DIABETES SUSCEPTIBILITY POLYMORPHISMS AND RISK OF PREDIABETES AND DIABETES COMPLICATIONS IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

Yu Yan

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Public Health (Epidemiology).

Chapel Hill
2009

Approved by:
Advisor: Dr. Kari E. North
Reader: Dr. Gerardo Heiss
Reader: Dr. Ronald Klein
Reader: Dr. Cynthia J. Girman
Reader: Dr. Ethan M. Lange

ABSTRACT

Yu Yan: Diabetes Susceptibility Polymorphisms and Risk of Prediabetes and Diabetes Complications in the Atherosclerosis Risk in Communities (ARIC) Study
(Under the direction of Dr. Kari E. North)

Transcription factor 7-like 2 (*TCF7L2*) has emerged as a consistently replicated susceptibility gene for type 2 diabetes, however, its association with prediabetes as quantified by impaired fasting glucose (IFG), and diabetes complications such as retinopathy has not been well characterized in population-based studies. Thus, we investigated the association between the *TCF7L2* rs7903146 polymorphism and two types of diabetes-related outcomes, IFG and retinal microvascular signs, in the Atherosclerosis Risk in Communities cohort.

The incident IFG analysis was conducted among 1,377 African American and 5,152 Caucasian participants without diabetes and IFG at baseline. IFG was defined as fasting glucose levels of 100–125 mg/dl. After adjusted for age, sex, and study center, the rs7903146 T risk allele was significantly associated with higher risk of IFG over 9 years of follow-up in Caucasians. Moreover, the association was stronger in Caucasians with obesity or high triglycerides. No association of the rs7903146 polymorphism and incident IFG was noted in African Americans, although we had limited power to assess this association.

We also evaluated the association between the rs7903146 polymorphism and retinal microvascular signs in 2,199 African American and 8,121 Caucasian participants in the ARIC cohort. After adjusting for age, sex, study center, and other covariates, *TCF7L2*

rs7903146 T risk allele was associated with increased risk of focal arteriolar narrowing in Caucasians with hypertension or without diabetes. No significant association of the rs7903146 polymorphism and retinal vascular signs was noted among African American individuals, although, again, we were limited in power to detect these associations.

In summary, our study replicates the association between the rs7903146 polymorphism and IFG risk in Caucasians and provides new evidence for interactions between *TCF7L2* and metabolic risk factors on the occurrence of IFG in Caucasians. Moreover, our study is the first to report an association with focal arteriolar narrowing in Caucasians with hypertension or without diabetes. Our study results contribute knowledge about the etiology of type 2 diabetes, and could be important for public health initiatives to encourage lifestyle changes in patients at risk of diabetes. Further research in other larger population-based studies will be needed to replicate our results.

ACKNOWLEDGEMENTS

This dissertation is the product of not only my own labors, but also the support and guidance given to me by many individuals over the past five years. I am deeply indebted to Dr. Kari North, my mentor, who has been most generous in giving her time, encouragement, support, and advice. She is not only a great mentor, but also a wonderful colleague and friend. I am indebted to the other members of my dissertation committee as well, Dr. Gerardo Heiss, Dr. Ronald Klein, Dr. Cynthia Girman, and Dr. Ethan Lange, for their guidance, assistance, and encouragement. This work would not have been possible without the important contributions from all committee members.

At this time, I cannot help but think of my former advisor, Dr. Harry Guess, who helped open the door for my entry to the University of North Carolina and supported me with his wisdom and advice ever since. He lives in my heart forever.

I would also like to thank Dr. Suzanne West, who assisted me in setting up this project and gave me tremendous help during the past five years.

Finally, I wish to recognize the constant support I have received from my parents, my husband Jing, my parents-in-law, as well as Nancy Colvin.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

AA	African American
A-C	Afro-Caribbean
ADA	American Diabetes Association
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9
A-I	American Indian
AIRg	acute insulin response to glucose
AP	attributable proportion due to interaction
APOE	Apolipoprotein E
ARIC	Atherosclerosis Risk in Communities
ARNT	aryl hydrocarbon receptor nuclear translocator
A/V	arterio-venous
BMI	body mass index
CAMK1D	calcium/calmodulin-dependent protein kinase 1D
CDC123	cell division cycle 123 homolog
CDKAL1	cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1
CDKN2A	cyclin-dependent kinase inhibitor 2A/2B (melanoma, p16, inhibits CDK4)
CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
CEPH	Genomic DNA samples obtained for a panel of 92 unrelated individuals chosen from Centre d'Etude du Polymorphisme Human (CEPH) pedigrees comprised of UTAH (93%), French (4%), and Venezuelan (3%) samples purchased from Coriell Cell Repository

CEU	30 mother-father-child trios from the CEPH collection, one of the populations studied in the HapMap project
CHD	coronary heart disease
CI	confidence interval
CRAE	central retinal artery equivalent
CRP	C-reactive protein
CRVE	central retinal venular equivalent
CVD	cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DI	disposition index
DZ	dizygotic
EGR2	early growth response 2
eNOS	endothelial nitric oxide synthase
FANCF	Fanconi anemia, complementation group F
FPG	fasting plasma glucose
FTO	fat mass and obesity associated
GAUC	glucose area under the OGTT curve
GDM	gestational diabetes mellitus
GLP-1	glucagon-like peptide 1
Grb10	growth factor receptor-bound protein 10
GWAS	genome-wide association study
HDL	high density lipoprotein
HHEX	hematopoietically expressed homeobox

HR	hazard ratio
HOMA-IR	homeostatis model assessment of insulin resistance
IAUC	insulin area under the OGTT curve
IFG	impaired fasting glucose
IGF2BP2	insulin-like growth factor 2 mRNA-binding protein 2
IGT	impaired glucose tolerance
IMT	intima-media thickness
IRS2	insulin receptor substrate 2
IS	insulin secretion
JAZF1	juxtaposed with another zinc finger gene 1
KCNJ11	potassium inwardly-rectifying channel, subfamily J, member 11
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1
LD	linkage disequilibrium
LDL	low density lipoprotein
LGR5	leucine-rich repeat-containing G protein-coupled receptor 5
LOD	logarithm of the odds
MAF	minor allele frequency
MODY	maturity onset diabetes of the young
MS	metabolic syndrome
MZ	monozygotic
NGT	normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung, and Blood Institute

NOTCH2	Notch homolog 2
OGTT	oral glucose tolerance test
OR	odds ratio
OR52H1	olfactory receptor, family 52, subfamily H, member 1
PAF	population attributable fraction
PCOS	Polycystic Ovary Syndrome
PPARG	peroxisome proliferator-activated receptor gamma
RALGPS2	Ral-A exchange factor
SENP1	sentrin specific peptidase 1
SLC30A8	solute carrier family 30 (zinc transporter), member 8
Si	insulin sensitivity
SNP	single nucleotide polymorphism
T2DM	type 2 diabetes
TCF7L2	transcription factor 7-like 2
THADA	thyroid adenoma associated
TSPAN8	tetraspanin 8
UBQLNL	ubiquilin-like
UK	United Kingdom
UKPDS	U.K. Prospective Diabetes Study
US	United States
VEGF	vascular endothelial growth factor
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHR	Waist –to-Hip ratio

Wnt	Wingless and Int
YRI	30 Yoruba mother-father-child trios in Ibadan, Nigeria, one of the populations studied in the HapMap project
ZBTB15	zinc finger and BTB domain containing 7B
ZNF659	zinc finger protein 659

CHAPTER I

INTRODUCTION

The rapid increase in the prevalence of hyperglycemia, type 2 diabetes (T2DM) and its complications imposes a major burden on the public health and significantly contributes to the high prevalence of cardiovascular disease in the United States and worldwide[1]. Identification and characterization of the genetic variants is important for the understanding of T2DM, and the etiology and pathogenesis of its complications.

Transcription factor 7-like 2 (*TCF7L2*), a Wingless and Int (Wnt) signaling-associated transcription factor located on chromosome 10q25, has emerged as a consistently replicated susceptibility gene for T2DM[2-4], possibly through the impairment of glucagon-like peptide-1-induced insulin secretion[5]. The T allele at single nucleotide polymorphism (SNP) rs7903146 located in intron 3 of *TCF7L2* confers risk for T2DM[6], however, its association with prediabetes phenotypes and retinopathy, one of the common complications of T2DM, has not been well characterized in population-based studies, especially in African Americans. Moreover, literature on *TCF7L2* gene–environment interaction assessment is limited.

The present study, conducted under approval of the University of North Carolina at Chapel Hill Institutional Review Board (see Appendix A), addresses the dearth of population-based studies examining the association between *TCF7L2* rs7903146 and prediabetes measured by impaired fasting glucose (IFG), and the association between *TCF7L2* rs7903146 and retinal vascular signs. Identifying susceptibility genes for diabetes-

related phenotypes and investigating the modification by metabolic risk factors on gene-diabetes-related phenotypes association contribute significant knowledge about the etiology of prediabetes, T2DM and retinopathy, and could have significant public health implications in patients at risk of diabetes, long before they develop frank diabetes. Given the recent rise in the prevalence of diabetes, such information may be important for public health initiatives to encourage lifestyle changes in such patients at risk. Here, we assess the relationship between SNP rs7903146 in *TCF7L2*, metabolic risk factors, and two types of diabetes-related endpoints (IFG and retinal microvascular phenotypes) using data from the ARIC Study, a community-based prospective cohort study of 15,792 males and females. The two manuscripts prepared for fulfillment of the Epidemiology doctoral program requirements are as follows:

Manuscript 1: Transcription Factor 7-Like 2 (*TCF7L2*) Polymorphism and Context-Specific Risk of Impaired Fasting Glucose in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study.

We investigated the effects of SNP rs7903146 *TCF7L2* on incident IFG in the context of other metabolic risk factors for diabetes. A total of 1,377 African American and 5,152 Caucasian participants without diabetes and IFG at baseline were selected from the entire ARIC cohort (n=15,792). Analyses were race-stratified and adjusted for age, sex, and ARIC study center. Hazard ratios (HR) and 95% confidence intervals (CI) of incident IFG were estimated by proportional hazard regression models. Gene–environment interaction testing was assessed on the multiplicative and additive scales between genotypes and different metabolic risk factors including obesity, elevated waist circumference,

hypertension, low HDL, high LDL, and high triglycerides. A Wald χ^2 test for significance of the estimated β-coefficient for the interaction term and the interaction contrast ratio (ICR) were employed to assess the departure from multiplicativity and additivity, respectively. This study addresses Aims 1 and 2 of the dissertation (see Chapter II).

Manuscript 2: Transcription Factor 7-Like 2 (*TCF7L2*) Polymorphism and Retinal Vascular Signs in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study.

We examined the association between SNP rs7903146 *TCF7L2* and retinal microvascular phenotypes and the extent to which hypertension and diabetes modified the association between gene-retinal phenotypes association in the ARIC cohort. A total of 2,199 African American and 8,121 Caucasian participants were selected from all eligible participants who returned for the third examination when retinal photography was first performed in 1993-1995 (n=12,887). Analyses were race-stratified and adjusted for age, sex, ARIC study center, current smoking, obesity, total serum cholesterol, total serum triglycerides, mean arterial blood pressure, and antihypertensive medication. Odds ratios (OR) and 95% CIs of prevalent retinal lesions (retinopathy, focal arteriolar narrowing, AV nicking) were estimated by logistic regression models; adjusted mean retinal vascular calibers for each genotype of rs7903146 were obtained under generalized linear models. A Wald χ^2 test for significance of the estimated β-coefficient for the interaction term (SNP × hypertension or SNP × diabetes) and the ICR were employed to assess the departure from multiplicativity and additivity, respectively. This study addresses Aims 3 and 4 of the dissertation (see Chapter II).

