the DIAGRAM+ meta-analyses

Chapter 8

ADAMTS13 activity as a novel risk factor for incident type 2 diabetes mellitus: a population-based cohort study

Thijs T.W. van Herpt
Paul S. de Vries
Symen Ligthart
Albert Hofman
M. Arfan Ikram
Mandy van Hoek
Eric J.G. Sijbrands
Oscar H. Franco
Moniek P.M. de Maat
Frank W.G. Leebeek
Abbas Dehghan

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ABSTRACT

Introduction: ADAMTS13 is a protease that breaks down von Willebrand factor (VWF) multimers into smaller, less active particles. VWF has been associated with an increased risk of incident type 2 diabetes mellitus. Here, we determine the association of ADAMTS13 activity and VWF antigen with incident diabetes.

Methods: The study included 5,176 participants of the Rotterdam Study, a prospective population-based cohort study. Participants were free of diabetes at baseline and followed up for more than 20 years. Cox proportional hazards models were used to examine the association of ADAMTS13 activity and VWF antigen with incident diabetes.

Results: ADAMTS13 activity was associated with an increased risk of incident diabetes (HR: 1.17; 95%CI: 1.08 to 1.27) after adjustment for known risk factors and VWF antigen. Although ADAMTS13 activity was positively associated with fasting glucose and insulin, the association with incident diabetes did not change when we adjusted for these covariates. ADAMTS13 activity was also associated with incident prediabetes after adjustment for known risk factors (HR: 1.11; 95%CI: 1.03, 1.19), while VWF antigen was not. VWF antigen was associated with incident diabetes, but this association was attenuated when adjusted for known risk factors.

Conclusions/interpretation: ADAMTS13 activity appears to be an independent risk factor for incident prediabetes and type 2 diabetes. As the association between ADAMTS13 and diabetes did not appear to be explained by its cleavage of VWF, ADAMTS13 may have an independent role in the development of diabetes.

INTRODUCTION

ADAMTS13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13) reduces the activity of von Willebrand factor (VWF) in platelet adhesion and aggregation by cleaving prothrombotic VWF multimers [1, 2]. Low ADAMTS13 levels and activity are associated with an increased risk of various thrombotic diseases, including ischemic stroke and myocardial infarction [3-8], as well as kidney disease [9]. Additionally, low ADAMTS13 activity may contribute to renal and cardiovascular complications of diabetes [10-12]. The association of ADAMTS13 with diabetes itself remains unexplored. Elevated levels of VWF have been associated with an increased risk of type 2 diabetes [13-17] which has been attributed primarily to VWFs role as a marker of endothelial dysfunction rather than its role in thrombosis [18]. However, VWF may also be associated with diabetes through its prothrombotic effect. This would be in line with emerging evidence that vascular disease may contribute to the development of diabetes [19]. Low ADAMTS13 activity and high VWF levels may exacerbate small vessel disease, which in turn may contribute to the development of diabetes [20-22]. If VWF is associated with diabetes through its prothrombotic function, then we expect ADAMTS13, with its antithrombotic function, to be inversely associated with the risk of diabetes. On the other hand, still little is known about the regulation of ADAMTS13 and its role as a marker of other physiological processes[23]. We previously showed that type 2 diabetes patients have higher ADAMTS13 activity than controls [8] [23], which is inconsistent with a mechanism involving VWF's prothrombotic function.

Nevertheless, the association may also reflect a response to diabetes, and studies on incident diabetes are needed to provide further insight into the direction of this association. In this study, we examined whether ADAMTS13 activity or VWF antigen levels associate with risk of type 2 diabetes in a large prospective population-based cohort study.

METHODS

Study description and population

The Rotterdam Study is a prospective population-based cohort study initiated in 1990 to study the determinants of several chronic diseases in older adults [24]. The first cohort (RS-I) includes 7,983 inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or older. The first examination took place between 1990 and 1993. The third visit, including 4,797 participants, took place between March 1997 and December 1999, and was used as the baseline in this study. The second cohort (RS-II) established between February 2000 and December 2001, includes another 3,011 inhabitants of

Ommoord who either reached the age of 55 years after the recruitment phase of RS-I or who had migrated into the research area. There were no eligibility criteria to enter the Rotterdam Study except age and residential area (postal code). The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All included participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Ascertainment of prediabetes and diabetes

iabetes, prediabetes and normoglycemia were defined according to the most recent WHO guidelines [25]. Prediabetes was defined as a fasting blood glucose between 6.0 mmol/L and 7.0 mmol/L or a non-fasting blood glucose between 7.7 mmol/L and 11.1 mmol/L (when fasting samples were absent); diabetes was defined as a fasting blood glucose higher than 7.0 mmol/L, a non-fasting blood glucose ≥ 11.1 mmol/L (when fasting samples were absent), or the use of blood glucose lowering medication. Prevalent and incident diabetes and prediabetes were ascertained using records kept by general practitioners, hospital discharge letters, pharmacy records, home interviews, and fasting glucose measurements performed at our research center during baseline and follow-up visits [26]. Information regarding the use of blood glucose lowering medication was derived from both home interviews and pharmacy records [26]. A schematic representation of the follow-up design is provided in Supplementary Figure 1. At baseline, more than 99% of the Rotterdam Study population was covered by the pharmacies in the study area. All potential events of prediabetes and diabetes were independently adjudicated by two study physicians, and in the case of disagreement consensus was sought with the help of an endocrinologist. Follow-up data is completed until January 1st 2012. Flowcharts detailing why prevalent cases of diabetes and prediabetes were classified as as such are shown in Supplementary Figures 2 and 3 respectively.

ADAMTS13 activity and VWF antigen measurements

Citrated plasma samples were collected at the third visit of RS-I and the baseline examination of RS-II, and stored at -80°C. Between June and October 2013, we measured ADAMTS13 activity using a kinetic assay based on the Fluorescence Resonance Energy Transfer Substrate VWF 73 (FRETS-VWF73) assay [27]. Plasma samples were measured against a reference curve of serial dilutions of pooled normal human plasma defined to have an ADAMTS13 activity of 1 IU/ml, and we express ADAMTS13 activity as a percentage of this. The ADAMTS13 activity of 6,258 participants was measured: 3,791 from RS-I, and 2,467 from RS-II

Between July and October of 2008, VWF antigen was determined in IU/ml with an in-house ELISA using polyclonal rabbit antihuman VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging [28]. The intra-assay coefficient of variation was 5.8% and the inter-assay coefficient of variation was 7.8%. VWF antigen was measured in 3,968 individuals from RS-I, and 2,561 individuals from RS-II.

In total, 5,176 participants with VWF and ADAMTS13 measurements also had a fasting glucose measurement and were free of diabetes at baseline, while 4,232 participants were free of prediabetes at baseline (Supplementary Figure 2 and 3).

Covariates

BMI was calculated by dividing weight in kilograms by height in meters squared. Information on current tobacco smoking was acquired from questionnaires. Lipid-lowering (statins, fibrates, and other lipid modifying agents), antihypertensive (diuretics, beta-blocking agents, ACE-inhibitors, calcium channel blockers), and antithrombotic medication use was assessed during a structured home interview. Blood pressure was measured twice by an oscillometric device after five minutes of rest and the mean was taken as the subject's reading. Serum total cholesterol and HDL-cholesterol levels were determined using an automated enzymatic method. Blood glucose levels were measured using the glucose hexokinase method (Instruchemie) [29]. Insulin levels were determined by metric assay (Biosource Diagnostics, Camarillo, CA). This assay has no cross-reactivity with either proinsulin or C-peptide. Serum alanine-aminotransferase (ALAT) levels were measured using a Merck Diagnostica kit on an Elan Autoanalyzer (Merck, Whitehouse Station, NJ, USA). White blood cell count was assessed in citrate plasma with a Coulter Counter T540 (Coulter Electronics, Hialeah, Florida, USA). C-reactive protein (CRP) was measured using CRPL3, an immunoturbidometric assay (Roche Diagnostics, Indianapolis, IN, USA). Prevalent CHD was defined as having a history of myocardial infarction or coronary revascularization procedures, as previously described [30].

Statistical Analysis

Statistical analyses were performed in SPSS version 21 (IBM Corp, Armonk, NY, USA) and R version 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria). Missing values for covariates (<5%) were imputed in SPSS using single imputation based on expectation maximization. VWF antigen, HDL cholesterol, CRP, ALAT, and fasting insulin were natural log-transformed. We used linear regression models to test the association of ADAMTS13 activity and VWF antigen with fasting glucose and fasting insulin. Individuals with prevalent diabetes were excluded in all analyses.

The association of ADAMTS13 activity and VWF antigen with incident diabetes was examined using Cox proportional hazards models. The assumption of proportional hazards was met. Three adjustment models were used. Model 1 was adjusted for age, sex, and cohort. Model 2 was additionally adjusted for HDL and total cholesterol, lipid-lowering medication, BMI, CRP, current smoking, antithrombotic medication, ALAT, white blood cell count, systolic blood pressure, antihypertensive medication, and prevalent CHD. Model 3 was additionally adjusted for fasting glucose and insulin. The assumption of proportional hazards was met for all models. In Model 1 ADAMTS13 activity and VWF antigen were tested separately, whereas in Models 2 and 3 the analysis of ADAMTS13 activity was adjusted for VWF antigen and vice versa. We examined the interaction between ADAMTS13 activity and VWF antigen on incident diabetes using a multiplicative interaction term, and adjusting for age, sex, and cohort. Results are shown per standard deviation (SD) of VWF antigen levels and ADAMTS13 activity. We also tested the association of ADAMTS13 activity quartiles with incident diabetes.

To test whether associations with incident diabetes were driven by participants with prevalent CHD, or users of lipid-lowering, antihypertensive, and antithrombotic medication, we excluded participants in each of these subgroups in a sensitivity analysis.

RESULTS

Baseline characteristics are shown in Table 1. In a median follow-up time of 11.2 years (interquartile range: 9.8, 12.6), 638 participants out of 5,176 participants that were free of diabetes at baseline developed diabetes (incidence rate: 12.4 per 1000 person years). As shown in Supplementary Figure 4, 36 of these individuals were diagnosed during follow-up because they started with insulin treatment, whereas 278 started using oral glucose-lowering medication. Another 324 participants were diagnosed with diabetes because of their fasting glucose levels. Of the 4,234 participants without prevalent prediabetes, 862 developed prediabetes (incidence rate: 21.1 per 1000 person years). As shown in Supplementary Figure 5, during follow-up 13 of these individuals started with insulin treatment, 118 started taking oral glucose-lowering medication, and 731 were diagnosed with prediabetes based on their fasting glucose levels.

Associations of ADAMTS13 activity and VWF antigen with incident diabetes are shown in Table 2. ADAMTS13 activity was associated with a 19% increased risk of incident diabetes per SD in the age and sex adjusted model (Hazard ratio [HR]: 1.19; 95% confidence intervals [95%CI]: 1.10 to 1.30), and this association remained unchanged when adjusting for potential confounders. Participants in the highest quartile of ADAMTS13 activity had a 46%

Table 1: Baseline characteristics of the study population.

	Mean (SD) or Percentage N = 5,176
Age (years)	69.0 (8.1)
Sex (female)	57.7
Body mass index (kg/m^2)	26.7 (3.8)
High-density lipoprotein cholesterol (mmol/L)	1.3 (1.12-1.60)
Total cholesterol (mmol/L)	5.9 (1.0)
Lipid-lowering medication use	11.4
Systolic blood pressure (mmHg)	142.1 (21.0)
Antihypertensive medication use	20.8
Alanine aminotransferase (U/L)	20.0 (16.0-26.0)
Current smoking	12.5
C-reactive protein (mg/L)	21.0 (10.0-44.3)
White blood cell count (10^9 cells/L)	6.7 (1.9)
Prevalent coronary heart disease	7.3
Prevalent prediabetes	18.2
Fasting glucose (mmol/L)	5.5 (0.5)
Fasting insulin (pmol/L)	71.36 (50.60-100.00)
Antithrombotic medication use	17.4
ADAMTS13 activity (%)	91.0 (17.2)
VWF antigen (IU/ml)	1.3 (0.6)

N=5176 Data are the mean (SD), percentage or median (interquartile range)

increased risk compared to participants in the lowest quartile (HR: 1.46; 95%CI: 1.15, 1.85). VWF antigen was associated with a 12% (HR: 1.12; 95%CI: 1.03 to 1.21) increased risk of incident diabetes per SD in the age and sex adjusted model. However, the increased risk was attenuated to 6% (HR: 1.06; 95%CI: 0.98 to 1.15) increased risk per SD after adjustment for additional covariates, and was 8% (HR: 1.08; 95%CI: 0.99 to 1.17) after further adjustment for fasting glucose and insulin.

Both ADAMTS13 activity and VWF antigen were positively associated with baseline fasting insulin, and ADAMTS13 activity was positively associated with baseline fasting glucose (Supplementary Table 1). Nevertheless, when we additionally adjusted for fasting glucose and insulin, the effect sizes did not change. These associations were robust to the exclusion of participants with prevalent CHD at baseline, and the exclusion of users of lipid-lowering, antihypertensive, and antithrombotic medication (Supplementary Table 2). There was an interaction between ADAMTS13 activity and VWF antigen with incident diabetes (*P*-value: 0.01). As shown in Figure 1, the association of ADAMTS13 activity with incident diabetes was strongest in the fourth quartile of VWF antigen (HR: 1.49: 95%CI: 1.27 to 1.75).

Furthermore, ADAMTS13 activity was also associated with an 11% (HR: 1.11; 95%CI: 1.03 to 1.19) increased risk of prediabetes per SD in Model 1, and this association was similar in Model 2 and 3 (Figure 2). In contrast, VWF antigen was not associated with incident prediabetes.

Table 2: Hazard ratios of ADAMTS13 activity on incident type 2 diabetes.

	Model 1		Model 2		Model 3	
	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value
Continuous (per SD)	1.19 (1.10, 1.30)	0.00003	1.17 (1.08, 1.27)	0.0001	1.17 (1.08, 1.27)	0.00009
Quartile 1 *(N_{cases}: 129)*	Reference		Reference		Reference	
Quartile 2 *(N_{cases}: 150)*	1.12 (0.88, 1.42)	0.4	1.10 (0.87, 1.39)	0.4	1.12 (0.88, 1.41)	0.4
Quartile 3 *(N_{cases}: 168)*	1.26 (1.00, 1.59)	0.05	1.31 (1.03, 1.65)	0.03	1.36 (1.08, 1.73)	0.01
Quartile 4 *(N_{cases}: 191)*	1.47 (1.16, 1.86)	0.001	1.46 (1.15, 1.85)	0.002	1.48 (1.17, 1.88)	0.001

ADAMTS13, A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13. SD, standard deviation. N_{cases}, number of incident diabetes cases. *Adjustments:* Model 1: Adjusted for age, sex, and cohort. Model 2: Additionally adjusted for VWF antigen, HDL and total cholesterol, lipid-lowering medication, body-mass index, CRP, former smoking, current smoking, antithrombotic medication, ALAT, white blood cell count, systolic blood pressure, antihypertensive medication, and prevalent CHD. Model 3: Additionally adjusted for glucose and insulin levels. HDL cholesterol, CRP, ALAT, and insulin were natural log transformed when used

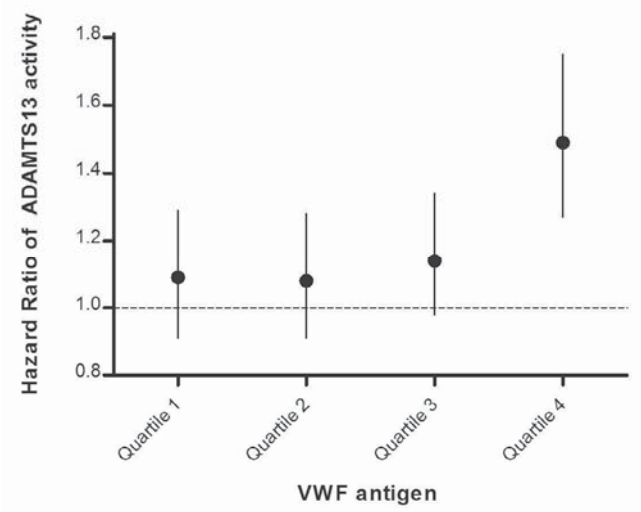

Figure 1: Hazard ratios of ADAMTS13 activity (per SD) for incident type 2 diabetes across quartiles of VWF antigen: interaction between ADAMTS13 and VWF. The vertical bars represent the 95% confidence intervals.

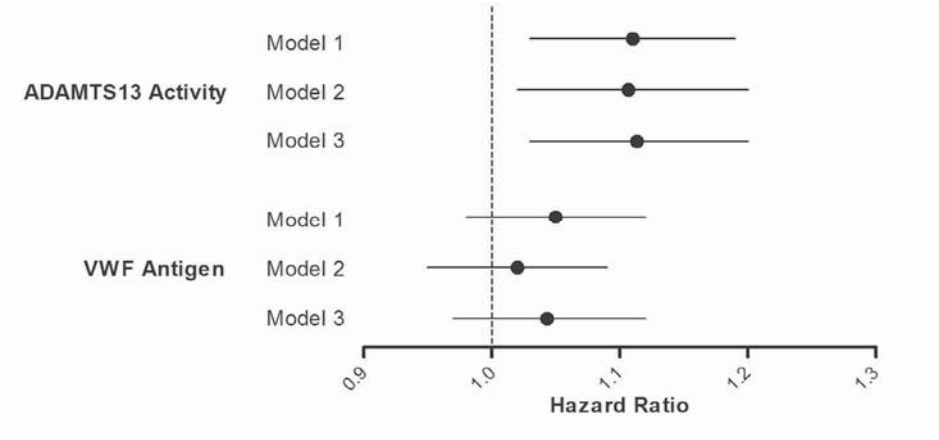

Figure 2: Hazard ratios of ADAMTS13 activity and log transformed VWF antigen (per SD) for incident prediabetes excluding participants with prediabetes at baseline (862 events in 4232 participants). The horizontal bars represent the 95% confidence intervals

DISCUSSION

In our study, ADAMTS13 activity was associated with an increased risk of incident diabetes, even after adjustment for other known risk factors, including VWF antigen, fasting glucose and fasting insulin. Furthermore, ADAMTS13 activity was associated with the incidence of prediabetes among participants with normoglycemia at baseline. VWF antigen was also associated with an increased risk of diabetes, but this association was attenuated after adjustment for known risk factors. These results suggest a role for ADAMTS13 in the occurrence of type 2 diabetes at an early stage before glucose levels rise.

To our knowledge, the association of ADAMTS13 with diabetes has not previously been studied with diabetes as the primary outcome, and we are the first to examine this association in a large prospective population-based cohort study. One cross-sectional study reported the association between ADAMTS13 and prevalent diabetes [12]. The researchers did not observe a statistically significant difference in ADAMTS13 levels between 86 cases of diabetes and 26 healthy controls. We previously observed that ADAMTS13 activity is 5% higher in participants with prevalent diabetes compared to participants without prevalent diabetes [31]. In the current study we excluded participants with prevalent diabetes, and instead focused on the risk of future diabetes. The results of this study are therefore largely independent from the results of previous cross-sectional study.

Our results for VWF are consistent with previous studies. VWF has been associated with incident diabetes in a range of studies [13-17], but in general the association weakened

after adjustment for confounders and became non-significant. In the Framingham Heart Study however, VWF remained significantly associated after adjustment for a wide range of potential confounders, including insulin resistance [14]. Similarly, in the Coronary Artery Risk Development in Young Adults study, VWF remained associated with insulin resistance after multivariable adjustment [32]. VWF is a marker of endothelial dysfunction, and this is thought to explain the association between VWF and diabetes [18]. We report an interaction between ADAMTS13 activity and VWF, with the largest effect of ADAMTS13 activity among participants in the highest quartile of VWF. This interaction suggests that the effect of ADAMTS13 is mainly present in individuals with advanced endothelial dysfunction.

The mechanism underlying the association of ADAMTS13 activity with diabetes remains unclear. Because the association was robust to the adjustment for baseline fasting glucose and insulin, and because ADAMTS13 activity was also associated with incident prediabetes, the possibility of reverse causation is limited. The latter association, for example, implies that high ADAMTS13 activity in individuals with healthy glucose metabolism is associated with the development the early subclinical stages of type 2 diabetes. However, the association between ADAMTS13 activity and diabetes is unlikely to be explained by its only robustly identified function as a cleaving protease of VWF, because in that case we would expect VWF (prothrombotic) and ADAMTS13 activity (antithrombotic) to be associated with diabetes in opposite directions. An alternative hypothesis is an additional proteolytic functionality of ADAMTS13 beyond VWF cleavage. After an initial interaction with globular VWF, ADAMTS13 undergoes a conformational change that increases its ability to break down VWF [33]. Recent research suggests that the conformational change not only increases the ability of ADAMTS13 to break down VWF, but also allows it to break down other proteins such as fibrinogen [34]. ADAMTS13 activity, as measured in our study, may partly reflect this process. As such, the observed association between ADAMTS13 activity and incident type 2 diabetes might be explained by the interaction of ADAMTS13 with one or more currently unknown proteins. Finally, the association could be explained by pathways responding to ADAMTS13. For example, there is preliminary evidence that ADAMTS13 upregulates the expression of vascular endothelial growth factor, a protein involved in angiogenesis that may contribute to the development of type 2 diabetes [35, 36]. ADAMTS13 may similarly activate other pathways that lead to the development of type 2 diabetes. However, since ADAMTS13 was discovered in 2001 most research has focused on its interactions with VWF and its role in thrombotic thrombocytopenic purpura. Therefore, we believe that further research is required to elucidate other pathways affected by ADAMTS13.

We measured ADAMTS13 activity using the FRETS assay, which is based on a synthetic peptide spanning the VWF cleavage site [27]. ADAMTS13 antigen is an alternative mea-

surement, which corresponds to the abundance of ADAMTS13. Future studies should investigate whether ADAMTS13 activity or antigen is most strongly associated to diabetes. If the association with diabetes is strongest with ADAMTS13 antigen, then the association of markers of ADAMTS13 gene expression, synthesis, secretion, and degradation with diabetes should be explored. Alternatively, a stronger association with ADAMTS13 activity points towards a downstream implication of VWF cleavage, albeit not the decreased activity of VWF itself.

The strengths of our study include the comprehensive assessment of incident diabetes and prediabetes, using medical records, linkage with pharmacies in the study area, and standardized blood glucose measurements at each of the follow up visits. Additionally, we used data from a well-characterized prospective population-based cohort study, which allowed us to correct for a wide range of covariates. We used a long follow up period, and adjusted for baseline fasting glucose and insulin to reduce the possibility of reverse causation. By also examining associations with incident prediabetes, we provide insight into the early development of subclinical disease.

The main limitation of our study is that, as in all observational studies, we cannot rule out residual confounding. In addition, we included individuals aged 55 years and older and effect estimates might not be generalizable to younger ages. A second limitation of this study is that we did not measure VWF activity or the ratio of small inactive VWF to large active VWF. Such measurements would provide insight into whether the observed association is related to the proteolysis of VWF by ADAMTS13.

In conclusion, we identified ADAMTS13 activity as a novel independent marker of incident diabetes, associated with both diabetes and prediabetes. Future research is necessary to confirm this association and to elucidate the biology underlying this association. Exploration of alternative mechanisms of ADAMTS13 beyond VWF cleavage is warranted as the association may not be explained by its antithrombotic function.

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SUPPLEMENTAL MATERIAL

Supplementary Table 1: Cross-sectional association of ADAMTS13 activity and VWF antigen (per SD) with fasting glucose and natural log transformed fasting insulin.