CHAPTER II

SPECIFIC AIMS

Our goal was to measure the associations between *TCF7L2* and prediabetes/retinal phenotypes using the Atherosclerosis Risk in Communities (ARIC) data. The ARIC study is an ongoing, bi-racial population-based longitudinal study of cardiovascular-related diseases in 15,792 males and females. Manuscript 1 addresses Aims 1 and 2, and Manuscript 2 addresses Aims 3 and 4.

The specific aims were as follows:

1) To estimate the association between SNP rs7903146 in *TCF7L2* and prediabetes as quantified by incident impaired fasting glucose (IFG).
    a) Proportional hazard regression modeling in which the association between SNP rs7903146 in *TCF7L2* and the hazard of incident IFG was estimated.
2) To estimate the extent to which metabolic risk factors including obesity, elevated waist circumference, hypertension, low HDL, high LDL, high triglyceride modified the association between SNP rs7903146 in *TCF7L2* and incident IFG.
    a) Proportional hazard regression modeling in which metabolic risk factors were evaluated as modifiers of the rs7903146 - incident IFG association.
3) To estimate the association between SNP rs7903146 in *TCF7L2* and retinal phenotypes including retinopathy, arteriovenous (AV) nicking, focal arteriolar narrowing, central retinal artery equivalent (CRAE) and the central retinal venular equivalent (CRVE).

a) Logistic regression modeling in which the association between SNP rs7903146 in *TCF7L2* and the odds of prevalent retinal microvascular signs (retinopathy, AV nicking, focal arteriolar narrowing) was estimated.
b) Generalized linear modeling in which adjusted mean retinal vascular calibers (CRAE, CRVE) for each genotype of rs7903146 were esimated.

4) To estimate the extent to which hypertension and diabetes modified the association between SNP rs7903146 in *TCF7L2* and retinal phenotypes.
   a) Logistic regression modeling in which hypertension and diabetes were evaluated as modifiers of the rs7903146 – prevalent retinal microvascular signs association.
   b) Generalized linear modeling in which hypertension and diabetes were evaluated as modifiers of the rs7903146 – CRAE / CRVE association.

CHAPTER III

BACKGROUND AND SIGNIFICANCE

Diabetes mellitus is a heterogeneous group of disorders characterized by hyperglycemia resulting from defects in insulin secretion and resistance to insulin action[7]. The two most common forms of diabetes mellitus are type 1 diabetes and T2DM. Both are caused by a combination of genetic and environmental risk factors. All forms of diabetes have serious effects on health. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision[7]. In addition to the consequences of abnormal metabolism of glucose, the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[7].

A. T2DM – a major public health concern

1. Definition of T2DM

T2DM is the most common form of diabetes mellitus and caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretary response[7, 8]. A diagnosis of T2DM is made if a fasting plasma glucose concentration is ≥7.0 mmol/l (≥126 mg/dl) or 2-hour plasma glucose concentration after a standard oral glucose challenge is ≥11.1 mmol/l (≥200 mg/dl)[7].

2. Pathogenesis of T2DM

T2DM is characterized by impaired insulin action (insulin resistance) and an insulin secretory defect as a result of impaired beta cell functioning[8]. Insulin resistance is a state in which the body does not respond to the action of insulin, even when enough insulin is being secreted [9]. Nearly 90% of T2DM patients are insulin resistant[10]. Insulin resistance, prediabetes, and T2DM are linked by a similar pathogenesis. Initially, insulin resistance causes an increase in insulin secretion appropriately from the beta cells of the pancreas. This compensatory mechanism results in euglycemia with elevated fasting and/or postprandial serum insulin levels. The beta cells continue to compensate by increasing insulin levels, resulting in hyperinsulinemia and maintaining glucose homeostasis for up to 7 years[11, 12]. As the beta cells eventually exhaust and insulin levels become too low to meet the requirement of skeletal muscles and liver tissues, a mild postprandial hyperglycemia develops. As insulin resistance increases and the progressive loss of beta cells function continues, more global defects in insulin secretion increase resulting in impaired fasting glucose (IFG).

3. Epidemiology of T2DM

T2DM is a major – and growing – worldwide public health concern. Globally, the prevalence of diabetes has increased dramatically over the past several decades although it is partly due to the diabetes definition changes[1, 13-15]. In 1997, the ADA proposed a lowered fasting blood glucose level from 140 to 126 mg/dl as a diagnostic sign of diabetes[16]. In 2003, the maximum normal levels had further been reduced from 110 to 100 mg/dl, resulting in a definition of IFG by a glycemia $\geq$100 and <126 mg/dl[17]. There were approximately 30 million individuals with T2DM in 1985, while by 1995, this number had escalated to 135 million[15, 18]. Furthermore, the total number of people with diabetes is projected to rise from

171 million in 2000 (2.8% of the world population) to 366 million in 2030 (4.4% of the world population)[1].

Diabetes mellitus imposes a major burden on the public health of the United States, where in 2002 it was the sixth leading cause of death[19] and was estimated to cost 92 billion dollars[20, 21]. T2DM accounts for 90% to 95% of diabetic individuals in the US[15, 21, 22]. Current predictions indicate that one in three Americans born in 2000 will develop T2DM; for Hispanics and African-Americans, the risk is almost one in two[23]. The highest prevalence of T2DM was found among Native Americans, particularly the Pima Indians who reside in Arizona[1]. T2DM is also known to be more predominant in Hispanics, Pacific Islanders, and African Americans than in Caucasians[22, 24]. In the ARIC study, the incidence of T2DM is 2.4-fold greater in African American women and 1.5-fold greater in African American men compared to their white counterparts[25]. Possible explanations include racial differences in socioeconomic status, adiposity, physical inactivity, and family history of diabetes[26].

4. Risk factors of T2DM

Approximately one-third of patients with T2DM may be undiagnosed[27]. Screening of asymptomatic individuals and individuals at high risk is recommended by American Diabetes Association (ADA) as an important strategy to the prevention and control of diabetes although the effectiveness of this strategy has not been determined[27, 28]. The ADA suggests screening be considered at any age if risk factors for diabetes are present, and recommends screening all individuals >45 years of age, regardless of their risk factor status[29]. It also recommends repeat screening at 3-year intervals.

Numerous epidemiological studies have identified the following major risk factors discussed below which are also criteria for screening: age, overweight (BMI>25 kg/m^2), first degree relative with diabetes, habitual physical inactivity, member of a high-risk ethnic population (e.g., A-A, Latino, Native American, Asian-American, Pacific islander), previously identified IFG or IGT, history of gestational diabetes or delivery of a baby weighing >9lb, hypertension, dyslipidemia, polycystic ovary syndrome and history of vascular disease[27, 30, 31].

Age

T2DM was known for years as "adult onset" emphasizing the prevalence of T2DM increases with age. Of persons less than 45 years, 45-64, 65-74, and 75 years or older, the prevalence per 100 population in 2004 were 1.2, 9.5, 18.1 and 15.7, respectively[32]. In 2004, the prevalence of diagnosed diabetes among people aged 45-64 years (9.5%) was approximately 8 times that of people less than 45 years of age (1.2%)[32]. The age of 45 years has been officially used as an important cut-off point in estimating the prevalence of T2DM, however the prevalence of T2DM in children and adolescents is rising at an alarming rate (e.g., approximately 4% in 1963 to 15% in 2000 among aged 6-19 years) which is estimated to increase if no effective measures taken to prevent obesity[33]. The ARIC study also identified age as an important risk factor for diabetes incidence[34].

Overweight

Overweight (BMI>25kg/m^2) plays a major role in the pathogenesis of T2DM by influencing insulin resistance. Obesity is also an independent risk factor for hypertension, dyslipidemia, and CVD which is the major cause of death in those with diabetes [27]. Among

people diagnosed with T2DM, 67% have a BMI ≥27 and 46% have a BMI ≥30 kg/m^2. Excess weight contributes to an estimated 70% of diabetes risk in the United States[35]. In ARIC, participants with a BMI ≥30 were more likely to have diabetes than those in lower BMI categories (22.4% versus 7.9%, $p < 0.01$) and the prevalence of diabetes increased with increasing BMI: 4.4% (BMI<20), 4.9% (20≤BMI≤24), 10% (25≤BMI≤29), and 22.4% (BMI≥30)[36]. In each category of BMI (18.5-<22, 22-<25, 25-<28, 28-<31, 31-<34, ≥34), African-American women had higher fasting insulin than Caucasian women ($P = 0.0003$), but not in men ($P = 0.2620$) in the ARIC cohort[37].

Two general mechanisms linking obesity and T2DM have been identified[11]. The first major mechanism involves the accumulation of fat in the liver and muscle mediates obesity-induced insulin resistance based on the following observations: experimental elevation of free fatty acids leads to insulin resistance; direct correlation between the lipid content of skeletal muscle and liver and insulin resistance; fatty acids and their metabolic products can reduce insulin signaling in muscle and liver at the cellular level. The second major mechanism is a group of peptides, made by fat cells, that decrease insulin sensitivity. It has been shown that adiponectin reduces insulin resistance and individuals with progressive obesity demonstrate reductions in adiponectin. Elevated levels of adipocytokines such as tumor necrosis factor-alpha, interleukin-6, and resistin are observed with obesity and these adipocytokines have been suggested to increase insulin resistance. Various adipose tissue beds produce different amounts of these peptides, perhaps adding to the regional differences these adipose depots make in their contributions to insulin resistance. Therefore, greater accumulation of fat in the body will increase insulin resistance, and increase the risk of developing T2DM.

First-degree relative with diabetes

It is well accepted that T2DM is an inherited disease. The Framingham Offspring Study found that the ORs for T2DM or prediabetes among offspring with maternal/paternal diabetes were 3.4 (95% CI: 2.3-4.9)/3.5 (2.3-5.2) and 2.7 (2.0-3.7)/1.7 (1.2-2.4), respectively, and among those with bilineal (maternal and paternal) diabetes were 6.1 (2.9-13.0) and 5.2 (2.6-10.5), respectively, when compared to individuals without parental diabetes[38]. In ARIC, parental history of diabetes has been suggested as an important predictor of incident diabetes[34]. In the Framingham Offspring Study, the offspring with maternal diabetes were more likely to have a mild slowly progressive form of glucose intolerance compared to offspring with paternal diabetes[38]. The Northern California Kaiser Permanente Diabetes Registry also reported excess maternal transmission of T2DM although the size of the excess was negligible in African-Americans and male offspring[39]. However, in a Korean cohort, excess paternal transmission of T2DM was observed in the offspring but not for maternal diabetes[40]. A review by Fetita *et al*. stated that intrauterine exposure in fetal to maternal hyperglycemia is associated with abnormal glucose homeostasis in offspring, which is demonstrated in animal models[41]. Mechanisms such as defects in pancreatic angiogenesis and innervation, or modification of parental imprinting, may be implicated, acting either independently or in combination[41].

Habitual physical inactivity

There is firm and consistent evidence that physical activity is inversely associated with T2DM[42-45]. A meta-analysis combining ten prospective cohorts of physical activity of moderate intensity and type 2 diabetes suggested that physical activities of moderate intensity such as brisk walking can substantially reduce the risk of type 2 diabetes[42]. The ARIC study

reported that the mean leisure time physical activity score was slightly higher in non-diabetic participants than diabetic participants in both races at baseline, and Caucasian participants had higher scores on average then African-American participants (unpublished data). To improve glycemic control and reduce the risk of cardiovascular disease (CVD) the ADA recommends at least 150min/week of moderate-intensity aerobic physical activity (50-70% of maximum heart rate) and/or at least 90min/week of vigorous aerobic exercise (>70% of maximum heart rate)[27]. Current evidence supports habitual physical inactivity and low cardiorespiratory fitness are involved in the progression to T2DM[46]. Physical inactivity can initiate and accelerate the pathogenesis of diabetes and subsequent morbidity and mortality. Conversely, regular physical activity can retard and even reverse the process[46]. In the Diabetes Prevention Program, the lifestyle-modification program with the goals of at least a 7 percent weight loss and at least 150 minutes of physical activity per week reduced the incidence of T2DM by 58% (95% CI: 48-66%)[47].