	ADAMTS13 activity		VWF antigen	
	β coefficient (95%CI)	*P*-value	β coefficient (95%CI)	*P*-value
Glucose				
Model 1	0.03 (0.01, 0.04)	0.001	0.01 (-0.00, 0.03)	0.08
Model 2	0.02 (0.01, 0.04)	0.003	-0.01 (-0.02, 0.01)	0.4
Insulin				
Model 1	0.06 (0.04, 0.07)	6×10^{-15}	0.07 (0.05, 0.08)	1×10^{-19}
Model 2	0.05 (0.04, 0.06)	4×10^{-15}	0.03 (0.02, 0.05)	2×10^{-8}

β coefficient refers to the 1 unit increase in fasting glucose (mmol/L) or insulin (natural log transformed pmol/L) per 1 standard deviation increase in VWF antigen or ADAMTS13 activity.

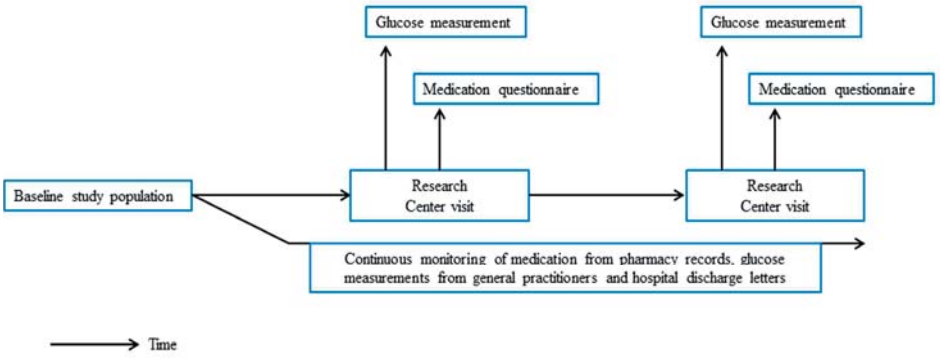
Adjustments: Model 1: Adjusted for age, sex, and cohort. Model 2: Additionally adjusted for HDL and total cholesterol, lipid-lowering medication, body-mass index, CRP, former smoking, current smoking, antithrombotic medication, ALAT, white blood cell count, systolic blood pressure, antihypertensive medication, and prevalent CHD. The analysis of VWF antigen was adjusted for ADAMTS13 activity and vice versa. HDL cholesterol, CRP, and ALAT were natural log transformed.

Supplementary Table 2: Association of ADAMTS13 activity and VWF antigen (per SD) with incident diabetes after exclusions based on disease and medication use at baseline*.

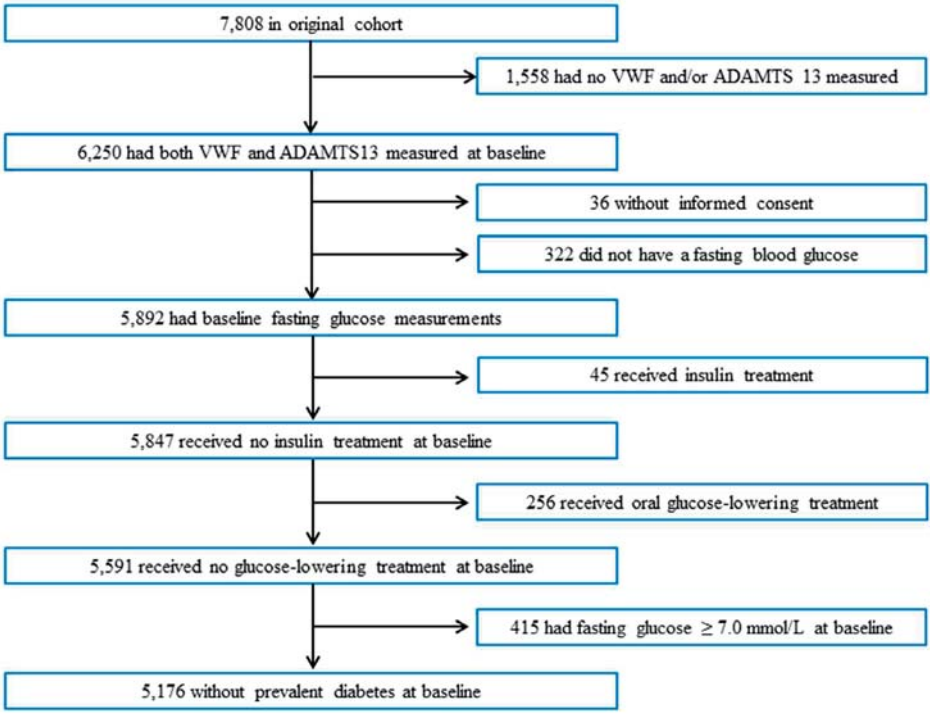
	ADAMTS13 Activity		VWF antigen	
	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value
Excluding cases of prevalent CHD: 565 events in 4674 participants				
Model 1	1.20 (1.10, 1.30)	0.00007	1.14 (1.05, 1.25)	0.002
Model 2	1.17 (1.07, 1.27)	0.0004	1.09 (1.00, 1.19)	0.06
Model 3	1.18 (1.08, 1.28)	0.0002	1.09 (1.00, 1.20)	0.05
Excluding antithrombotic medication users: 490 events in 4062 participants				
Model 1	1.20 (1.09, 1.32)	0.0001	1.14 (1.04, 1.25)	0.006
Model 2	1.15 (1.05, 1.27)	0.002	1.07 (0.98, 1.18)	0.1
Model 3	1.16 (1.06, 1.27)	0.001	1.09 (0.99, 1.20)	0.08
Excluding lipid-lowering medication users: 526 events in 4372 participants				
Model 1	1.18 (1.08, 1.29)	0.0005	1.12 (1.03, 1.23)	0.01
Model 2	1.15 (1.05, 1.26)	0.002	1.05 (0.96, 1.15)	0.3
Model 3	1.15 (1.05, 1.25)	0.002	1.07 (0.98, 1.17)	0.2
Excluding antihypertensive medication users: 415 events in 3837 participants				
Model 1	1.19 (1.07, 1.32)	0.001	1.12 (1.01, 1.24)	0.03
Model 2	1.20 (1.08, 1.33)	0.0005	1.06 (0.96, 1.18)	0.2
Model 3	1.20 (1.08, 1.33)	0.0005	1.11 (1.00, 1.23)	0.05

*Exclusions were based on non-imputed variables.

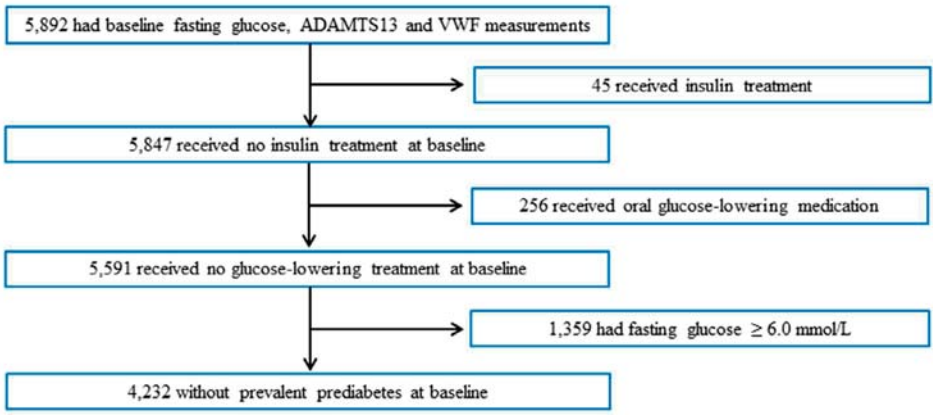
Adjustments: Model 1: Adjusted for age, sex, and cohort. Model 2: Additionally adjusted for HDL and total cholesterol, lipid-lowering medication, body-mass index, CRP, current smoking, antithrombotic medication, ALAT, white blood cell count, systolic blood pressure, antihypertensive medication, and prevalent CHD. The analysis of VWF antigen was adjusted for ADAMTS13 activity and vice versa. Model 3: Additionally adjusted for glucose and insulin levels. HDL cholesterol, CRP, ALAT, and insulin were natural log transformed when used.



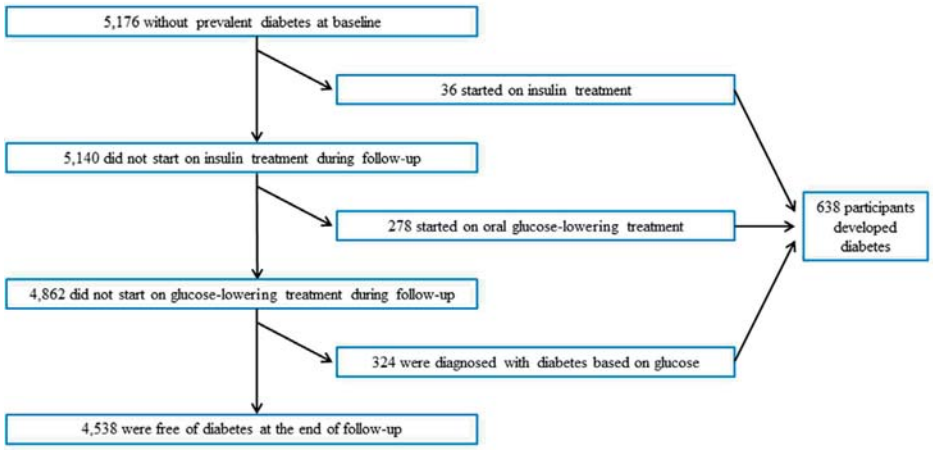
Supplementary Figure 1: Schematic representation of the follow-up design.



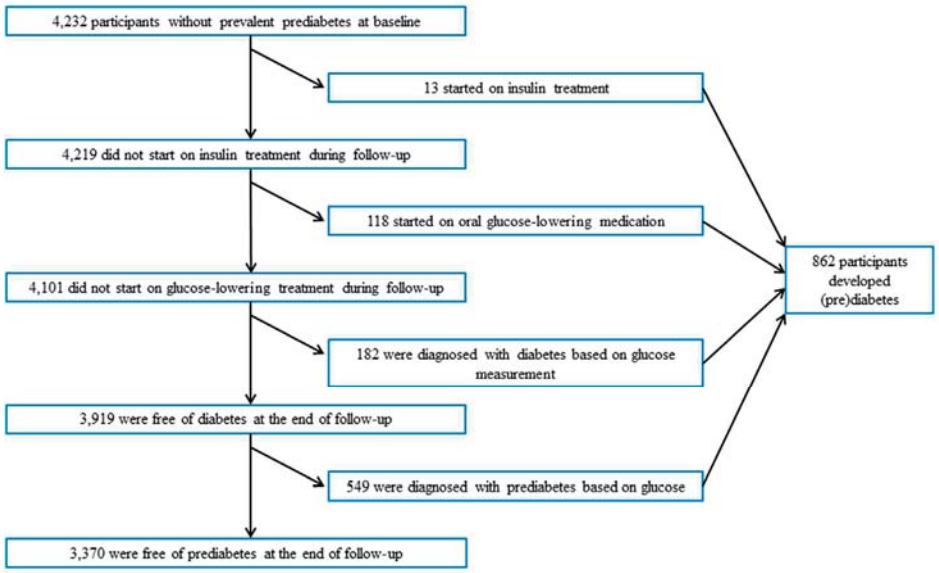
Supplementary Figure 2: Selection of the study population at baseline for incident diabetes follow-up.



Supplementary Figure 3: Selection of the study population at baseline for incident prediabetes follow-up.



Supplementary Figure 4: Follow-up of incident diabetes events in the study population.



Supplementary Figure 5: Follow-up of incident prediabetes events in the study population.