High-risk ethnic population

Minorities in the United States exhibit a higher prevalence of diabetes compared to the white population. According to the National Diabetes Fact Sheet, United States, 2005, among people aged 20 years or older non-Hispanic blacks, Hispanic/Latino Americans, American Indians and Alaska Natives, and Asian Americans and Pacific Islanders were 1.8, 1.8, 2.2, and 1.5 times as likely to have diagnosed diabetes as non-Hispanic whites (www.cdc.gov, 2005). In the ARIC cohort, the incidence of T2DM is 2.4-fold and 1.5-fold higher in African American women and men, respectively, compared to their white counterparts [25]. Furthermore, African Americans bear a disproportionate burden of morbidity and mortality associated with T2DM[35, 48]. The high prevalence of T2DM in African

Americans can be attributed, in part, to high prevalence of obesity, physical inactivity and insulin resistance. Other contributing factors, such as lower social economic status and access barriers to health care, may negatively impact the African-American group[26, 48, 49]. The third National Health and Nutrition Examination Survey (NHANES III) data were examined for racial and ethnic differences in health care access and health outcomes for patients with T2DM [50]. Small differences by race and ethnicity were identified.

IFG and IGT

IFG and IGT are used to characterize a "prediabetes" state, an intermediate category between normoglycemia and diabetes. IFG is now defined as fasting plasma glucose (FPG) between 100 and 125 mg/dL (between 5.6 and 6.9 mmol/l) with the lower threshold changed from 110 to 100 mg/dL[7, 51]; IGT is defined as a postprandial blood glucose between 140 to 199 mg/dL (between 7.8 to 11.0 mmol/l) after a 75-g glucose load on the oral glucose tolerance test (OGTT)[7]. There are many who disagreed with dropping the threshold for IFG from 110 mg/dL to 100 mg/dL[52, 53]. The ADA stated that changing the IFG cut point to 100 mg/dl (5.6 mmol/l) would optimize its sensitivity and specificity for predicting future diabetes[51], but studies suggested that IFG with the cutoff at 110 mg/dL is more likely to confer risk of postchallenge hyperglycemia[52]. In addition, IFG with the cutoff at 100 mg/dL does not predict mortality below 126 mg/dL[53]. In effect, the dropping of the threshold increases the prevalence of IFG, but with potentially low predictive value, and few studies have documented the value of lower threshold.

According to the National Health and Nutrition Examination Survey (NHANES) (1999-2002) data, the crude prevalence of IFG among adults aged ≥20 years in the US was

26.0% in 1999–2002[54]. The overall standardized prevalence in non-Hispanic blacks (17.7%) was significantly lower than that in non-Hispanic whites (26.1%, $P = 0.0007$) and Mexican Americans (31.6%, $P < 0.00001$), a pattern consistent across all ages[54]. The ARIC study results suggested that African Americans have higher fasting glucose than Caucasians[55].

The natural history of both IFG and IGT is variable, with ~25% progressing to diabetes, 50% remaining in their abnormal glycemic state, and 25% reverting to normal glucose state over an observational period of 3–5 years[56]. Individuals with other diabetes risk factors such as obesity are more likely to progress to diabetes[56]. It takes up to 10 years for individuals with prediabetes generally to develop T2DM with beta-cell abnormalities found long before frank T2DM[8]. Multiple studies have shown that IGT is more prevalent than IFG and that there is limited overlap between them[57-64]. The incidence of diabetes is highest in individuals with both IFG and IGT compared to isolated IFG or isolated IGT. Isolated IGT appears to better predict diabetic cases than isolated IFG [59, 64-68]. A FPG of 5.7 mmol/l is closer to a 2-hour glucose value of 7.8 mmol/l in terms of sensitivity and specificity of predicting future diabetes[59, 64]. There is no threshold value of IFG in terms of future diabetes and cardiovascular risks, as these risks increase continually with increasing FPG[69].

Different pathophysiologic mechanisms in glucose homeostasis have been suggested in isolated IFG and isolated IGT individuals[56]. Isolated IFG and isolated IGT individuals differ in their site of insulin resistance[70]. Hepatic insulin resistance and normal muscle insulin sensitivity are predominantly demonstrated in isolated IFG individuals whereas individuals with isolated IGT have normal to mildly reduced hepatic insulin sensitivity and moderate to severe muscle insulin resistance. Both muscle and hepatic insulin resistance are manifested in

individuals with both IFG and IGT. IFG and IGT individuals also differ in the insulin secretion pattern[70]. Individuals with isolated IFG only have a defect in early-phase insulin secretion response during the OGTT whereas individuals with isolated IGT appear to have a defect in early-phase insulin secretion but also a severe defect in late-phase insulin secretion. The combinational hepatic insulin resistance and early-phase insulin secretion defect in isolated IFG leads to fasting hyperglycemia, and the combined muscle and hepatic insulin resistance and defective late insulin secretion results in hyperglycemia after a oral glucose load.

The ARIC study evaluated practical strategies involving fasting glucose, clinical rules, and the oral glucose tolerance test (OGTT) for the detection of undiagnosed diabetes, IFG and IGT[71]. Screening with FPG using the conventional IFG cut point (FPG≥6.1mmol/l) identified 68.8% of the diabetic cases but only 28.1% of the IFG/IGT cases. Two screening strategies obtained the best results—detecting >85% of the cases of diabetes, 58% of the cases of IFG/IGT, and 52% of the cases of IGT: the first one used an FPG cut point of 6.1 mmol/l and then applied a clinical detection rule to those below this cut point; the second one used an FPG cut point of 5.6 mmol/l and then applied an OGTT to those with FPG <6.1 mmol/l. The ARIC study results suggested that FPG-based screening strategies complemented by clinical detection rules and/or an OGTT, are effective and practical in the detection of hyperglycemic states.

Gestational Diabetes Mellitus (GDM)

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy[72]. The prevalence of GDM is about 4% of all the pregnancies in the U.S.

although the range is much wider (1-14%) depending on the population studied, as well as the prevalence of obesity [73, 74]. The Nurses' Health Study identified increasing age, BMI, weight gain, cigarette smoking, and non-European ethnicity as predictive factors for GDM[74]. A study by Dooley *et al*. demonstrated that the relative risk for GDM was higher in black (1.81, 95% CI 1.13, 2.89), and Hispanic (2.45, 95% CI 1.48, 4.04) women than in white women[75]. The rate of developing diabetes after GDM varies, ranging from ~5% during the subsequent 3-6 months to 47% at 5-year follow-up; it was reported that ~40% of women previously diagnosed as GDM develop diabetes by 15-years of follow-up[76]. The development of subsequent diabetes is influenced by the degree of obesity prior to pregnancy, insulin requirements during pregnancy and higher glucose values during OGTT[76]. Based on the ARIC study results that the relative risk for diabetes was higher for African American women vs. Caucasian women than it was for African American men vs. Caucasian men [25], Kahn and Williamson proposed that the differential exclusion of gestational diabetes with respect to race may be one possible explanation[77]. A Caucasian woman with GDM is more likely to be diagnosed during pregnancy than an African American woman. It is possible that Caucasian women with a known history of GDM might have been excluded from the study, whereas African American women with an unrecognized history of GDM might have been included. Unfortunately, the ARIC study did not collect specific information on GDM.

Hypertension

Hypertension is often associated clinically with diabetes either as part of the metabolic syndrome or as a manifestation of diabetic nephropathy and the coexistence of these two conditions synergistically increases in the risk of life-threatening cardiovascular events [78-80]. Hypertension has been suggested as an independent risk factor for diabetes. In

ARIC cohort, the risk of developing diabetes was 2.4-fold greater in hypertensive individuals than in those that were normotensive after adjusting for obesity[81]. The ARIC study also found that the prevalence of hypertension than in African Americans (72% in diabetics vs. 52% non-diabetics) was higher in Caucasians (51% in diabetics vs. 25% in non-diabetics) (unpublished data). Elevated systemic blood pressure accelerates the progression of both microvascular and macrovascular complications in diabetes. Vasoactive hormone pathways, e.g. the renin-angiotensin-aldosterone system, appear to play a pertinent role in the progression of diabetes and diabetic complications[79].

Several secondary or *post hoc* trials involving patients with hypertension or cardiovascular disease have suggested that agents that block or inhibit the renin–angiotensin system may prevent diabetes[82]. However, the prospective trial, the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study[83] found that among persons with prediabetes, the use of ramipril (an angiotensin converting enzyme inhibitor to treat hypertension) for 3 years does not significantly reduce the incidence of diabetes or death but does significantly increase regression to normoglycemia. The variations in study design, participants, diabetes diagnosis, and the duration of follow-up may explain these findings.

Dyslipidemia

Reduced HDL-cholesterol levels and increased triglyceride concentrations are the key characteristics of dyslipidemia in T2DM patients[84, 85]. A similar pattern was observed in the ARIC study. The mean HDL-cholesterol levels (mg/dl) for diabetic vs. non-diabetic participants at baseline were 49 vs. 56 in African Americans, and 41 vs. 51 in Caucasians,

respectively; the mean triglyceride levels (mg/dl) were 152 vs. 105 in African Americans, and 212 vs. 130 in Caucasians, respectively (unpublished data), suggesting dyslipidemia is a risk factor for diabetes. Elevated triglyceride rich lipoproteins contribute to increased availability of free fatty acids in the liver and raised levels of free fatty acids generate lipotoxicity, which decreases insulin secretion induced by glucose and then worsens the insulin resistance. Consequently, the increased triglyceride causes the reduction of HDL cholesterol[86, 87]. Several post-hoc analyses of subgroups of diabetic subjects from large clinical trials suggest a beneficial effect of lipid-lowering therapy (e.g. statins) in T2DM, for example, a reduction of macrovascular complications[88-91].

Polycystic Ovary Syndrome (PCOS)

PCOS is characterized by chronic anovulation and hyperandrogenism[92]. Large cohort studies have demonstrated that the prevalence of glucose intolerance is as high as 40% in PCOS women when the WHO criteria are used[93-95]. It is estimated that approximately 20% of impaired glucose tolerance and 40% of T2DM are attributed to PCOS in reproductive-aged women[96]. A study by Ehrmann *et al.* suggested that African-American PCOS women had significantly higher insulin levels ($P < 0.05$) and were significantly more insulin resistant ($P < 0.05$) than Caucasian women with PCOS, however, fasting glucose and 2-h glucose levels were similar between African-American and Caucasian PCOS women[97]. The majority of women with PCOS have peripheral insulin resistance, and insulin resistance plus beta-cell dysfunction plays an important role in the consequent development of T2DM and CVD[98].

Inflammation and endothelial dysfunction

T2DM and atherosclerotic vascular disease may arise from a "common soil"[99] with common antecedent factors[100]. These factors have shown a strong correlation with markers of inflammation and endothelial dysfunction[101]. The ARIC study found that the haemostatic variables, especially associated with inflammation and endothelial dysfunction, such as factor VII [OR: 1.4 (95% CI: 1.1–1.6)], fibrinogen [1.2; (1.0–1.5)], factor VIII [1.8 (1.3–2.3) in women], and von Willebrand [1.4 (1.1–1.8) in women] are related to incidence of T2DM, after adjusting for age, sex, race, study center, family history of diabetes, fasting glucose, physical activity, and smoking[102]. ARIC findings support a role for inflammation and endothelial dysfunction in diabetes pathogenesis. Other studies found elevated levels of C-reactive protein (CRP) and other markers of inflammation manifested in patients with T2DM, suggesting atherosclerosis and T2DM may share the same inflammation origin[103-105].