PART V

General discussion

Chapter 9

General discussion

Given the ongoing global epidemic of obesity and obesity-related diseases, research on this topic is of vital importance. Both metabolic syndrome and type 2 diabetes are obesity-related health conditions. In this thesis, I studied the incidence and prevalence of metabolic syndrome, type 2 diabetes, and associated cardiovascular disease and mortality. Furthermore, both the prevalence of well-known type 2 diabetes-associated risk alleles, a novel hematological substance as a risk marker and a novel candidate gene were investigated. Here, I will discuss the findings of my research, methodological issues, potential implications for clinical practice and directions for future research.

9.1 MAIN FINDINGS

PART II: METABOLIC SYNDROME IN MODERN SOCIETY: DEFINITIONS AND PREDICTIVE ABILITY

Metabolic syndrome is a combination of risk factors for diabetes mellitus and cardiovascular disease that often cluster together. Although considered a useful tool in categorizing patients, its added value for prediction of cardiometabolic events above its individual components was unclear (1–3), especially since there are several definitions of metabolic syndrome (4–7). In part II of this thesis, we have investigated the prevalence and longitudinal hazard ratios for cardiometabolic endpoints of different definitions and components of metabolic syndrome in a prospective population-based study.

The clinical value of metabolic syndrome and risks of cardiometabolic events and mortality in the elderly: the Rotterdam Study (Chapter 3)

Since the introduction of insulin resistance in type 2 diabetes by Himsworth (8) and the postulation of insulin resistance as the underlying cause of dyslipidemia and hypertension clustered in the ‘insulin resistance syndrome’ by Reaven in 1988 (9), there has been much debate on the metabolic syndrome. The debate primarily questions the added value of the metabolic syndrome in prediction and treatment of cardiovascular disease and diabetes (10). Also, there is still no concluding evidence of a common pathophysiological pathway that has been postulated some decades ago. Finally, the numerous definitions of metabolic syndrome make it challenging to compare population estimates. In our studies, we found metabolic syndrome to be highly prevalent with prevalence estimates of 19.4 to 42.4%, depending on the definition, in line with previous reports in middle-aged and elderly populations in the USA and Europe (11–17). The high variability in prevalence between the three definitions reflects the inhomogeneity of metabolic syndrome definitions. While the EGIR-definition has a more restrictive nature towards the selection of insulin-resistant non-diabetic patients, the IDF and AHA/NHLBI emphasize obesity. Since the prevalence and incidence of obesity are rising, we have found higher prevalence estimate

of metabolic syndrome according to these latter definitions. We have investigated each definition's hazard ratio for cardiometabolic disease: metabolic syndrome has a strong, mainly hyperglycemia-based, risk associations with incident diabetes and only weak associations with cardiovascular disease and mortality. However, after correction for its components, none of the definitions remained significantly associated with cardiometabolic endpoints. Which leads us to the following considerations. First, our results add to the evidence that metabolic syndrome has no additional value on top of its components in risk association for cardiometabolic disease in the general population. This has been observed previously (1–3,18,19). In line, single glucose measures were more predictive for incident diabetes than a diagnosis of metabolic syndrome (1,20,21). In our study, a diagnosis of metabolic syndrome had only a weak association with CVD-events which disappeared after correction for its own components. We already knew that metabolic syndrome is more related to diabetes than to CVD-events (2) and metabolic syndrome is outperformed by risk-assessment tests of the prediction of cardiovascular disease (2,20,22–24). This could be a result of weakening by dichotomization since the components of metabolic syndrome are continuous variables losing valuable information when forced into dichotomous values. It could also be the result of a lack of risk factors for cardiovascular disease not included in the definitions of metabolic syndrome like smoking, age and low-density lipoprotein (LDL) cholesterol. Our results re-emphasize the limited clinical applicability of metabolic syndrome in risk prediction of cardiovascular disease and diabetes. There are also no consequences for treatment when a patient is diagnosed with metabolic syndrome. Hypertension, diabetes and dyslipidemia will be treated according to the current guidelines (25–29). An effective treatment option to target all components at once is improving lifestyle. Fasting blood glucose, blood pressure, waist circumference and triglycerides improve simultaneously when reducing body weight (30–32). Lifestyle modification should be the primary focus of a physician when faced with each separate metabolic entity instead of a therapeutic response on a diagnosis of metabolic syndrome. After lifestyle, residual risk for cardiovascular disease needs to be treated with appropriate drugs (10).

The metabolic syndrome did not have an additional value in risk prediction and treatment, but it may be useful in creating awareness in both physician and patient. Since the components are more often found in combination than chance would dictate (33), a physician will be triggered to look for other risk factors when faced with one component. Furthermore, a diagnosis of metabolic syndrome can provide a physician with a more powerful communication tool in order to make a patient aware, take actions and be more adherent to therapy compared to the message of each separate risk factor (34). By clarifying a patient's perceived risk, one can increase chances of participating in lifestyle interventions (35,36).

Apart from its role in risk prediction and risk communication, the metabolic syndrome can be of importance from a pathophysiological perspective. Numerous studies have aimed to explain the coherence of cardiovascular risk factors such as insulin resistance, central obesity and hypertension. Targeting the initiating events that lead to metabolic syndrome can prevent or delay the onset of cardiovascular disease.

Initially, insulin resistance was seen as the underlying cause of metabolic syndrome (9,37). The cause of the insulin resistance itself, however, remains to be clarified. One explanation was the abdominal fat and oversupply of fatty acids in central obesity. Adipose tissue is both metabolically and immunologically active (38), secreting cytokines and adipokines (39,40). Due to nutritional excess, adipocytes undergo hypertrophy that can lead to cells that outgrow their blood supply (41), leading to inflammation, secretion of cytokines like interleukin (IL)-6 and decreased levels of adiponectin and cell stress with increased ROS production (42–44). So a state of lipid oversupply in peripheral tissues leads to chronic inflammation (45,46), leading to impaired insulin signalling (47–51). In metabolic syndrome, increased levels of inflammation have been found in the liver, intestines, and adipose depots (52–54). Obese subjects with metabolic syndrome and weight gaining subjects have higher levels of oxidative stress biomarkers compared to obese subjects without metabolic syndrome and lean subjects (55–58). Treatment of inflammation with salicylate leads to improved insulin sensitivity and lipid profiles in prediabetic and type 2 diabetes patients (59–61). Another factor that is related to the metabolic syndrome could be related to the microbiome**.** Studies have already shown an association between fecal microbiota and type 2 diabetes and obesity (62). Modifying the gut microbiota can be an approach to target metabolic syndrome (63).

Both lifestyle modification (31,64,65), gastric bypass (66,67), medication that directly interferes with pro-inflammatory pathways, the microbiome or the relieve of oxidative stress could stop or slow down the deteriorating effects of metabolic syndrome. Although the metabolic syndrome is not as useful in clinical practice for prediction purposes, through pathophysiological research, we can find potentially new therapeutic targets to prevent obesity-associated disease.

PART III: TYPE 2 DIABETES MELLITUS: LIFETIME RISK AND DISEASE COURSE

In the year 2035, more than half a billion people worldwide are expected to live with diabetes (68). Preventive strategies are very important in averting this epidemic. An effective risk communication strategy from the physician to the patient in the prevention of prediabetes and diabetes is vital. Lifetime risks reflect precise estimates of an individual's risk of developing a disease throughout a lifespan. Patients prefer lifetime risk over 10-year and 20-year risk estimates and absolute risk over relative risk. The absolute risks are

clear and simplistic presentations of risks of disease and projections of long-term benefits of preventive measures are possible, which are more likely to affect a patient's perception of effectiveness (69,70). In part III of this thesis, we have assessed lifetime risk of prediabetes, diabetes and insulin dependency in a prospective, population-based cohort study (Chapter 4). Furthermore, we have assessed the effect of applying two different glucose cut-off values to define prediabetes on prevalence and lifetime risk estimates to develop type 2 diabetes (chapter 5).

Lifetime risk of progression from prediabetes to type 2 diabetes: a prospective cohort study (Chapter 4)

A raised blood glucose level just below the threshold for a diagnosis of type 2 diabetes is a major risk factor for diabetes (71–73) referred to as prediabetes. Prediabetes increases the risk for diabetes but also for cardiovascular disease, cancer and mortality (74–76). Previous analysis has shown that patients evolve through a prediabetic state to eventually become an incident case of diabetes (77). By applying both pharmacological and non-pharmacological interventions in prediabetes, patients can lower their diabetes-risk (73–76)(71,73,78–83), thus averting a diagnosis of diabetes. Since fasting and post-load glucose levels increased linearly no less than 6 years before a diagnosis of diabetes (77), screening for prediabetes could be relevant in the prevention of diabetes.

Due to our study, we now know that at age 45, one in three individuals in the Netherlands will develop diabetes, one in two will develop prediabetes and that three out of four individuals that have glucose ranges in the prediabetes range at age 45 will progress to diabetes. Our results came from a large, prospective, community-dwelling population, and indicate the magnitude of the health issue we face. We are the first to address lifetime risk of diabetes, prediabetes and progression from prediabetes to diabetes based on data retrieved from active prospective follow-up and actual measurements instead of cross-sectional data (84) and simulated models based on questionnaires (85). We see a resemblance of our percentage of progression from prediabetes to diabetes (70 vs 75%) (79–82)(71,73,86) in US-based research, which strengthens our belief in the vast extent of this health issue.

Although it is a well-known risk factor for diabetes, we noted a substantial obesity-related increase in lifetime risk on prediabetes, diabetes and insulin use. By providing these estimates, including the obesity-relation, the communication between patient and physician could be substantially improved. From literature, it is clear that by increasing the impact of risk-communication as a physician, therapy-adherence will improve (87). The impact that risk communication has on a patient's behaviour depends on its presentation: absolute risk has more impact than relative risk (88). Alternative presentations, such as 30-year risk

or lifetime risk have more impact than 10-year risk and are therefore recommended by the American Heart Association (89) for young people, as they respond better to these estimates (90). The estimates we present here are easy to interpret for both the experts and lays and will thereby facilitate clear communication.

Lifetime risk to progress from prediabetes to type 2 diabetes among women and men: a comparison between American Diabetes ASsociation and World Health Organization diagnostic criteria (Chapter 5)

The American Diabetes Association (ADA) and the World Health Organisation (WHO) define prediabetes differently (91,92). The ADA definition has a lower threshold of 5.6mmol/L for fasting glucose and diagnoses a larger portion of the population with prediabetes compared to the WHO's threshold of 6.1 mmol/L. In our population-based study, 39% of all participants and half the elderly had ADA-defined prediabetes, comparable to Vistisen 2018 (93). These high prevalence estimates raise questions on the definition of prediabetes since it is used to identify high-risk individuals eligible for interventions to prevent diabetes. In studies such as the DPP 19% of prediabetes returned to normoglycemia within 10 years using lifestyle, medication or both (78), through treatment of pioglitazone 48% returned to normoglycemia in 2 to 4 years (94) and metformin showing a risk reduction of 18% in 15 years after randomization (81,95). However, since up to half our population is identified as a high-risk individual, we question the feasibility of ADA-prediabetes screening and prevention of diabetes. We evaluated this question from two perspectives: 1) the prevention of incident diabetes and 2) the prevention of cardiovascular disease.

Lowering the cut-off value of ADA-prediabetes is based on results from progression to diabetes among Pima-Indians and a study from the island of Mauritius (96,97). The ADA's motivation for the lower cut-off value was to improve the prediction of diabetes risk (91). Indeed, numerous trials and meta-analyses have found diet, exercise and drug-based interventions to be successful in reducing the risk of diabetes with 40 to 60% in high-risk individuals (79,83,98). In the follow-up studies, it became clear that this was merely a delay in the onset of diabetes by two to four years than prevention as such (95,99–101). Recent meta-analyses confirm the effectiveness of both diet and physical activity- interventions in high-risk individuals in decreasing progression rates of diabetes (80,102,103). However, there is no evidence that the effectiveness of prevention strategies increases by lowering the glucose cut-off value. Furthermore, results from prevention studies show that effectiveness is related to the intensity and the length of programs (102,103). Doubling the prevalence of high-risk individuals will increase the need for high-intensity prevention programs and will put a major burden on the health care budget. In our population, a 45-year old individual with ADA- prediabetes has a lifetime risk of 51.7% to develop diabetes, whereas at age 75 a lifetime risk of 29.4% remains. So at age 45, half the high-risk

individuals and at age 75, almost two-thirds will never develop diabetes regardless of a costly prevention program.