5. Genetics of T2DM

Evidence for a genetic component to T2DM comes from several sources: animal models, familial aggregation, and gene mapping studies. All of these lines of evidence support a genetic etiology of T2DM, but also shed light on the complexity and heterogeneity of T2DM.

Animal models

Due to the limited availability of human tissues, animal models of diabetes have become very useful in providing valuable insights into the etiology of T2DM. Studies in animal models have aided in the identification of genes that are functionally important in the pathophysiology of T2DM.

Studies in C57BL/6J mice with IGT found that the loss-of-function mutation in the gene encoding nicotinamide nucleotide transhydrogenase was significantly associated with glucose intolerance and less insulin secretion through the impairment of mitochondrial ATP production[106]. The activation of uncoupling protein 2 will prevent glucose-dependent closure of K_{ATP} channels and consequently beta-cell activity and insulin secretion are impaired. This is a clear example of a promising candidate gene discovered from a mouse model of diabetes. Genes that have arisen from animal models such as *ARNT*[107], and *IRS2*[108] now warrant testing in genetic and functional studies in human beings.

Familial aggregation

1). Family studies

Family studies compare the disease prevalence within family members of a proband to that expected in the general population. A higher prevalence within family members is expected because of an increased number of shared genes between family members. For T2DM, the prevalence is increased in individuals who have a first degree relative with the condition. The lifetime risk of T2DM is 70% in offspring of both diabetic parents, whereas the risk is about 40% if only one parent is diabetic[109]. The ARIC study also suggested a parental history of diabetes as an important predictor of incident diabetes in middle-aged adults[34]. In addition, young-age onset T2DM seems to be more familial than late-age onset diabetes. In Pima Indians, the offspring of parents that have been diagnosed as diabetic individuals prior to the age of 45 have a higher prevalence of diabetes compared to the offspring of parents that developed diabetes after the age of 45[110]. A study in South Asian individuals reported similar findings[111].

2). Twin studies

Familial clustering suggests a genetic component for T2DM, as do twin studies. Twin studies are employed to assess the extent to which familial aggregation of disease can be accounted for by inherited genetic factors. In twin studies, the concordance rates for the presence of disease under investigation are estimated and compared in monozygotic (MZ) and dizygotic (DZ) twins. Because MZ twins share the identical genes and DZ twins share half of genes on average, and as both types of twins tend to share most of their environment, increased concordance rates in MZ twins compared with DZ twins are indicative of shared genetic factors predisposed to the disease. For T2DM, estimates for concordance rates varied ranging from 0.20 to 0.91 in MZ, while 0.10–0.43 in DZ twins[112-118]. Although these studies varied regarding sample sizes, ethnicity, study design (proband-based or population-based), disease definition and age distribution, concordance rates were consistently higher in MZ twins than in DZ twins across all studies. Based on the fact that the high concordance rates in MZ twins could reflect a correlation of intrauterine environment, and as the "equal environments assumption" in twin studies might not always hold true due to the increasing sharing of environment risk factors post-natally, the results of twin studies warrant cautious interpretation. It was estimated that the age-adjusted concordance rate in MZ twins may be up to 70-80% for T2DM[119]. Despite the caveats in twin studies, the evidence from familial aggregation still supports that a genetic component plays an important role in the etiology of T2DM.

Gene mapping studies

The inheritance patterns for T2DM are complex. Because of its complexity, with both gene-gene and gene-environment interactions, the identification of susceptibility SNPs for

T2DM has not been easily achieved. Although the progress is slow, over the last decade, researchers have embarked on linkage scans and candidate gene studies in an attempt to discover genes impacting on the risk of T2DM. The most significant findings from linkage studies, and association studies including genome-wide association studies are discussed below.

1). Linkage studies

Initial linkage studies focused on target regions in the genome with prior suggested association with disease, or regions known to harbor genes that were plausibly functional for disease predisposition[120-122]. Later, technologies advances in genome mapping enabled researchers to perform linkage scans spanning the entire genome with 5-10cM intervals. Multiple linkage studies including genome-wide linkage studies were conducted in a variety of populations and identified a number of regions demonstrating at least suggestive evidence for linkage [logarithm of the odds (LOD)>2], but only a few regions have shown significant evidence for linkage in a single scan (LOD score >3.6), or consistent replication across scans[123].

One of the earliest significant linkage peaks was at chromosome 2q37.3[124]. The gene calpain 10 (*CAPN10*)[125] encoding an intracellular calcium-dependent cysteine protease[126] was discovered 4 years after the locus was first mapped. Physiological studies suggested that variations in *CAPN10* activity affected insulin secretion[126]. However, given the inconsistency of results across linkage studies, association studies, and meta-analysis results[127-138], widespread acceptance of *CAPN10* as a T2DM predisposing gene has been lacking. These inconsistent results with respect to the *CAPN10* gene could be related to population-specific

environmental triggers, gene-gene interactions, or population-specific patterns of linkage disequilibrium (LD)[139].

Despite these difficulties with linkage analysis, a number of regions have been replicated in multiple populations[123]. A region on chromosome 1q21-1q25 has been observed in multiple distinct populations[140-145], other regions with most evidence for loci are chromosome 12q24[146-150], and chromosome 20[148, 151-154]. Additional regions showing significant linkage (LOD>3.6) in the initial scan that are supported by at least one other study (LOD>1.0) include 3q24, 3q28, 10q26, and 18p11[123].

While some of the loci have shown at least moderate support from several populations, no single locus shows strong linkage evidence in multiple populations. This suggests that T2DM is a polygenic disease and no T2DM susceptibility locus has a strong effect in most populations. Some of putative loci may be type I errors. Other possible causes may include population heterogeneity, and different gene-gene and gene-environment interactions in each population studied. It is also possible that the lack of consistency is because of a large number of genes involved, each with a small effect, and many studies have been underpowered to detect all of these genes involved.

2). Association studies

Association studies investigate the relationship between disease status and a particular allele, genotype or haplotype of genetic marker/s. A case-control study design is utilized by most association studies in which the prevalence of a putative disease marker is compared among persons with a disease (cases) to persons without the disease (controls). For T2DM, given the two major mechanisms including insulin secretion defects and insulin resistance in

the development of T2DM, most candidate gene studies have focused on genes that encode proteins in the pathways of glucose-induced insulin secretion from the beta-cells, peripheral insulin-induced glucose uptake in muscle and fat, and insulin regulation of liver gluconeogenic pathways.

To date, a large number of association studies have been undertaken in T2DM, but only a handful have been reproduced in multiple samples and generated consistent results. A number of causes may contribute to the poor reproducibility: poor study design (poor matching of cases and controls, a wide usage of convenience samples), limited sample size, limited number of markers typed, population heterogeneity, gene-gene and gene-environment interactions, etc. Despite these difficulties, there is now compelling evidence that common variants in the *TCF7L2*[2], *PPARγ*[155-157], *KCNJ11* (in Caucasians only) [127, 158-162] genes influence susceptibility to T2DM. Other possible genes, such as the *HNF4α*, have been inconsistently associated and meta-analysis of these association studies are warranted [163-166]. Other genes with less well-established impact on T2DM are *IRS1*[167], *ABCC8*[163, 168], *HNF1A*[167] and *INS* [163, 169].

The *PPARγ* (peroxisome proliferator-activated receptor-γ)[155-157, 170, 171] gene has been widely studied because it is important in adipocyte and lipid metabolism. It is also a target for the hypoglycemic drugs known as thiazolidinediones. The *PPARγ* gene substantially decreases insulin sensitivity and increases the risk of T2DM. This gene is quite common in most populations, especially in Caucasians, with a population attributable risk of ~25%. In ARIC, the Pro12Ala variant in *PPARγ* gene was not significantly associated with diabetes [OR: 0.64 (95%CI: 0.34–1.20); P=0.16] in African American participants, but the Pro/Ala

genotype was associated with markers of greater insulin sensitivity including lower insulin levels (P = 0.001), lower HOMA-IR (P = 0.005), and lower diastolic blood pressure (P = 0.02) among nonobese African Americans[170].

The *KCNJ11* (potassium inwardly-rectifying channel, subfamily J, member 11) [127, 158-162, 171] gene is part of the ATP-sensitive potassium channel, which plays a key role in regulating the release of hormones, such as insulin and glucagon, in the beta cells. Mutation in *KCNJ11* can affect the potassium channel's activity and insulin secretion, ultimately leading to the development of T2DM. *KCNJ11* is now the target for the sulphonylurea class of drugs used routinely in the treatment of T2DM. A recent meta-analysis conducted in Caucasians suggested that the E23K variant is significantly associated with T2DM [EK vs. EE: 1.12 (1.01-1.23); KK vs. EE: 1.44 (1.17-1.78)][161]. However, the only large-scale study in African Americans (n=1173) suggested that *KCNJ11* is inversely associated with T2DM [OR: 0.69 (0.49–0.99)] indicating the *KCNJ11* is a primarily susceptible gene to T2DM in Caucasians[162]. *ABCC8* is a sulfonylurea receptor that is located on the same chromosome locus 11p15.1 as *KCNJ11*[172]. Studies suggest that *ABCC8* influences insulin secretion through the ATP-sensitive potassium channels as well[172].

HNF4A, one of the genes primarily associated with the maturity onset diabetes of the young (MODY), encodes an orphan hormone nuclear receptor that, together with other HNF genes such as *HNF1A*, constitutes part of a network of transcription factors controlling gene expression in pancreatic β-cells, liver, and other tissues[163, 167]. In β-cells, these transcription factors regulate insulin secretion. Evidence for *HNF4A* and T2DM has been conflicting and a meta-analysis is warranted [163-166]. Studies suggest that *HNF-1α* Ala98Val polymorphism is

associated with a significant reduction in post-OGTT serum insulin and C-peptide levels among Caucasians [173].

IRS1 is a protein that plays a pivotal role in insulin and cytokine signalling via the phosphatidylinositol-3-kinase pathway[174]. Functional studies have shown impaired insulin signalling and impaired insulin secretion associated with this gene[167]. Furthermore, the gene is associated with insulin resistance [174].

INS encodes the hormone preproinsulin, which upon proteolytic cleavage generates mature insulin and C-peptide[163]. Evidence for the association between *INS* and T2DM is not conclusive and a role for *INS* in T2DM predisposition has not been definitively established [163].

3). Genome-wide association studies (GWAS)

The genome-wide association study (GWAS) is an increasingly popular approach to greatly enhance our understanding of the genetic basis of common and complex diseases such as T2DM[171, 175]. Companies such as Affymetrix and Illumina have utilized major advances in technology to develop high-throughput genetic arrays that can capture information from the majority of common variations in the human genome[171]. These chips can analyze approximately 300 - 2,500,000 SNPs. With the low genotyping cost per-SNP and the presence of well-designed large cohort and case-cohort studies[175], this technology has facilitated rapid progress in genetic research of T2DM.

According to the Genome.gov (accessed on February 05, 2009), a total of ten GWAS on T2DM-related traits with at least 100,000 SNPs assayed in the initial stage have been

published[176]. All GWAS were performed in the Caucasian population. **Table 1** lists SNPs with p-values < 1.0×10^{-5} from these ten GWAS. The research progress by Frayling reviewed six GWAS that were published by September, 2007 and provided convincing evidence for six new gene regions involved in T2DM in Caucasians[171] plus five known gene regions[171]: *CDKAL1* (CDK5 regulatory subunit-associated protein 1-like 1), *CDKN2* (cyclin-dependent kinase inhibitor 2A), *FTO* (fat mass and obesity-associated), HHEX (haematopoietically expressed homeobox)-*IDE* (insulin-degrading enzyme), *IGF2BP2* (insulin-like growth factor 2 mRNA-binding protein 2), *KCNJ11*, *PPARG*, *SLC30A8* (solute carrier family 30 (zinc transporter), member 8), *TCF2* (transcription factor 2, hepatic), *TCF7L2*, and *WFS1* (Wolfram syndrome 1). In addition, rs9300039 in the chromosome 11 has been identified to be associated with increased risk of T2DM ($P = 4 \times 10^{-7}$) in a Finnish GWAS[177]. In 2008, Zeggini et al. performed a meta-analysis of three T2DM GWA scans comprising of 10,128 European individuals and detected six previously unknown loci ($P < 10^{-8}$)[178]: *JAZF1* (juxtaposed with another zinc finger gene 1), *CDC123* (cell division cycle 123 homolog)-*CAMK1D* (calcium/calmodulin-dependent protein kinase 1D), *TSPAN8* (tetraspanin 8)-*LGR5* (leucine-rich repeat-containing G protein-coupled receptor 5), *THADA* (thyroid adenoma associated), *ADAMTS9* (ADAM metallopeptidase with thrombospondin type 1 motif, 9), and *NOTCH2* (Notch homolog 2). Another two GWAS discovered 4 SNPs within *KCNQ1* (potassium voltage-gated channel, KQT-like subfamily, member 1) to be associated with increased risk of T2DM in East Asian (Japanese, Singaporean) and European (Danish) populations ($P < 10^{-8}$)[179, 180]. Together, common variation in 19 gene regions altered the risk of T2DM in Caucasians with a level of statistical confidence based on GWAS findings (**Table 2**).

Common variants in *TCF7L2* emerged as one of the top signals and each T allele copy from rs7903146 conferred substantially higher risk than all of the other 10 gene variants indicating *TCF7L2* may be the most important T2DM gene in Caucasians[171]. *TCF7L2* encodes a transcription factor that is expressed in the fetal pancreas and plays a significant role in the WNT signalling pathway[171]. One of its targets is HHEX that encodes a transcription factor with a key role in pancreatic development. The *HHEX–IDE* locus has shown to be associated with reduced insulin secretion[171].

CDKN2B lies next to its close relative *CDKN2A*. The overexpression of *CDKN2A* results in decreased islet proliferation in ageing mice[181].

We know little about *CDKAL1*, but it is highly expressed in human islets[182]. *CDKAL1* shares homology with the CDK5 regulatory-subunit-associated protein-1 gene (CDK5RAP1), a known inhibitor of CDK5 activation. CDK5 may downregulate insulin expression through the formation of p35–CDK5 complexes and then reduce beta-cell function[171].

Table 1. Results from ten genome-wide association studies on T2DM-related traits

First Author (year)	Initial Sample Size	Disease/ Trait	Region	Reported Gene(s)	SNP	MAF in Controls	p-Value	OR	95% CI
Meigs(2007) [183]	1,087 individuals	Diabetes related insulin traits	14q12	Intergenic	rs2877832	NR	2.00E-06	NR	NR
			13q21.33	Intergenic	rs2066219	NR	9.00E-06	NR	NR
			14q12	Intergenic	rs2877832	NR	3.00E-06	NR	NR
			7p15.1	*CPVL*	rs10486607	NR	8.00E-06	NR	NR
		Incident diabetes	2q32.3	*TMEFF2*	rs10497721	NR	7.00E-07	NR	NR
Salonen (2007) [184]	500 cases, 497 controls	Type 2 diabetes	10q25.2	*TCF7L2*	rs7903146	NR	5.00E-08	1.71	[1.41-2.08]
			2q12.1	Intergenic	rs6712932	NR	6.00E-06	1.52	[1.27-1.82]
Saxena (2007) [185]	1,464 cases, 1,467 controls	Type 2 diabetes	10q23.33	*HHEX*	rs1111875	0.53	6.00E-10	1.13	[1.08-1.17]
			9p21.3	*CDKN2A,CDKN2B*	rs10811661	0.83	8.00E-15	1.20	[1.14-1.25]
			6p22.3	*CDKAL1*	rs7754840	0.31	4.00E-11	1.12	[1.08-1.16]
			3q27.2	*IGF2BP2*	rs4402960	0.29	9.00E-16	1.14	[1.11-1.18]
			3p25.2	*PPARG*	rs1801282	0.86	2.00E-06	1.14	[1.08-1.20]
			8q24.11	*SLC30A8*	rs13266634	0.65	5.00E-08	1.12	[1.07-1.16]
			10q25.2	*TCF7L2*	rs7903146	0.26	1.00E-48	1.37	[1.31-1.43]
			11p15.1	*KCNJ11*	rs5219	0.47	7.00E-11	1.14	[1.10-1.19]
Scott (2007) [177]	1,161 cases, 1,174 controls	Type 2 diabetes	3p25.2	*PPARG*	rs1801282	0.82	2.00E-06	1.14	[1.08-1.20]
			10q23.33	*HHEX*	rs1111875	0.52	6.00E-10	1.13	[1.09-1.17]
			11p12	*Intergenic*	rs9300039	0.89	4.00E-07	1.25	[1.15-1.37]
			9p21.3	*CDKN2A, CDKN2B*	rs10811661	0.85	8.00E-15	1.20	[1.14-1.25]
			6p22.3	*CDKAL1*	rs7754840	0.36	4.00E-11	1.12	[1.08-1.16]
			3q27.2	*IGF2BP2*	rs4402960	0.30	9.00E-16	1.14	[1.11-1.18]
			11p15.1	*KCNJ11*	rs5219	0.46	7.00E-11	1.14	[1.10-1.19]
			10q25.2	*TCF7L2*	rs7903146	0.18	1.00E-48	1.37	[1.31-1.43]
			8q24.11	*SLC30A8*	rs13266634	0.61	5.00E-08	1.12	[1.07-1.16]
Sladek(2007) [186]	1,380 cases, 1,323 controls	Type 2 diabetes	10q23.33	*HHEX*	rs1111875	0.40	3.00E-06	1.19	[0.82-1.56]
			10q25.3	*TCF7L2*	rs7903146	0.30	2.00E-34	1.65	[1.28, 2.02]
			8q24.11	*SLC30A8*	rs13266634	0.30	6.00E-08	1.18	[0.69-1.67]
Steinthorsdottir(20	1,399 cases,	Type 2	10q25.2	*TCF7L2*	rs7903146	0.30	2.00E-10	1.38	[NR]

Table 1. Results from ten genome-wide association studies on T2DM-related traits

First Author (year)	Initial Sample Size	Disease/ Trait	Region	Reported Gene(s)	SNP	MAF in Controls	p-Value	OR	95% CI
07) [187]	5,275 controls	diabetes	8q24.11	*SLC30A8*	rs13266634	0.67	3.00E-06	1.15	[1.08-1.22]
			6p22.3	*CDKAL1*	rs7756992	0.26	8.00E-09	1.20	[1.13-1.27]
Timpson(2008) [188]	1,924 cases, 2,938 controls	Type 2 diabetes	16q12.2	*FTO*	rs8050136	NR	2.00E-17 (obese)	1.30	[1.23-1.39]
			10q25.2	*TCF7L2*	rs7903146	NR	9.00E-30 (non-obese)	1.49	[1.39-1.59]
			11p15.1	*KCNJ11*	rs5219	NR	5.00E-07 (obese)	1.19	[1.11-1.27]
			8q24.11	*SLC30A8*	rs13266634	NR	7.00E-06 (non-obese)	1.18	[1.10-1.27]
			9p21.3	*CDKN2B*	rs10811661	NR	7.00E-07 (non-obese)	1.26	[1.15-1.38]
			10q25.2	*TCF7L2*	rs7903146	NR	6.00E-16 (obese)	1.31	[1.23-1.40]
			6p22.3	*CDKAL*	rs10946398	NR	7.00E-07 (non-obese)	1.18	[1.11-1.26]
			11p15.1	*KCNJ11*	rs5219	NR	1.00E-09 (non-obese)	1.25	[1.16-1.34]
WTCCC(2007) [189]	1,924 cases, 2,938 controls	Type 2 diabetes	6p22.3	*CDKAL1*	rs9465871	0.18	3.00E-07	1.18	[1.04-1.34]
			16q12.2	*FTO*	rs9939609	0.40	2.00E-07	1.34	[1.17-1.52]
			10q25.2	*TCF7L2*	rs4506565	0.32	5.00E-12	1.36	[1.20-1.54]
			4q27	NR	rs7659604	0.38	9.00E-06	1.35	[1.19-1.54]
			3p14	NR	rs358806	0.80	3.00E-06	1.16	[1.03-1.33]
			12q15	NR	rs1495377	0.50	7.00E-06	1.28	[1.11-1.49]
			12q13	NR	rs12304921	0.15	7.00E-06	2.50	[1.53-4.09]
Zeggini (2008) [178]	4,549 cases, 5,579 controls	Type 2 diabetes	7p15.1	*JAZF1*	rs864745	0.50	5.00E-14	1.10	[1.07-1.13]
			3p14.1	*ADAMTS9*	rs4607103	0.76	1.00E-08	1.09	[1.06-1.12]
			12q13.2	*DCD*	rs1153188	0.73	2.00E-07	1.08	[1.05-1.11]
			3p25.2	*SYN2, PPARG*	rs17036101	0.93	2.00E-07	1.15	[1.10-1.21]
			6p22.3	*CDKAL1*	rs6931514	NR	1.00E-11	1.25	[1.17-1.33]
			10q23.33	*HHEX*	rs5015480	NR	7.00E-08	1.17	[1.11-1.24]

Table 1. Results from ten genome-wide association studies on T2DM-related traits

First Author (year)	Initial Sample Size	Disease/ Trait	Region	Reported Gene(s)	SNP	MAF in Controls	p-Value	OR	95% CI
			16q12.2	*FTO*	rs8050136	NR	7.00E-06	1.15	[1.09-1.22]
			10q25.2	*TCF7L2*	rs7903146	NR	3.00E-23	1.37	[1.28-1.47]
			11p15.1	*KCNJ11*	rs5215	NR	4.00E-07	1.16	[1.09-1.23]
			9p21.3	*CDKN2A,CDKN2B*	rs7020996	NR	2.00E-07	1.26	[1.15-1.38]
			3q27.2	*IGF2BP2*	rs4402960	NR	8.00E-08	1.17	[1.10-1.25]
			6p21.1	*VEGFA*	rs9472138	0.28	4.00E-06	1.06	[1.04-1.09]
			1p12	*NOTCH2, ADAM30*	rs10923931	0.11	4.00E-08	1.13	[1.08-1.17]
			2p21	*THADA*	rs7578597	0.90	1.00E-09	1.15	[1.10-1.20]
			10p13	*CDC123,CAMK1D*	rs12779790	0.18	1.00E-10	1.11	[1.07-1.14]
			12q21.1	*TSPAN8,LGR5*	rs7961581	0.27	1.00E-09	1.09	[1.06-1.12]
Zeggini (2007) [190]	1,924 cases, 2,938 controls	Type 2 diabetes	16q12.2	*FTO*	rs8050136	0.60	1.00E-12	1.17	[1.12-1.22]
			10q23.33	*HHEX*	rs5015480	0.43	6.00E-10	1.13	[1.08-1.17]
			3q27.2	*IGF2BP2*	rs4402960	0.32	9.00E-16	1.14	[1.11-1.18]
			9p21.3	*CDKN2A/B*	rs10811661	0.83	8.00E-15	1.20	[1.14-1.25]
			6p22.3	*CDKAL1*	rs10946398	0.32	4.00E-11	1.12	[1.08-1.16]
			9p21.3	*CDKN2B*	rs564398	NR	1.00E-07	1.12	[1.07-1.17]
			3p25.2	*PPARG*	rs1801282	NR	2.00E-06	1.14	[1.08-1.20]
			11p15.1	*KCNJ11*	rs5215	NR	5.00E-11	1.14	[1.10-1.19]
			10q25.2	*TCF7L2*	rs7901695	NR	1.00E-48	1.37	[1.31-1.43]
			8q24.11	*SLC30A8*	rs13266634	0.30	5.00E-08	1.12	[1.07-1.16]