Although prediabetes is indisputably associated with cardiovascular disease, studies and meta-analyses on prevention of prediabetes found no reduction in microvascular (104), cardiovascular disease and mortality (80,99,102,105–108). Although there was a 50% reduction in retinopathy in the Da Qing study, there was no effect on the incidence of nephropathy and neuropathy (109). In the same follow-up study, Zhang reported a 38% and 20% reduction in all-cause and cardiovascular mortality, respectively (100). This is surprising since the result was only seen in women and the diabetes onset in this study was delayed by only 3.6 years. No other trial study has reported similar effects on cardiovascular disease and mortality. To explain the results of prediabetes prevention on cardiovascular disease, I would like to address a study by Vistisen et al. (93) in which subgroups of cardiovascular disease and all-cause mortality in the Whitehall study were studied. They found that there was no optimal cut-off point in fasting glucose to predict cardiovascular disease. Furthermore, no association in prediabetes between fasting glucose and cardiovascular disease after adjustment for cardiovascular risk factors and demographics remained. Prevention of prediabetes lacking risk reduction for CVD can, therefore, be a result of mere clustering of risk factors in prediabetes then the risk associated with the sole entity itself (93). Remains to be said that there is no additional evidence that a lower glucose cut-off improves the effect of prediabetes prevention on cardiovascular morbidity and mortality.

In addition, one has to realize that interventions even in high-risk populations are more effective in trial-based setting compared to the real-world (103,110–114).

The enormous prevalence estimate of prediabetes in our population and the various results in the effectiveness of prevention in prediabetes in a variety of endpoints make us question whether lowering the glucose cut-off value, as has been done in ADA-definition, would be beneficial in determining prevention policy. We would merely recommend research on preventive interventions in type 2 diabetes at population level or health care system instead of a screen-and-treat policy (112,115). Until then, we follow Dutch guidelines and propose WHO-defined glucose cut-off values for prediabetes screening. Our and other results on prediabetes still calls for extensive research and long-term economic evaluations of each preventive effort to determine which suits the obesity epidemic best.

PART IV: RISK FACTORS AND COMPLICATIONS IN TYPE 2 DIABETES

The pathogenesis of type 2 diabetes is heterogeneous and contains both genetic and environmental factors. Apart from well-known risk factors such as family history (116),

ethnicity (117), obesity (117,118) and impaired glucose tolerance (72), there is a constant search for new associated risk factors and risk alleles. We aim to contribute to this search by performing the DIAGENE-study (chapter 6), a study on prevalence and risk associations of vascular complications in both primary care and outpatient setting of patients with type 2 diabetes. Furthermore, we have performed a candidate-gene study on polymorphisms in proline transporter SLC6A20 and susceptibility to Type 2 diabetes (chapter 7). Finally, we have investigated whether ADAMTS13 activity is independently associated with incident prediabetes and type 2 diabetes (Chapter 8).

The DiaGene Study; the prevalence of macro- and microvascular disease (Chapter 6)

Despite the preventive treatment of cardiovascular risk factors in patients with type 2 diabetes (25,26,119–124) a substantial portion of patients with type 2 diabetes will develop vascular complications (76,125–127). By collecting the DiaGene cohort our aim was not only to analyze the layout of diabetes care in the Netherlands but also to assess the burden of vascular disease in the diabetes population and elucidate potential new metabolic, genetic and environmental risk factors. Furthermore, we aimed to confirm the association of well-known genetic risk variants with type 2 diabetes in our population.

One major characteristic of Dutch diabetes care is the subdivision between primary and outpatient hospital care (128,129). Due to national referral agreements, there were noticeable differences between the patients in the primary care setting and outpatient clinic, in the DiaGene Study. The complicated nature of diabetes in the outpatient clinic was reflected by a younger age of onset, longer duration of diabetes, higher HbA1c, more insulin treatment, a greater proportion of low eGFR and significantly more prevalent microvascular and macrovascular complications. However, both lipids and blood pressure were significantly lower and thus better treated compared to primary care. The prevalence estimates of both microvascular and macrovascular complications are comparable to other diabetes populations in the Netherlands, UK and France. As for our genetic analyses, we have confirmed the prevalence and odds-ratios of well-known T2D-risk variants. These had the same direction of association to type 2 diabetes as earlier reported (130–132). We use these results as proof of a comparable genetic background of our type 2 diabetes population compared to other genetic studies. Up to his point, our study is mainly exploratory and confirmatory. However, the DiaGene study already proved its value by participating in a large meta-analysis of the DIAGRAM-consortium in which new type 2 diabetes-risk alleles where discovered (133). Furthermore, we are planning multi-layer omics on top of genome-wide association analyses to further elucidate determinants of T2DM and its complications. The availability of prospective data improves the quality of our analyses, and will a focus during the forthcoming years. Recently, our group has published on glycan patterns and their association with type 2 diabetes (134,135).

SLC6A20; incident type 2 diabetes (Chapter 7)

SCL6A20 is a member of the solute carrier family 6 (SLC6) which has 20 members (136). From a biological perspective in type 2 diabetes, SLC6A20 is interesting since it is a proline transporter and therefore, an essential component in the proline metabolism (136). Since proline is synthesized from dietary glutamate, which is one of the major gluconeogenic precursors in the kidney (137) and small intestines (138) it might influence type 2 diabetes risk. In a previous study, performed by Patterson et al. in non-human primates (139), SLC6A20 had reduced activity in the kidneys of primates and mice with type 2 diabetes. In humans, the relation of SLC6A20 with type 2 diabetes remains unclear. Therefore, we studied genetic variance in the SLC6A20 gene in relation to type 2 diabetes in a prospective population-based study as discovery population and an additional multi-ethnic replication cohort. In this study, we found the A allele of rs1306384 in the SLC6A20 gene to have a highly significant association with type 2 diabetes. We were the first to describe this polymorphism in relation to type 2 diabetes. Since the era of genome-wide association studies and meta-analyses of genome-wide association studies, focusing on one particular part in the genome in a relatively small number of participants might appear as outdated. Given the timeframe, however, we have selected a locus within a high potential causal pathway for type 2 diabetes. Furthermore, we have replicated our results in a meta-analysis of two different multi-ethnic populations and corrected for multiple testing. In a large-scale genome-wide meta-analysis on genome-wide association studies in type 2 diabetes by morris in 2012 (131), the variant has not been related to diabetes risk, despite the highly significant P-value in our meta-analysis. This could be an effect of age. In an age-stratified analysis that we performed in our population, we found a stronger association of the SNP with diabetes in the old compared to the young persons of the population. The large meta-analysis of Morris includes almost 150,000 individuals but of different age categories, and this could be the reason that the SNP did not reach significance.

In other words, the genetic variance of SLC6A20 can manifest itself at high age through interaction with ageing or being part of a degenerative process. The latter could be an effect of epigenetic factors being more present at high age. Therefore, we concluded that our results are not generalizable to younger populations.

ADAMTS13 activity; associated risk of diabetes and prediabetes (Chapter 8)

ADAMTS13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif member 13) was discovered in 2001 and is best known for its function in cleaving von Willebrand factor (VWF) multimers (140,141) . Relatively low ADAMTS13 activity is associated with an increased risk of vascular disease (142–145) and vascular complications in T2D (146,147) and elevated levels of VWF have been associated with type 2 diabetes (142–145). We, therefore, hypothesized that ADAMTS13 levels are inversely associated with the risk of

diabetes. We based our hypothesis on the biological interaction of VWF and ADAMTS13 in thrombosis potentially exacerbating small vessel disease, which could contribute to the development of diabetes (148–151).

Our results, however, contradict the hypothesis mentioned above. In our prospective population-based study, ADAMTS13 activity was associated with an increased risk of incident diabetes and prediabetes after adjustment for known risk factors. Since we are the first to find evidence to suggest a causal role for ADAMTS13 in type 2 diabetes, we have to put our results in perspective. In earlier work, we had already found 5% higher levels of ADAMTS13 activity in participants with prevalent diabetes compared to participants without diabetes (145,152). Previous cross-sectional studies found no difference (147) or lower levels of ADAMTS13 activity in a small study on type 1 diabetes (153). An interesting observation from the latter study was a positive correlation between HbA1c-levels and ADAMTS13 activity. The authors proposed that it reflects high levels of metabolic stress with an increase of hepatic production and release of ADAMTS13. Indeed, ADAMTS13 is primarily synthesized (154) in the liver and in cases of acute liver injury or liver failure a dramatic decrease in circulating levels of ADAMTS13 takes place (155). The upregulation of ADAMTS13 activity was previously observed in mice models of diabetes (156) and rat models of cholestasis and steatohepatitis (157). However, the aforementioned results suggest reverse causation in the association of ADAMTS13 with incident diabetes.

In our population-based setting, however, we find reverse causation to be unlikely since ADAMTS13 is associated to both incident prediabetes and diabetes in a prospective setting and this association is robust to adjustment for fasting glucose and fasting insulin. The underlying mechanism of the association of ADAMTS13 with incident diabetes remains to be explored. Since the association we found is opposite to what we expected based on its known function as a cleaving protease, we hypothesize the association to be mediated through alternative functionalities of ADAMTS13 or through pathways that respond to ADAMTS13, one of which is regulation of inflammation (158,159). A study by Chauhan found a role for ADAMTS13 in down-regulation of inflammation in skin and vein samples in mouse models (160). Alternative functionalities have been described for ADAMTS13 in the degradation of extracellular matrix (159), regulating the permeability between brain and blood (161) and having pro- or anti-angiogenic effects through regulation of VEGF activity (162). In research done by Gandhi et al., ADAMTS13 was found to reduce early atherosclerosis in ApoE -/- mice (163,164) and plays a role in the remodeling of brain tissue after cerebral ischemia (165–167). De Meyer found ADAMTS13 to reduce inflammatory responses after myocardial infarction (168). In a study by Wolters et al. low ADAMTS13 activity was associated with risk of dementia during a follow-up period of 15 years(169). One other association was found in ADAMTS13 -/- mice in which diabetes was induced. These mice suffered

from premature death that was non-thrombotic and thus possibly through an alternative pathway (170). Hence, there is plenty of evidence that suggests additional pathways to be affected by ADAMTS13 beyond its role in thrombotic thrombocytopenic purpura.

Therefore, we believe that further research is necessary to replicate our findings and to elucidate the underlying causal pathways. Finding the causality between ADAMTS13 and diabetes would be important for biological understanding in itself but also to find possible new therapeutic targets. Furthermore, ADAMTS13 appears to include a risk portion that is not yet covered by current risk factors in diabetes. By establishing ADAMTS13 as a risk factor and adding it to the current set of risk factors, diabetes risk prediction can be improved. So being said, ADAMTS13 could be a potential biomarker in the setting of diabetes.

9.2 METHODOLOGICAL TOPICS

There are some methodological issues in this thesis that need to be addressed to evaluate the results of my research properly.

STUDY POPULATIONS

The DiaGene study

The DiaGene study is a multi-centre, extensively phenotyped type 2 diabetes cohort study with concurrent inclusion of diabetes-free individuals. The population was included in all four medical centers in the city of Eindhoven with the intention to invite all patients with type 2 diabetes. Eventually, we included 1886 patients from an estimated total of 10000 patients with type 2 diabetes in the Eindhoven region. We cannot measure or calculate the representability of our study sample. The selection of patients was not related to exposure or outcome. However, the possibility of a form of selection bias needs to be considered. In a population-based study, there is a chance of the so-called ‘healthy volunteer effect’ or ‘healthy worker effect’ that causes an underestimation of disease risk estimations since there is a selection towards healthy participants that have lower risks compared to the ‘real’ population (171). However, the prevalence estimates of vascular complications in the DiaGene in comparison to other populations of type 2 diabetes do not lead us to believe there has been a strong effect of healthy volunteer selection bias in our study.