Abbreviations: CI, confidence interval; MAF, minor allele frequency; NR, not reported; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 2. Details of 19 T2DM gene regions

Example variant	Closest gene	Mode of identification	Previous evidence	p value	Additional evidence from human physiology
rs1801282 (P12A)	*PPARG*	Candidate	Monogenic + drug target	$2x10^{-6}$	Nothing consistent
rs5215 (E23K)	*KCNJ11*	Candidate	Monogenic + drug target	$5x10^{-11}$	Alters insulin secretion in general population
rs7901695	*TCF7L2*	Region-wide	None	$1x10^{-48}$	Alters insulin secretion in general population
rs4430796	*TCF2*	Candidate	Monogenic	$8x10^{-10}$	Nothing consistent
rs10010131	*WFS1*	Candidate	Monogenic	$1x10^{-7}$	Nothing consistent
rs1111875	*HHEX–IDE*	GWAS	Some, e.g. HHEX KO mouse has disrupted pancreatic development	$7x10^{-17}$	Early studies indicate altered insulin secretion in general population
rs13266634	*SLC30A8*	GWAS	None	$1x10^{-19}$	Early studies indicate altered insulin secretion in general population
rs10946398	*CDKAL1*	GWAS	None	$2x10^{-18}$	Early studies indicate altered insulin secretion in general population
rs10811661	*CDKN2A–2B*	GWAS	Some – CDKN2A KO mouse has reduced islet proliferation	$8x10^{-15}$	Nothing consistent
rs4402960	*IGF2BP2*	GWAS	Some — binds insulin-like growth factor mRNA	$9x10^{-16}$	Nothing consistent
rs8050136	*FTO*	GWAS	None	$1x10^{-12}$	Alters BMI in general population
rs9300039	*Intergenic*	GWAS	None	$4x10^{-7}$	Nothing consistent
rs864745	*JAZF1*	GWAS	None	$5x10^{-14}$	Nothing consistent
rs12779790	*CDC123-CAMK1D*	GWAS	None	$1x10^{-10}$	Nothing consistent
rs7961581	*TSPAN8-LGR5*	GWAS	None	$1x10^{-9}$	Nothing consistent
rs7578597	*THADA*	GWAS	None	$1x10^{-9}$	Nothing consistent
rs4607103	*ADAMTS9*	GWAS	None	$1x10^{-8}$	Nothing consistent
rs10923931	*NOTCH2*	GWAS	None	$4x10^{-8}$	Nothing consistent
rs2283228	*KCNQ1*	GWAS	None	$3x10^{-12}$	Nothing consistent

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; GWAS, genome-wide association study; KO, knockout; N/C, not captured; LF-B3, variant hepatic nuclear factor.

JAZF1 encodes a transcriptional repressor of *NR2C2* (nuclear receptor subfamily, group C, member 2). It has been shown that mice deficient in *NR2C2* have growth retardation, perinatal and early postnatal hypoglycemia[178].

Less is known about the other genes. *IGF2BP2* binds to the key growth and insulin signaling molecule insulin-like growth factor 2 (IGFII) and is also expressed in the pancreatic islet[181]. *SLC30A8* is a pancreatic beta-cell specific zinc transporter[171]. *FTO* gene is related to increased adiposity, which seems to explain its association with diabetes[191]. *KCNQ1* is expressed in pancreatic islets[178]. *CDC123* has a role in cell cycle regulation[178], and *TSPAN8* is a cell-surface glycoprotein expressed in carcinomas of pancreas[178].

The GWAS in American Indians suggested that SNPs on chromosome 3 near zinc finger protein 659 (*ZNF659*), chromosome 11 near Fanconi anemia, complementation group F (*FANCF*), chromosome 11 near zinc finger and BTB domain containing 7B (*ZBTB15*), and chromosome 12 near sentrin specific peptidase 1 (*SENP1*) confered susceptibility to younger-onset T2DM[192]. SNPs in or near four genes that showed evidence for association with T2DM in Mexican Americans: rs979752 and rs10500641 near ubiquilin-like (*UBQLNL*) and olfactory receptor, family 52, subfamily H, member 1 (*OR52H1*) on chromosome 11, rs2773080 and rs3922812 in or near Ral-A exchange factor (*RALGPS2*) on chromosome 1, and rs1509957 near early growth response 2 (*EGR2*) on chromosome 10[193]. In the Amish, the strongest T2DM association signal was observed on chromosome 7 in a functionally relevant candidate gene, *Grb10* (growth factor receptor-bound protein 10), an adaptor protein that regulates insulin receptor signaling[194]. None of these common variants identified in Caucasians was observed in American Indians, Mexican Americans, and Amish. It is

possible that the relatively sparse density of the 100K SNP panel in these 3 GWAS compared to the GWAS SNP panel in Caucasians may fail to capture those common predisposing genes [194]. It is also possible that those T2DM susceptibility genes in Caucasians may not play a significant role in diabetes in other ethnic populations[192, 193].

6. *TCF7L2* and T2DM

Of the T2DM susceptibility genes so far identified by GWAS, the SNP rs7903146 within *TCF7L2* has emerged as having by far the most pronounced effect on diabetes risk[171]. As one of the target genes of this dissertation work, I will discuss this gene, *TCF7L2*, in detail below.

The discovery of TCF7L2

The deCODE Genetics group published a genome-wide linkage scan for T2DM in 2003[195]. The authors reported suggestive evidence for linkage to a region in chromosome 10q with the LOD score of 1.69. When the interaction between the linkage peaks at 10q and 5q34, another suggestive region, the LOD score at 10q increased to 4.06 if the analysis was restricted to families with a negative score at 5q34.

In February 2006, Grant *et al.* (2006) [2] reported a common microsatellite in the *TCF7L2* gene region (DG10S478) that was associated with T2DM in an Icelandic population, with a convincing replication of this finding in two additional Caucasian samples. DG10S478 marker is within a well-defined linkage disequilibrium (LD) block of 92.1kb and no other known genes reside within this LD block. Individuals heterozygous (38% of the population) and homozygous for the at-risk DG10S478 variant (7% of the population) had (prevalence) relative risks of 1.41 (95%CI: 1.17-1.70) and 2.27 (1.70, 3.04), respectively in the Icelandic

population. Importantly, they replicated the findings in additional samples from the US and Denmark. A population attributable risk of 21% was estimated in the three combined Caucasian populations. Grant *et al.* (2006) reported five SNPs, which showed an association with T2DM as well, with the strongest correlation to DG10S478 were rs12255372 (r^2=0.95), rs7903146 (r^2=0.78), rs7901695 (r^2=0.61), rs11196205 (r^2=0.43), and rs7895340 (r^2=0.42) and recommended rs12255372 and rs7903146 be included in any replication effort[2]. Although some SNPs showed slightly higher estimated relative risks and lower p values in one or two of the cohorts, none demonstrated a stronger association to T2DM than DG10S478 when the results for all three cohorts were combined. The association of T2DM with the at-risk variant was reportedly not modified by body mass; although carriers of the at-risk variant appeared to have an earlier age of onset.

Confirmation of the original findings

Since Grant *et al.*[2], common variants in the *TCF7L2* gene have been compellingly associated with T2DM in subsequent replication studies[4], however, among the large number of SNPs showing associations with T2DM, there are no obvious functional candidates. **Table 3** reviewed the characteristics of variants within *TCF7L2* that have been investigated in subsequent replication efforts.

Association studies

Since the first association study by Grant et al.[2], a large number of papers have been published. A meta-analysis of 10 association studies suggested that *TCF7L2* rs7903146 T allele was associated with T2DM (OR: 1.46; 95% CI: 1.42-1.51)[4]. There are also data from

other ethnic populations. For example, *TCF7L2* is associated with T2DM in studies of individuals of Indian[196], Japanese[135, 197], Mexican-American[198], West African[199], Moroccan[4],

Table 3. Characterization of *TCF7L2* intron variants from replication studies.

Variant*	*TCF7L2* location†	Genomic position‡	Relative position§	MAF (Population)** Caucasian	MAF (Population)** African-American
DG10S478	Intron 3	114460845-114461228	.	0.27 (Iceland); 0.26 (Denmark); 0.25 (USA)	.
rs10885390	5' to gene	114630787	-117.552	0.29	0.35
rs12573128	Intron 3	114720787	-27.552	0.11	0.17
rs11196175	Intron 3	114726604	-21.735	0.28	0
rs7895307	Intron 3	114733951	-14.388	0.41	0.22
rs7079711	Intron 3	114735778	-12.561	0.22	0.46
rs4074718	Intron 3	114738606	-9.733	0.42	0.16
rs11196181	Intron 3	114739008	-9.331	0.07	0
rs17747324	Intron 3	114742493	-5.846	0.19	0.02
rs7901695	Intron 3	114744078	-4.261	0.28	0.47
rs4506565	Intron 3	114746031	-2.308	0.27	0.47
rs7903146	**Intron 3**	**114748339**	**0**	**0.25**	**0.29**
rs10885402	Intron 3	114751686	3.347	0.42	0.10
rs6585198	Intron 3	114752226	3.887	0.40	0.12
rs7896811	Intron 3	114756707	8.368	0.13	0.16
rs4132670	Intron 3	114757760	9.421	0.27	0.28
rs6585200	Intron 3	114758598	10.259	0.41	0.10
rs6585201	Intron 3	114758772	10.433	0.41	0.13
rs12354626	Intron 3	114762418	14.079	0.03	0
rs7904519	Intron 3	114763916	15.577	0.41	0.10
rs10885405	Intron 3	114767660	19.321	0.42	0.13
rs10885406	Intron 3	114767713	19.374	0.42	0.11
rs10787472	Intron 3	114771286	22.947	0.42	0.11
rs11196192	Intron 3	114772277	23.938	0.05	0.04
rs7924080	Intron 3	114777001	28.662	0.42	0.09
rs12243326	Intron 3	114778805	30.466	0.21	0.29
rs7077039	Intron 3	114779066	30.727	0.41	0.14
rs7100927	Intron 3	114786037	37.698	0.40	0.10
rs11196199	Intron 3	114786107	37.768	0.17	0.16
rs17685538	Intron 3	114787461	39.122	0.18	0
rs11592706	Intron 3	114788975	40.636	0.03	0
rs7895340	Intron 4	114791515	43.176	0.40	0.13
rs11196200	Intron 4	114791927	43.588	0.41	0.13
rs11196203	Intron 4	114795849	47.51	0.17	0.15
rs11196205	Intron 4	114797037	48.698	0.41	0.23
rs10885409	Intron 4	114798061	49.722	0.42	0.13
rs12255372	Intron 4	114798892	50.553	0.22	0.27
rs12265291	Intron 4	114800229	51.89	0.42	0.10

Table 3. Characterization of *TCF7L2* intron variants from replication studies.