Conversely, individuals that experience a disease or could be more likely to participate in a study on that specific disease. However, as the DiaGene study has a prospective design for vascular complications, participants are considered not to take their decision based on

future events to happen, thus making participation selection based upon this argument less likely (172).

There are some limitations concerning data in the DiaGene study. First, although we have retrieved biometrical data at the time of inclusion, the majority of laboratory measurements are collected within 6 months prior or after the moment of inclusion. For some measurements, this interval lies within a year.

Finally, we did have to rely on meticulous record-keeping in medical files for data on micro- and macrovascular events. For macrovascular events in primary care, self-reported data were used, which we validated with data from hospital-based participants. We found an actual underestimation for myocardial infarction based on self-reported data compared to hospital discharge data. This is a phenomenon that has been described before (173). Although we acknowledge all the aforementioned, the quality of data is inherent to the cohort study set-up and will be comparable to other studies that have the same design. Furthermore, we have had two independent investigators to collect the data and reach consensus on discrepancies so that we have optimized data-reliability.

The Rotterdam Study

The Rotterdam Study is a large prospective population-based study with long-term follow-up and high-quality data on incident prediabetes and type 2 diabetes using general practitioner's records, electronic linkage with pharmacy dispensing records, hospital discharge letters and glucose measurements from each participant's study centre visit (174). Furthermore, through the active follow-up system, there is a comprehensive assessment of metabolic syndrome, incident diabetes, cardiovascular disease and mortality. Although there may be certain healthy-volunteer effect studies that require active participation, the chances of selection bias are low since it is a random selection of the population, and having a high participation rate (174). The study population is mainly European-Caucasian and has a relatively high age since the original study was set up to investigate diseases of the elderly. Results drawn from studies performed in the Rotterdam Study population should, therefore, be applied with caution to the European population, which is changing dynamically. One should be even more careful to apply our results to other ethnic populations.

Genetic association

In the era of genome-wide analyses, there is discussion and criticism on the specific candidate-gene approaches, as is described in chapter 7. One of the main issues is the lack of power in studies that perform candidate-gene analysis. The other point of criticism is the external validity in the sense of replication.

In the SLC6A20-study, we chose to analyze a polymorphism in a biologically plausible pathway and replicated it in a multi-ethnic population. By doing so, we increased power and addressed the issue of replication.

Although we have made a substantial effort to address issues related to candidate-gene studies, we acknowledge the limited power of candidate-gene studies and realize that in the context of multigenetic and multifactorial disease the study design belongs for large part to the past. At the time of writing, even after genome-wide association studies with increasing sample sizes and meta-analyses have discovered 128 distinct signals at 113 loci independently associated with type 2 diabetes (130,132,175,176), only 5-20% of the overall predisposition of type 2 diabetes is explained (175,177). Among others, there is a major contribution of genomics to the understanding of T2D disease risk. The incomplete overlap between the loci identified with glycemic traits and T2D showed that the risk of T2D is not entirely driven by the effects on glucose levels in healthy individuals. Even more, most T2D loci have primary roles in ß-cell function and less in insulin resistance (178). Eventually, this caused a paradigm shift in the understanding of the disease pathophysiology from primarily insulin resistance to ß-cell dysfunction. There are global efforts to perform sequencing studies with increasing sample size in search of less common or rare variants that explain heritability of type 2 diabetes (130,175). Other omic layers, like epigenomics may give further insight into the genetic architecture and the etiology of type 2 diabetes (176).

Lifetime risk approach

Lifetime risk estimation is a proven method to provide patients, physicians and policymakers with a risk estimate that is comprehensive but easy to interpret, thereby promoting efficient risk communication. For this reason, absolute risks are preferred over relative risks in providing effective risk communication to patients (88); and lifetime risk estimates are preferred over 10-year absolute risks in the patient-physician communication relation.

In chapter 4 and 5, we discuss the lifetime risk estimate of diabetes in the Netherlands. We have used a modified survival analysis in which there is a separate event when someone is death without ever having developed diabetes. This method has been applied in previous studies (179,180). Since the method accounts for the competing risk of death, it does not overestimate the remaining lifetime risk of diabetes in comparison to the Kaplan-Meier estimate. We calculate and mention lifetime risk for diabetes, however, only calculated the lifetime risk for diabetes at the age of 45. So one can, therefore, claim that we did not have a true estimate of diabetes risk. However, given the fact that the cumulative incidence before the age of 45 is low (181), the clinically relevant incidence resides in particular at young middle-age.

9.3 CLINICAL IMPLICATIONS

One of the primary goals of this thesis was to investigate the clinical applicability of the metabolic syndrome. Although we are not the only one to have addressed this issue, we now know that the metabolic syndrome does not add additional value in risk association to the diagnosis of its separate components when evaluating cardiometabolic endpoints. In other words, one does not treat a patient differently when someone is 'diagnosed' with metabolic syndrome, since each component has its own most effective treatment strategy. Furthermore, we readdress the variability in diagnosing the metabolic syndrome, which is reflected by the specific metabolic syndrome populations that emerge when applying AHA-NHLBI definition versus the EGIR-definition.

This thesis addresses the gigantic scale of type 2 diabetes in The Netherlands. One in three will develop diabetes; one in eleven will use insulin in their lifetime. By doing so, we have delivered a very profound and clear signal to preventive organizations in the Netherlands, both commercially and government-based. This is one of the most serious health issues we have ever faced. Furthermore, these clear estimates will have their effects in the doctor's office since the delivery of absolute lifetime risks will improve patient adherence and compliance to therapy.

We deliver another clear message in diabetes prevention by providing lifetime risks of diabetes in individuals having prediabetes. By showing an immense prevalence of ADA-defined prediabetes in our population and limited lifetime risk of diabetes, we question the feasibility of ADA-defined prediabetes in a screen-and-treat policy.

By giving a comprehensive description of the prevalence and complications of type 2 diabetes in the Netherlands and the different lines of care, I believe to have re-addressed the importance of diabetes research in the Netherlands. A substantial residual risk remains to be reduced through both pharmacological and lifestyle interventions. Furthermore, the DiaGene study has created a foundation for future omic studies such as our recent glycomic research that has been published by Lemmers et al. and Dotz et al. (134,135). Such omic layers are providing us with new pathophysiological insights, that can be used for preventive and therapeutic strategies. Also some of these omic layers may harbor valuable biomarkers to allow for early intervention to prevent disease or improve the outcome.

Last but not least, our work has created at least new hypothesis-generating results with respect to risk factors for type 2 diabetes. Although I am well aware that these risk factors are not directly clinically applicable at this moment, it is valuable to have mentioned them in this section. Hopefully, my optimism will stimulate the further clinical testing of variance

in proline metabolism in relation with the pathophysiology of type 2 diabetes. Furthermore, although we know ADAMTS13 for its cleaving function in von Willebrand multimers, there have been numerous new functions of ADAMTS13 published. My unexpected result that ADAMTS13 increased the risk of diabetes, offers a focus on the yet unknown possible functions of ADAMTS13 in future human research.

9.4 DIRECTIONS FOR FUTURE RESEARCH

Metabolic syndrome

Although the metabolic syndrome has lost its value as a separate clinical entity to be diagnosed in general patient populations, it may still have pathophysiological value for the underlying mechanism to be discovered. The way obesity and insulin resistance are related to hypertension, dyslipidemia and hyperglycemia remains to be explored and if so, offers possible therapeutic targets.

Genetic studies on type 2 diabetes

There has been a lot of genetic research in type 2 diabetes. From linkage studies, to candidate-gene approach, and even genome-wide association studies, whole-genome sequencing studies with increasing sample sizes. A total of 128 signals at 113 loci have been independently associated with type 2 diabetes.(130,175,178). Although increasingly more variants have been identified, the impact on clinical-decision-making has been modest. At the moment the disease-risk associated with genetic testing is still outperformed by risk factors that are more readily available and already incorporated in clinical assessment (182,183). Also, genetic risk counseling showed no significant effect on self-reported motivation or adherence in prevention programs for individuals having a high-risk for type 2 diabetes mellitus (184). Due to incomplete overlap of risk loci, glycemic traits and T2D on the one hand and primary roles in ß-cell function for most T2D loci on the other hand, genomic studies did cause a shift in disease pathophysiology insights from primary insulin resistance to ß-cell dysfunction (178). This shows that the field of genomics, together with other omic layers, is able to unravel new genes and biological mechanisms that ultimately can be a target for therapy. So, although not directly useful in clinical prediction, genomics can potentially play a major role in personalized precision medicine. I therefore advocate to increase collaboration between study groups to maximize population sizes in order to find more genetics variants. Furthermore, I would recommend adding omic layers such as glycomics, proteomics and microbiomics since this can increase the level of findings of biologically higher complexity. These efforts will eventually lead us to a better understanding of type 2 diabetes.

Studies on risk factors for type 2 diabetes

There is a long and complicated history of risk factor studies in type 2 diabetes. Although we have investigated risk factors on an epidemiological level, I believe future research in type 2 diabetes should mainly focus on a fundamental level. This should focus on alternative functionalities of ADAMTS13 and the way it is related to type 2 diabetes. Is it purely a factor in endothelial dysfunction or does it have additional functionalities in vascular remodelling that can be of importance in the disease-pathophysiology of disease?

Studies on prevention of type 2 diabetes

We have made it even more clear that type 2 diabetes is of cardinal importance in future health care. New prevention strategies are needed to control this growing epidemic. The prevention strategies focusing on specific risk factors can be considered to some extent as personalized medicine. The question is whether specific risk factors are needed to determine your treatment, given the general beneficial effects of lifestyle management on cardiovascular and diabetes risk. Therefore, apart from screen and treat strategies, we advocate a population-based approach directed at both the individuals and the environment. By integrating our medical research in governmental policy on obesity and type 2 diabetes prevention, one does not patronize but effectively prevent the disease from happening.

9.5 CONCLUDING REMARKS

The current thesis presents a variety of research on prevalence, lifetime risk, and conventional and genetic risk factors of type 2 diabetes and metabolic syndrome. In this chapter, I discussed the results, methodological issues and proposed future directions for research. Although aware of my humble position in nature's inscrutability, I believe the contents of this thesis will help fighting today's metabolic threats.

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Summary

A global pandemic of obesity-associated diseases such as type 2 diabetes, hypertension and dyslipidemia is emerging due to ageing, increased food-consumption and sedentary behavior. Research on this topic is of vital importance for more insight into the magnitude of the problem, associated risk factors and potential therapeutic targets to reduce disease burden.

Part I of the thesis starts with a general introduction on type 2 diabetes, obesity and metabolic syndrome. Chapter 1 furthermore contains an overview of risk factors for type 2 diabetes and its complications. It concludes with a section on lifetime risk estimation and the role of prediabetes in prevention of type 2 diabetes. Subsequently, the aims and scopes of this thesis are discussed in chapter 2.

In part II of the thesis, we studied the clinical value of the metabolic syndrome. Chapter 3 contains a study on prevalence of metabolic syndrome according to different definitions and risk of cardiometabolic endpoints in a community-dwelling population. We performed this study because controversy remained on whether a diagnosis of metabolic syndrome has added value on top of its individual components. Furthermore, various definitions of metabolic syndrome exist and our aim was to provide insight into the effect of different definitions on prevalence estimates and disease risk association. In our population, we conclude that metabolic syndrome is a highly prevalent condition with variability depending on which definition of metabolic syndrome is applied. Metabolic syndrome was associated with incident type 2 diabetes mellitus, coronary heart disease, stroke, cardiovascular mortality and all-cause mortality. However, all significant associations disappeared after correcting metabolic syndrome for its individual components. We conclude that metabolic syndrome does not have additional value on top of individual components for clinical practice.

Lifetime risk of type 2 diabetes and prediabetes is the subject of part III of this thesis. They provide an estimation of the cumulative risk of developing a disease during an individual's remaining lifespan. In chapter 4, we studied lifetime risk of type 2 diabetes in the Rotterdam Study. Through the data-analysis in a prospective population-based cohort setting with long-term follow-up and extensive data on both parameters of glucose metabolism and pharmacology, we here provided a unique setting for estimation of the burden of elevated blood glucose levels in the context of overall survival. The estimates of lifetime risk are easy to interpret for both experts and lays and will thereby facilitate communication. We found that the lifetime risk at age 45 to develop prediabetes is one in two and that 75% of study subjects that developed prediabetes progress to diabetes during their lifetime. A staggering one-in-three of the population at age 45 will develop diabetes throughout their lifetime. Of these, 50% will become insulin-dependent. We conclude that our results

demonstrate the immense burden of impaired glucose metabolism on our society and underline the importance of effective prevention strategies. The aspect of prevention is discussed in chapter 5. Here, we compare lifetime risk to progress to diabetes between the definition of prediabetes according to the World Health Organization (WHO) and the American Diabetes Association (ADA), the latter having a lower glucose cut-off value, in both women and men. We found that ADA-criteria for prediabetes diagnosed up to half of the population as prediabetes, more than doubling the prevalence estimates of prediabetes generated by WHO-criteria in each age category. At age 45, approximately half the individuals diagnosed with prediabetes according to ADA-definition and approximately two-thirds of WHO-defined prediabetes will eventually develop diabetes. Women with prediabetes had higher lifetime risk to progress to diabetes compared to men probably due to their worse metabolic state at baseline and this lifetime risk was substantially lower in women and men with ADA prediabetes as compared to WHO prediabetes. Therefore we conclude that ADA criteria result in the addition of relatively low risk individuals to the prediabetes diagnosis. More research is needed into the optimal sex- and age-specific prediabetes cutoff for the most efficient and cost-effective preventive strategies.

Part IV of this thesis handles with risk factors for type 2 diabetes and its complications on both epidemiological, biochemical and genetical level. Chapter 6 gives a description of the DiaGene study, which is a multicenter T2DM cohort study collected in the Netherlands in both primary and secondary care. The main purpose of the DiaGene Study is to study genetic, biochemical and environmental determinants of T2DM and its complications. In total, 1886 patients with type 2 diabetes and 854 controls were included. From their data, we concluded that considerable rates of macrovascular and microvascular complications are present in the Dutch diabetes population despite treatment. These prevalence rates are comparable to other type 2 diabetes populations. Apart from prevalence of complications, we describe 11 well-known type 2 diabetes genetic risk variants (in TCF7L2, PPARG-P12A, KCNJ11, FTO, IGF2BP2, DUSP9, CENTD2, THADA, HHEX, CDKAL1, KCNQ1) and observe similar associations compared to literature. Since we have genetic and biochemical data combined with an extensively phenotyped population, this study is well-suited to further elucidate pathophysiological disease mechanisms. One genetic association in type 2 diabetes is further discussed in chapter 7, where we have assessed the influence of genetic variance in the proline transporter SLC6A20 by performing a candidate-gene study. Since SLC6A20 is an essential component of proline metabolism and therefore can influence glucose homeostasis, we hypothesized that variants in the SLC6A20 gene influence type 2 diabetes risk in our population. Our study resulted in several findings. At first, the minor alleles of rs13062383, rs10461016 and rs2286489 increased the risk of Type 2 diabetes in the discovery cohort. However, in an Asian replication population and subsequent meta-analysis, a highly significant association of rs13062383 with Type 2 diabetes remained.

Therefore, we concluded that rs13062383 in SLC6A20 increased the susceptibility to type 2 diabetes in populations with different genetic backgrounds. In chapter 8, we report our study on the association of ADAMTS13-activity and VWF-antigen with incident diabetes. ADAMTS13 is a protease that breaks down von Willebrand factor (VWF) multimers into smaller, less active particles. VWF has been associated with an increased risk of incident type 2 diabetes mellitus. In our large prospective population-based study, ADAMTS13 activity was associated with an increased risk of incident diabetes and prediabetes after adjustment for known risk factors and VWF antigen. Therefore we concluded that ADAMTS13 activity appears to be an independent risk factor for incident prediabetes and type 2 diabetes. Since the association cannot be explained through its known function, an alternative hypothesis is an additional proteolytic functionality of ADAMTS13 beyond VWF cleavage.

Finally, part IV of the thesis contains the general discussion in chapter 9. Herein, I discuss the results in the context of present-day literature, methodological issues and proposed future directions for research. Summarizing the conclusions of this thesis are 1) the metabolic syndrome does not add additional value in risk association to the diagnosis of its separate components when evaluating cardiometabolic endpoints. 2) The scale of type 2 diabetes in The Netherlands is gigantic. One in three will develop diabetes, one in eleven will use insulin in their lifetime. 3) There is an immense prevalence of ADA-defined prediabetes in our population and limited lifetime risk of diabetes. Further research is needed on the feasibility of ADA-defined prediabetes in a screen-and-treat policy. 4) SLC6A20 is a genetic risk variant associated with type 2 diabetes and ADAMTS13 is an independent risk factor for incident type 2 diabetes and prediabetes.

The scientific field of obesity-associated diseases is dynamic and contains many challenges that need to be solved. Through this thesis, we contribute to the improvement of identification, prevention and treatment of type 2 diabetes and associated diseases.

Samenvatting

Er is een wereldwijde epidemie van ziektes die geassocieerd zijn met obesitas zoals diabetes mellitus type 2, hypertensie en dyslipidemie. Dit komt onder andere door vergrijzing, voedingsgewoonten en een inactieve leefstijl. Onderzoek naar dit onderwerp is van levensbelang want we hebben inzicht nodig in de exacte grootte van het probleem, de risicofactoren die geassocieerd zijn met de verschillende ziektes en mogelijke therapeutische opties om de ziektelast te verminderen.

Deel 1 van dit proefschrift start met een algemene introductie over diabetes type 2, obesitas en het metabool syndroom met ook een overzicht van risicofactoren voor diabetes type 2 en de complicaties van deze ziekte. Hoofdstuk 1 eindigt met uitleg over epidemiologische levenslange risicoberekeningen en de rol van het vaststellen van prediabetes bij de preventie van diabetes type 2. Naast een algemene introductie wordt in hoofdstuk 2 het doel van dit proefschrift uiteengezet.

In deel 2 van dit proefschrift hebben we de klinische waarde van het metabool syndroom bestudeerd. Hoofdstuk 3 beschrijft hoe vaak het metabool syndroom voorkomt en hoe dit varieert afhankelijk van welke definitie men kiest. Tevens kijken we naar het verband tussen metabool syndroom en het risico op cardiometabole ziektes in de algemene bevolking. We hebben deze studie verricht omdat er controverse bestond over of het metabool syndroom toegevoegde waarde heeft bovenop de losse componenten waaruit het syndroom bestaat. Daarnaast zijn er verschillende definities van het metabool syndroom, waarbij ons doel was meer inzicht te verkrijgen in het effect dat het toepassen van de verschillende definities heeft op de prevalentiecijfers en risico associatie. Op basis van onze resultaten concluderen we dat het metabool syndroom in onze populatie een hoge prevalentie kent met hoge variabiliteit afhankelijk van verschillende afkapwaardes van de definities. Het metabool syndroom was in onze studie geassocieerd met de incidentie van diabetes type 2, coronaire hartziekten, cerebrovasculaire aandoeningen, cardiovasculaire mortaliteit en mortaliteit door alle oorzaken. Echter verdwijnen al deze associaties nadat we het metabool syndroom corrigeren voor de individuele componenten waaruit het is opgebouwd. We concluderen dan ook dat het metabool syndroom geen toegevoegde waarde heeft bovenop de componenten waaruit het is opgebouwd voor voorspelling van cardiometabole ziektes in de klinische praktijk.

Het levenslange risico op het krijgen van diabetes type 2 en prediabetes staat centraal in deel III van dit proefschrift. Deze levenslange risicoberekening geeft een schatting van het cumulatieve risico dat een persoon loopt om een ziekte in zijn of haar leven te krijgen. In hoofdstuk 4 hebben we het levenslange risico op diabetes type 2 in de Rotterdam Studie onderzocht. Door deze specifieke analyse toe te passen in een prospectief cohort van de algehele populatie met een lange follow-up en uitgebreide gegevens over glucose

metabolisme en medicatiegebruik, hebben we een unieke en kwalitatief hoogwaardige schatting kunnen geven van hoe vaak stoornissen van het glucose metabolisme voorkomen We hebben gevonden dat er op een leeftijd van 45 jaar, een levenslang risico van 50% is om prediabetes te ontwikkelen en dat 75% van de personen die prediabetes hebben, ook diabetes ontwikkelen in hun leven. Uiteindelijk ontwikkelt 1 op 3 personen met een leeftijd van 45 jaar, diabetes tijdens de rest van hun leven. Van deze diabetici zal de helft insulineafhankelijk worden. Door onze berekeningen van levenslang risico zorgen we voor makkelijk te interpreteren risicoschattingen voor zowel zorgverleners als leken. Hiermee bevorderen we de kwaliteit van risico-communicatie in de spreekkamer. Daarnaast hebben we met onze studie de grootte van het probleem in Nederland duidelijk gemaakt en pleiten we voor onderzoek naar effectievere preventieve strategieën.

Het preventieve aspect wordt verder besproken in hoofdstuk 5. Hier vergelijken we het levenslange risico op progressie naar diabetes type 2 tussen twee definities van prediabetes in zowel vrouwen als mannen. Dit zijn de definities van de World Health Organization (WHO) en de American Diabetes Association (ADA), van welke de laatste een lagere glucose afkapwaarde heeft. Uit onze resultaten bleek dat de ADA-definitie de helft van de populatie diagnosticeert als prediabeet en dat in elke leeftijdscategorie de prevalentiecijfers het dubbele waren van de WHO-definitie. Op een leeftijd van 45 jaar zal ongeveer de helft van de individuen met een ADA-prediabetes diagnose uiteindelijk diabetes ontwikkelen ten opzichte van tweederde van de individuen met een WHO-prediabetes diagnose. Vrouwen met prediabetes hebben een hoger levenslang risico om diabetes te ontwikkelen in vergelijking met mannen, hetgeen waarschijnlijk het gevolg is van hun slechtere metabole toestand bij het begin van de studie follow-up. Dit levenslang risico op het ontwikkelen van diabetes was lager bij zowel vrouwen en mannen met ADA prediabetes in vergelijking met WHO-prediabetes. Hieruit concluderen we dat een ADA-definitie resulteert in het selecteren van individuen met een lager risico op het ontwikkelen van diabetes, waarbij het de vraag is of bij al deze personen preventieve interventies effectief, noodzakelijk en kosteneffectief zullen zijn. We stellen dan ook voor dat er nader onderzoek moet plaatsvinden naar de optimale afkapwaarde met aandacht voor zowel leeftijd als geslacht binnen de prediabetes definitie waarmee de meest effectieve en efficiënte selectie van hoog-risico patiënten kan worden gemaakt

Deel IV van dit proefschrift gaat over epidemiologische, biochemische en genetische risicofactoren voor het krijgen van diabetes type 2 en de complicaties die met diabetes type 2 gepaard gaan. Hoofdstuk 6 geeft een beschrijving van de DiaGene studie, een multicenter diabetes type 2 cohort studie uit de eerste en tweede lijn in Nederland. Het belangrijkste doel van de studie is het analyseren van genetische, biochemische en omgevingsfactoren die het ontstaan van diabetes type 2 en haar complicaties beïnvloeden. Uit de data die

we verkregen, concludeerden we dat er ondanks behandeling een aanzienlijk aantal macro- en microvasculaire complicaties voorkomt in de diabetespopulatie in Nederland. De prevalentiecijfers zijn vergelijkbaar met andere studiepopulaties van diabetes type 2. Los van de prevalentie van complicaties, beschrijven we 11 bekende genetische risico varianten (TCF7L2, PPARG-P12A, KCNJ11, FTO, IGF2BP2, DUSP9, CENTD2, THADA, HHEX, CDKAL1, KCNQ1) en zien we dezelfde associaties als eerder beschreven is in de literatuur. We concluderen dat onze studie, waarin we genetische en biochemische data combineren met een uitgebreid gefenotypeerde populatie, geschikt is om verder onderzoek te doen naar pathofysiologische mechanismen van diabetes type 2.