Variant*	*TCF7L2* location†	Genomic position‡	Relative position§	MAF (Population)** Caucasian	MAF (Population)** African-American
rs11196208	Intron 4	114801305	52.966	0.42	0.13
rs7077247	Intron 4	114802060	53.721	0.42	0.15
rs12718338	Intron 4	114803036	54.697	0.40	0.14
rs11196213	Intron 4	114811544	63.205	0.43	0.31
rs3750804	Intron 4	114823840	75.501	0.31	0.06
rs11196228	Intron 4	114854287	105.948	0.07	0
rs911768	Intron 4	114864761	116.422	0.03	0.01
rs290494	Intron 4	114875861	127.522	0.19	0.03
rs3814573	Intron 4	114888083	139.744	0.41	0.08
rs1555485	Intron 4	114902524	154.185	0.20	0
rs290483	Intron 10	114905204	156.865	0.42	0.31

*From published studies. †Intronic location from Ensemble ENST00000347863 (126). ‡Genomic position on chromosome 10 in NCBI Build 35. §Genomic position (in kilobytes) relative to rs7903146. **from HapMap project data except for DG10S478. (http://www.hapmap.org). **CEPH**, Genomic DNA samples obtained for a panel of 92 unrelated individuals chosen from Centre d'Etude du Polymorphisme Human (CEPH) pedigrees comprised of UTAH (93%), French (4%), and Venezuelan (3%) samples purchased from Coriell Cell Repository; **CEU**, 30 mother-father-child trios from the CEPH collection, one of the populations studied in the HapMap project; **MAF**, minor allele frequency; **YRI**, 30 Yoruba mother-father-child trios in Ibadan, Nigeria, one of the populations studied in the HapMap project.

French[186], Amish[200] and Finnish[201] ancestry, but not among the Pima Indian population[202]. Regarding the lack of association in Pima Indians, the authors hypothesized that other highly prevalent, unidentified genetic or environmental risk factors interacted with variants in *TCF7L2* may result in no overall association in this population[202]. **Table 4** reviewed the association studies that examined the association between *TCF7L2* gene variations and T2DM and related traits in diverse populations.

It is worth noting that association studies in African Americans generated conflicting results. In the African-American participants of the Diabetes Prevention Program[203], an Afro-Caribbean sample from the UK population [204] and an African-American sample from Arkansas, US[205], the effect estimates were either close to the null or had a similar magnitude as those of their Caucasian counterparts. Notably, no statistically significant associations

between *TCF7L2* variants and T2DM were noted, possibly because of inadequate sample size. In contrast, the association between SNP rs7903146 of *TCF7L2* and T2DM was replicated in families from West Africa (RR=1.45, 95% CI 1.19-1.77)[199] and Moroccans (OR=1.56, p<0.0001)[4]. Interestingly, differences in minor allele frequency across these 5 studies cannot explain these finding discrepancies. Compared to their Caucasian counterparts, populations with large amounts of African ancestry exhibit greater genetic diversity and some diabetes susceptibility variants may be maintained at higher or lower frequencies in these populations, when compared to populations with predominantly European ancestry [206]. Furthermore, gene-gene and gene-environment interactions do play an important role in the pathogenesis of T2DM and may explain these study discrepancies.

A few studies assessed the association between *TCF7L2* and metabolic syndrome[207, 208]. No association with metabolic syndrome defined by the International Diabetes Federation [OR: 1.08 (0.90-1.28)] or National Cholesterol Education Program [OR: 1.01 (0.83-1.23)] criteria was noted in a population-based sample from the Cooperative Health Research in the Region of Augsburg survey [207]. Among patients aged ≥65 years with diabetes or impaired fasting glucose, carriers of the rs7903146 T allele (risk allele) were less likely to have two or more metabolic syndrome features [OR: 0.55 (0.30-0.99)] [208]. When metabolic syndrome features were studied separately, T allele carriers had an inverse association with hypertension [OR: 0.65 (0.35-1.20)], abdominal obesity [0.67 (0.36-1.20)], high triglycerides [0.58 (0.33-0.99)], and low HDL [0.67 (0.37-1.21)][208].

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
Barber (2007)[209]	UK females and males; Finnish females	Case-control	UK: 2834; Finnish: 1700	PCOS	OR	0.95-1.10	.	.
Bielinski (2008)[210]	Caucasian and African American adults	Cohort	13369	CHD, CVD, stroke, all cause mortality	HR	0.92-1.12	.	Age, gender, race, smoking, BMI
Bodhini (2007)[211]	Asian Indian females and males	Case-control	2069	T2DM	OR	1.29-1.56	.	Age, gender, and BMI
Cauchi (2006) [212]	French females and males	Case-control	4866	T2DM	OR	1.60-1.89	31%-37.7%	.
				CHD, severe retinopathy, severe nephropathy	.	No evidence of association	.	.
				BMI	.	Reverse association in the T2DM group ($p=8.0*10^{-3}$)	.	.
Cauchi (2006) [213]	French females and males	Prospective	4976	T2DM and IFG	HR, OR	T2DM: 1.19-1.37 T2DM&IFG: 1.14-1.20	10.4%-13.3%	.
Cauchi (2007)[4]	Moroccan females and males (cases: BMI<30; controls: BMI<27)	Case-control	931	T2DM	Allelic OR	1.56	.	.
	Austrian females and males	Case-control	1563	T2DM	Allelic OR	1.52		
Cauchi (2007)[214]	French Caucasian females and males	Case-control	6385	T2DM, Obesity	OR	1.14-1.88	.	Age and gender
Chandak (2006) [215]	Indian females and males	Case-control	1354	T2DM	OR	1.39-2.28	.	.
Dahlgren (2007)[216]	Swedish elderly males	Cohort	1142	T2DM	OR	1.88-2.15	.	.
Damcott (2006)	Amish females and	Case-control	618	T2DM	OR	0.69-1.57	.	Age, sex and

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
[200]	males							pedigree structure.
De Silva (2007)[217]	UK females and males	community-based, cases enriched case-control	community-based: 2586 case enriched: 2700	T2DM	OR	1.16-2.47	17-27%	No adjustment
Duan (2007)[218]	French cardiac females and males	Case-control	1037	T2DM	OR	1.52-2.48	.	No adjustment
Elbein (2007) [205]	Europid females and males	Case-control	378	T2DM	OR	1.46-1.72	.	.
	African American females and males	Case-control	554	T2DM	OR	0.93-1.10	.	.
Field (2006) [219]	UK Caucasian females and males	Case-control	13795	T1DM	OR	0.90-0.99	.	.
Fisher (2009)[220]	German aged 35–65 years	Case–cohort	3042	T2DM	HR	1.51		Age, gender, BMI, sports activity, smoking, energy intake etc.
Florez (2006)[203]	US Caucasian, A-A, Hispanic, Asian, American Indian with IGT	Clinical trial	W: 1671 A-A: 605 H: 497 A: 128 A-I: 82	T2DM	HR	1.00-1.55	6%-11%	.
Folsom (2008)[221]	US Caucasian and African American females and males	Cohort	13117	Colon cancer	HR	1.25	17%	Age, gender, study center and other covariates
Grant (2006) [2]	Icelandic, Danish and US Caucasian females and males	Case-control	Iceland: 2116 Denmark: 767 US: 891	T2DM	RR	1.37-3.29	17%-28%	Relatedness
Groves (2006) [222]	UK Caucasian females and males	Case-control	Population-based: 4732 Family-based: 388	T2DM	OR	1.30-1.90	~16%	.

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
Guo (2007)[202]	Pima Indian females and males	Case-control	Population-based: 3501 Family-based: 1037	T2DM	OR	1.02-1.04	.	Age, gender, birth year, and family membership
Hayashi (2007) [197]	Japanese females ad males	Case-control	2694	T2DM	OR	1.30-4.35	.	.
Helgason (2007)[199]	Danish whites, Icelandic whites, and West Africans	Case-control	Danish:3549; Icelandic: 11135; African: 1069	T2DM	OR	1.20-1.49	.	No adjustment
Horikoshi (2007) [223]	Japanese females and males	Cross-sectional	2029	T2DM	OR	1.18-1.69	~3%	Age, gender, and BMI
Humphries (2006) [204]	UK European whites, Indian Asians and Afro-Caribbean females and males	Prospective and Cross-sectional	W: 3999 I-A: 1150 A-C; 629	T2DM	HR, OR	HR: 1.25-1.61 OR: 1.05-2.11	.	Age, center, tyiglyceride, CRP, systolic blood pressure and BMI.
Kimber (2007) [224]	UK European whites females and males	Case-control	6516	T2DM	OR	1.35-2.11	18.9%	Age, gender and obesity
Kirchhoff (2008)[225]	German Caucasians at increased risk of diabetes	Cross-sectional	1065	continuous traits	.	.	.	.
Korner (2007)[226]	German Caucasian children	Case-control	1312	Obesity, glucose and insulin measures	OR	0.78 in obese children	.	.
Kottgen (2008)[227]	Caucasian and African Americans	Cohort	ARIC: 11061	chronic kidney disease	HR	1.17-1.20	.	Age, gender, center, diabetes, fasting glucose, systolic BP, antihypertensive medication intake, BMI, smoking
Kunika (2008)[228]	Japanese females and	Cross-	2877	T2DM	OR	1.59	.	.

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
	males	sectional						
Lehman (2007) [198]	Mexican Americans females and males	Family-based prospective	545	T2DM	HR	0.92-1.15	.	relatedness
Loos (2007)[229]	Europid non-diabetic females and males	population-based	1697	continuous traits	.	.	.	.
Lyssenko (2007)[230]	Swedish and Finnish females and males	Prospective	Swedish: 7061; Finnish: 2651	T2DM	OR	1.27-3.17	.	Age, time of follow-up, BMI, gender and family history of DM
Mayans (2007) [231]	Sweden females and males	Matched case-control	1792	T2DM	OR	1.08-1.47	.	No adjustment
Marquezine (2008)[232]	a Brazilian cohort of patients with known coronary heart disease; a general Brazilian cohort	Cohort	CHD patients: 560; General residents: 1449	T2DM	OR	CHD patients: 1.57; General residents: 1.15	.	Age and gender
Marzi (2007)[207]	German females and males	Case-control; cross-sectional	C-C: 2369; C-S: 1404	T2DM; MS	OR	T2DM: 1.16-2.00; MS: 1.01-1.08	.	Age, gender and BMI
Melzer (2006)[208]	Italian females and males >=65 years	Cross-sectional	1155	T2DM; IFG	OR	1.06-1.64	.	Age and gender
Munoz (2006) [233]	US Caucasian and A-A nondiabetic females aged 7-57 years	Cross-sectional	W: 138 A-A: 118	Si, AIRg, DI	*	.	.	Age, BMI, percent fat mass and ethnicity.
Ng (2007)[234]	Hong Kong Chinese females and males	Case-control	852	T2DM	OR	1.27-2.11	.	.
Palmer (2008)[235]	Hispanic American and African American non-diabetic females and males	Prospective cohort	H-A: 1268 A-A: 581	Continuous traits	.	.	.	Age, gender, center and BMI
Qu (2007)[236]	Mixed European	Family-based	2658	T1DM	†	.	.	.