Een nieuwe genetische associatie bij diabetes type 2 wordt besproken in hoofdstuk 7, waarin we de invloed van genetische variatie binnen de proline transporter SLC6A20 onderzoeken door het verrichten van een kandidaat-gen studie. Omdat SLC6A20 een essentieel onderdeel is van proline metabolisme en glucose homeostase beïnvloedt, was onze hypothese dat varianten in het SLC6A20 gen het risico op het krijgen van diabetes type 2 beïnvloedt. Onze studie heeft ons verschillende bevindingen opgeleverd. Allereerst zagen we dat de minor allelen rs13062383, rs10461016 en rs2286489 het risico op het krijgen van diabetes type 2 verhogen. Daarnaast zagen we dat in een Aziatische replicatie studie en meta-analyse alleen de associatie van rs13062383 hoogst significant bleef. Zodoende concludeerden we dat rs13062383 in SLC6A20 het risico op het krijgen van diabetes type 2 in populaties met verschillende genetische achtergrond verhoogt.

In hoofdstuk 8 beschrijven we een studie die de associatie onderzocht van ADAMTS13-activiteit en von-Willebrand factor (VWF)-antigen met incidente diabetes type 2. We hebben dit onderzocht omdat ADAMTS13 een protease is dat vWF-multimeren verwerkt tot kleinere, minder actieve onderdelen. VWF is in het verleden al geassocieerd met een verhoogd risico op het krijgen van diabetes type 2. In onze studie bleek ADAMTS13-activiteit geassocieerd te zijn met een verhoogd risico op het krijgen van zowel diabetes als prediabetes na correctie voor bekende risicofactoren en VWF-antigeen. We hebben hierdoor geconcludeerd dat ADAMTS13-activiteit een onafhankelijke risicofactor voor het krijgen van prediabetes en diabetes type 2.

Tot slot bevat deel IV van dit proefschrift de algemene discussie in hoofdstuk 9. Hierin bediscussieer ik de resultaten van de onderzoeken in de context van de huidige literatuur, maar ook methodologische kwesties en voorstellen voor toekomstig onderzoek. Ik heb enkele conclusies van dit proefschrift te benoemen: 1) het metabool syndroom heeft geen toegevoegde waarde ten opzichte van de componenten waaruit het is opgebouwd bij risicopredictie van cardiovasculaire en metabole ziekte. 2) de schaal waarop diabetes type 2 in Nederland voorkomt is gigantisch; 1 op de drie ontwikkelt diabetes, 1 op 11

zal insulineafhankelijk worden in zijn of haar leven. 3) Door het toepassen van de ADA-definitie ontstaat er een enorme prevalentie van prediabetes met een relatief gelimiteerd levenslang risico op het krijgen van diabetes. We betwijfelen hiermee de haalbaarheid van ADA-prediabetes in een screen-en-behandelstrategie. 4) SLC6A20 is een genetische variant welke geassocieerd is met diabetes type 2, evenals ADAMTS13-activiteit, hetgeen ook geassocieerd is met prediabetes. Het wetenschappelijk onderzoek bij ziektes geassocieerd met obesitas is zeer dynamisch en kent veel uitdagingen die nog moeten worden opgelost. Met deze thesis, dragen we bij aan een verbetering van identificatie, preventie en behandeling van diabetes type 2 en geassocieerde ziektes.

List of publications

LIST OF PUBLICATIONS IN CHRONOLOGICAL ORDER [IMPACT FACTOR]

1. **van Herpt TTW,** Ligthart S, van Hoek M, Lieverse AG, Ikram MA, Sijbrands EJG, Dehghan A, Kavousi M. Lifetime risk to progress from prediabetes to type 2 dibaetes among women and men: a comparison between American Diabetes Association and World Health Organization diagnostic criteria. *in revision at BMJ Open Diabetes Research and Care*. [3.2]
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3. **Van Herpt TTW*,** Lemmers RFH*, Van Hoek M*, Langendonk JG, Erdtsieck RJ, Bravenboer B, Lucas A, Mulder MT, Haak HR, Lieverse AG, Sijbrands EJG. Introduction of the DiaGene study: clinical characteristics, pathophysiology and determinants of vascular complications of type 2 diabetes. *Diabetol Metab Syndr* 2017; 19(9):47. [2.4]
4. **Van Herpt TTW*,** de Vries PS*, Ligthart S, Hofman A, Ikram MA, Van Hoek M, Sijbrands EJG, Franco OH, de Maat M, Leebeek FW, Dehghan A. ADAMTS13 activity as a novel risk factor for incident type 2 diabetes mellitus: a population-based cohort study. *Diabetologia* 2017; 60(2):280-286. [6.2]
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Mannen van het HS; Jan, Joost en Rick (Face, red). Een prachtige studententijd hebben we mogen voortzetten in een mooi leven als man, vader en uiteraard HS-mattie. Jan/Jean: een jachtvliegtuig dat gewapend met onophoudelijke energie, chirurgische vakkundigheid, een overontwikkelde emotionele radar en een bak aan intelligentie iedere donkere wolk weer opklaart. Bedankt voor jouw vriendschap, je bent een gouden gast met een gouden toekomst samen met Michelle en Jantje! Joost/Josh/Kolossos: de mate waarin jij zonder angst of aannames nieuwe avonturen instapt is bewonderenswaardig. Naast jouw sociale capaciteit en vingervlugge handigheid beschik je over een beukharde processor. We delen onze liefde voor Sacha Baron Cohen en reaguren ons een ongeluk waarvoor dank. Ik hoop dat we nog veel avonturen mogen beleven samen! Alle geluk en liefde voor jou, Lot, Vins en Mels! Face, ontzettende vent die je bent. Vanaf het moment dat je mijn treinmaatje was, ben je een geweldige maat. Ik hou enorm van je nuchtere commentaar en prettige adviezen. Zelf het Midden-Oosten kun je met een vlijmscherpe gortdroge analyse verhelderen. Dat we nog maar lang mogen genieten van onze vrouwkes, kinders, weekendjes en fessa's.

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Curriculum Vitae

Thijs van Herpt was born on July 19th 1984 in Helmond. After graduating Cum Laude for his gymnasium on Pleincollege Eckart in Eindhoven, he studied medicine at Maastricht University. The last year of his internship he completed under supervision of Prof. Dr. H.R. Haak in Máxima medical center in Eindhoven where he started as part-time resident not in training and researcher after which he moved to Rotterdam in 2011 to perform full-time research at the departments of vascular internal medicine and epidemiology of the Erasmus MC. In 2013, he started his training to become an internist in the Havenziekenhuis with Dr. P.J. Wismans in Rotterdam. After this period in Rotterdam, he continued his training in the Reinier de Graaf Gasthuis Delft (Dr. H. Boom and Dr. E.F. Posthuma) and returned to Erasmus MC in 2016 under supervision of Dr. S.C.E. Klein Nagelvoort-Schuit. In 2017, he was enrolled in the sub specialism of Acute Internal Medicine under supervision of Dr. J. Alsma. During and after his last year of training, he worked as a senior resident and internist in Amphia hospital in Breda. From March 2020 onward, he is enrolled in a fellowship training program Intensive Care in Maastricht University Medical Center under supervision of Dr. D.C.J.J. Bergmans. Thijs has a wife and a son, called Mandy and Niek. In his spare time, he hikes, runs marathons or cycles across the Dutch, Flemish and French countryside.

Thijs van Herpt werd geboren op 19 juli 1984 te Helmond. Na het Cum Laude behalen van zijn gymnasiumdiploma aan het Pleincollege Eckart in Eindhoven begon hij aan de studie geneeskunde aan de Universiteit Maastricht. Hij doorliep zijn coassistentschappen waarvan hij in het laatste jaar zijn artsexamen in 2008 volbracht in het Máxima Medisch Centrum Eindhoven onder de hoede van Prof. Dr. H.R. Haak. Hier kwam hij ook te werken als parttime arts-assistent en parttime arts-onderzoeker. In 2011 verhuisde hij naar Rotterdam om fulltime onderzoek te doen bij de afdelingen vasculaire geneeskunde en epidemiologie van het Erasmus MC waarna hij in 2013 begon met de opleiding tot internist. In eerste instantie onder de bezielende leiding van Dr. P.J. Wismans in het Havenziekenhuis te Rotterdam. Vervolgens heeft hij in 2014 de opleiding voortgezet in het Reinier de Graaf Gasthuis te Delft (opleider Dr. H. Boom en Dr. E.F.M. Posthuma) om in september 2016 weer terug te keren naar het Erasmus MC (opleider Dr. S.C.E. Klein Nagelvoort-Schuit). Hij heeft zich vanaf 1 mei 2017 gespecialiseerd in het aandachtsgebied Acute Geneeskunde (opleider J. Alsma). Vanaf 1 juli 2019 was hij werkzaam als Internist Acute Geneeskunde in het Amphia ziekenhuis in Breda waarna hij vanaf 1 maart 2020 een fellowship Intensive Care volgt in het Maastricht Universitair Medisch Centrum onder begeleiding van Dr. D.C.J.J. Bergmans. Hij heeft een vrouw en zoon, genaamd Mandy en Niek. In zijn vrije tijd wandelt hij, loopt hij marathons of fietst hij door het Nederlands, Vlaams en Franse landschap.

PhD Portfolio

Naam	Thijs van Herpt
Department	Internal Medicine
Research school	Cardiovascular Research School Erasmus University Rotterdam (COEUR)
Promotor	Prof. dr. E.J.G. Sijbrands, Prof.Dr. O.H. Franco
Copromotor	Dr M. van Hoek, Dr. A.G. Lieverse
PhD-period	2008-2020

PhD training	**Year**	**ECTS**
General academic skills		
Evidence Based Medicine	2010	0.3
Biomedical English Writing and Communication	2015	3.0
Research skills		
COEUR		
- Lectures: 11	2009-2013	1.1
- Vascular Clinical Epidemiology	2010	0.7
- Molecular biology in atherosclerosis and cardiovascular research	2014	1.5
NIHES		
- Introduction to Data-analysis (ESP03)	2010	1.0
- Principles of Research in Medicine (ESP01)	2010	0.7
- Case-Control studies (ESP40)	2011	0.7
- Regression analyses (ESP09)	2011	1.9
- Repeated Measurements (CE08)	2011	1.4
Molmed Courses		
- SNP's and human disease	2009	2.0
- SNP course	2010	2.0
- Biomedical research techniques	2011	1.5
Other		
- MMC Evidence Based Medicine	2010	0.2
- MMC Statistics and SPSS	2009	0.2
Symposia and conferences		
Wetenschapsdagen Interne Geneeskunde, Antwerpen*	2010-2012	1.2
American Diabetes Association 75th scientific session, Boston *	2015	1.5
American Diabetes Association 76th scientific session, New Orleans *	2016	1.2

Internal Medicine research symposia, Rotterdam*	2016	0.6
* *Poster presentation*		
Presentations and teaching		
Presentations at the Internal Medicine Departments of EMC		2.1
Poster and oral presentations at symposia		1.6
Teaching		5.1
Total		**30.6**