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
	females and males							
Raitakari (2007)[237]	Finnish healthy children and adolescents	Prospective cohort	1663	IFG	OR	1.1-2.9	9%	Age, gender, waist, physical activity, and insulin
Rees (2008)[238]	A UK-resident South Asian cohort of Punjabi ancestry	Case-control	1268	T2DM	OR	1.31	.	.
Saadi (2008)[239]	Emirati females and males	Cross-sectional	368	Prediabetes, T2DM, MS	OR	Prediabetes/T2DM: 1.16-1.28	.	BMI, waist circumference
Salonen (2007)[184]	Caucasian females and males	GWAS	997	T2DM	OR	1.64-1.71	.	.
Saxena (2006) [240]	Scandinavia, Poland and US females and males	Family-based case-control	8310	T2DM	OR	1.40	.	.
Schafer (2007)[241]	German non-diabetic females and males	Cross-sectional	1110	Continuous traits	.	.	.	.
Scott (2006) [201]	Finnish females and males	Case-control	2104	T2DM	OR	1.01-1.39	.	.
Scott (2007)[177]	Finnish females and males	GWAS	2335	T2DM	OR	1.37	.	.
Shaat (2007) [242]	Scandinavia pregnant women	Case-control	1881	GDM	OR	1.49-2.05	.	Age?
Sladek (2007) [186]	French females and males	Case-control	5511	T2DM	OR	1.65-2.77	28%	.
Thorsby (2008)[243]	Norway females and males	Cross-sectional	2949	T2DM	OR	1.47-1.61	.	diabetes in family, waist, physical activity, BMI, SBP and HDL
Vliet-Ostaptchouk (2006) [244]	Dutch Breda females and males	Case-control	1422	T2DM	OR	1.29-1.96	10%	Age, sex, BMI
Watanabe (2007)	Mexican Americans	Family-based	572	GDM	‡	.	.	.

Table 4. Review of association studies examining the relationship between *TCF7L2* polymorphisms and T2DM and related traits.

Author (year)	Study Population	Study Design	Sample Size	Outcome	Measure	Estimates	PAF	Covariate adjustments
[245]	females and males	case-control						
Wang (2007)[246]	Finnish females and males	Clinical trial; cross-sectional; study on offspring of T2DM patients	C-T: 507; C-S: 1766 men; Offspring: 238 nondiabetics	T2DM, continuous traits	HR; OR	HR: 1.14-1.71; OR: 1.96-3.10	.	HR: Age, gender, BMI, and FPG; OR: Age, BMI
Weedon (2006) [247]	UK Caucasian females and males	Case-control	6077	T2DM	OR	1.48	.	.
Zhang (2006) [248]	US Caucasian females and males aged 30-75 years	Case-control	3520	T2DM	OR	1.42-1.99	18.7%	Age, race, time of blood draw, fasting status, physical activity, smoking, family history of diabetes and history of hypertension.

Abbreviations: A = Asian; A-A = African American; A-C = Afro-Caribbean; A-I = American Indian; AIRg = acute insulin response to glucose; DI = disposition index; H = Hispanic; I-A = Indian Asian; IFG = impaired fasting glucose; IGT = impaired glucose tolerance; MS = metabolic syndrome; NGT = normal glucose tolerance; OR = odds ratio; PAF = population attributable fraction; PCOS = polycystic ovary syndrome; Si = insulin sensitivity; T2DM = T2DM mellitus; W = White.

*ANCOVA and ordinary least-squares regression were used to compare the difference between genotypes.

†Transmission ratio=369/342 (X^2=1.0, p=0.311). SNP rs7903146 has no association with T1D.

‡Linear modeling was used to compare quantitative traits between genotypes under an additive genetic model.

Among the large number of SNPs showing associations with T2DM, there are no obvious functional candidates. The SNP rs7903146 T allele has shown strongest association with T2DM and remains the most likely candidate, however, rs7903146 is located in an intron with no obvious mechanism by which it affects the activity of *TCF7L2*[6]. Since no coding SNPs are correlated with rs7903146, it is likely that rs7903146 is closest to the unidentified functional variant [6] and the causal variant acts through affecting the expression of *TCF7L2*[249]. Because of this and in order to avoid the multiple comparison problem, my dissertation work on the associations between *TCF7L2* and prediabetes/retinal abnormalities/characteristics will focus on SNP rs7903146 only. **Table 5** reviewed association studies examining the relationship between SNP rs7903146 and diabetes-related discrete traits such as T2DM, IFG, IGT.

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
T2DM						
Bodhini (2007)[211]	Asian Indian females and males	1031/1038	T/T vs. C/C	1.50 (1.08-2.08)	Age, gender, and BMI	
			C/T vs. C/C	1.44 (1.18-1.76)	Age, gender, and BMI	
Cauchi (2006) [212]	French females and males	4734/4998*	T vs. C	1.69 (1.55-1.83)	N/A	
		1218/1439	T/T vs. C/C	2.86	N/A	
		1936/2268	C/T vs. C/C	1.66	N/A	
Cauchi (2006) [213]	French females and males	278/8868*	T vs. C	1.19 (0.92-1.53)	N/A	Baseline analysis
		364/5850	T vs. C	1.37 (1.10-1.70)	N/A	Incident T2DM over 9ys
		642/5850	T vs. C	1.30 (1.10-1.55)	N/A	Incident and Prevalent T2DM
Cauchi (2007)[4]	Moroccan females and males (cases: BMI<30; controls: BMI<27)	516/415	T vs. C	1.56 (1.92-1.89)?	N/A	
Cauchi (2007)[214]	French Caucasian females and males		T vs. C	1.88 (1.69-2.10)	Age and gender	BMI<30
				1.56 (1.33-1.84)	Age and gender	30<=BMI<40
				1.24 (1.03-1.50)	Age and gender	BMI>=40
	Austrian females and males	486/1075	T vs. C	1.52 (1.29-1.78)	N/A	
Chandak (2006) [215]	Indian females and males	2010/753*	T vs. C	1.46 (1.22-1.75)	N/A	
		532/239	T/T vs. C/C	2.17 (1.44-3.28)	N/A	
		814/365	C/T vs. C/C	1.39 (1.08-1.78)	N/A	
Dahlgren (2007)[216]	Swedish elderly males	168/1770	T/T vs. C/C	2.15 (1.20-3.85)	N/A	
			C/T vs. C/C	1.88 (1.32-2.67)	N/A	
Damcott (2006) [200]	Amish females and males	137/142	T/T vs. C/C	1.46 (p=0.07)	Age, sex and pedigree structure	
De Silva (2007)[217]	UK community-based females and males	487/2099	T vs. C	1.32 (1.13-1.52)	N/A	
			T/T vs. C/C	1.92 (1.38-2.65)	N/A	
			C/T vs. C/C	1.16 (0.94-1.43)	N/A	
	UK case enriched females and	487/2099	T vs. C	1.58 (1.38-1.80)	N/A	

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
	males					
			T/T vs. C/C	2.47 (1.82-3.34)	N/A	
			C/T vs. C/C	1.65 (1.35-2.02)	N/A	
Elbein (2007) [205]	Europid females and males	190/183	T vs. C	1.72 (1.25-2.37)	N/A	
	African American females and males	369/186	T vs. C	1.05 (0.80-1.37)	N/A	
Florez (2006) [203]	US Caucasian, A-A, Hispanic, Asian, American Indian with IGT	382/.	T/T vs. C/C	1.55 (1.20-2.01)	.	
		560/.	C/T vs. C/C	1.00 (0.84-1.19)	.	
	US Caucasians with IGT	198/.	T/T vs. C/C	1.62 (1.16-2.25)	.	
		300/.	C/T vs. C/C	1.03 (0.81-1.30)	.	
	US A-As with IGT	<79/.	T/T vs. C/C	1.20 (0.66-2.17)	.	
		126/.	C/T vs. C/C	1.09 (0.76-1.56)	.	
	US Hispanics with IGT	<76/.	T/T vs. C/C	2.26 (1.14-4.50)	.	
		90/.	C/T vs. C/C	0.89 (0.56-1.41)	.	
	US Asians with IGT	<37/.	T/T vs. C/C	0.92 (0.11-7.48)	.	
		<37/.	C/T vs. C/C	0.55 (0.23-1.33)	.	
	US American Indians with IGT	<30/.	T/T vs. C/C	NA	.	
		<30/.	C/T vs. C/C	1.15 (0.35-3.78)	.	
Grant (2006) [2]	Icelandic, Danish and US Caucasian females and males	1630/1780	T vs. C	1.54 (1.39-1.70)	Relatedness	
	Icelandic Caucasian females and males	1066/788	T vs. C	1.50 (1.31-1.71)	Relatedness	
	Danish Caucasian females and males	214/498	T vs. C	1.46 (1.15-1.85)	No adjustment	
	US Caucasian females and males	350/494	T vs. C	1.71 (1.40-2.09)	No adjustment	
Groves (2006) [222]	UK Caucasian females and males	2001/2476	T vs. C	1.36 (1.24-1.48)	No adjustment	
		1041/1392	T/T vs. C/C	1.90 (1.54-2.33)	No adjustment	
		1731/2259	C/T vs. C/C	1.35 (1.19-1.53)	No adjustment	
Guo (2007)[202]	Pima Indian females and males	578/459	T vs. C	1.04 (0.82-1.32)	Age, gender, birth year	Population-

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
					and family membership	based
		1561/1940	T vs. C	1.02 (0.68-1.54)	Age, gender, birth year and family membership	Family-based
Hayashi (2007) [197]	Japanese females ad males		T vs. C	1.30 (1.00- 1.68)		
Helgason (2007)[199]	Danish whites	1149/2400	T vs. C	1.49 (1.34-1.66)	No adjustment	
	Icelandic whites	1185/9950	T vs. C	1.47 (1.33-1.62)	No adjustment	
	West Africans	621/448	T vs. C	1.45 (1.19-1.77)	No adjustment	
Horikoshi (2007) [223]	Japanese females and males	1205/824	T vs. C	1.69 (1.21-2.36)	Age, gender and BMI	
		.	T vs. C	2.02 (1.28-3.21)	.	The analysis restricted to those with BMI lower than the median
		.	T vs. C	1.32 (0.81-2.17)	.	The analysis restricted to those with BMI higher than the median
Humphries (2006) [204]	UK European whites females and males	1459/2493	T vs. C	1.54 (1.35-1.76)	No adjustment	
		794/1492	T/T vs. C/C	2.11 (1.69-2.63)	No adjustment	
		1266/2296	C/T vs. C/C	1.43 (1.25-1.64)	No adjustment	
	UK Indian Asians females and males	837/300	T vs. C	1.53 (1.17-2.00)	No adjustment	
		426/189	T/T vs. C/C	1.64 (1.03-2.63)	No adjustment	
		741/274	C/T vs. C/C	1.50 (1.14-1.99)	No adjustment	
	UK Afro-Caribbean females and males	307/311	T vs. C	1.26 (0.92-1.73)	No adjustment	
		171/187	T/T vs. C/C	1.32 (0.74-2.33)	No adjustment	
		277/285	C/T vs. C/C	1.25 (0.90-1.75)	No adjustment	
Kimber (2007) [224]	UK European whites females and males	3225/3291	T/T vs. C/C	2.03 (1.67-2.47)	Age, gender and obesity	

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
			C/T vs. C/C	1.36 (1.21-1.52)	Age, gender and obesity	
Lehman (2007) [198]	Mexican Americans females and males	.	T/T vs. C/C	1.24	Relatedness	p=0.03
		.	C/T vs. C/C	1.09	Relatedness	
Lyssenko (2007)[230]	Swedish females and males	1422/5639	T vs. C	1.35 (1.23-1.48)	Age, time of follow-up, BMI, gender and family history of DM	
			T/T vs. C/C	1.47 (1.15-1.89)	Age, time of follow-up, BMI, gender and family history of DM	
			C/T vs. C/C	1.57 (1.37-1.80)	Age, time of follow-up, BMI, gender and family history of DM	
	Finnish females and males	150/2501	T vs. C	1.43 (1.10-1.87)	Age, time of follow-up, BMI, gender and family history of DM	
			T/T vs. C/C	3.17 (1.54-6.52)	Age, time of follow-up, BMI, gender and family history of DM	
			C/T vs. C/C	1.48 (1.04-2.12)	Age, time of follow-up, BMI, gender and family history of DM	
Mayans (2007) [231]	Sweden females and males	872/857	T vs. C	1.42 (1.21-1.69)	No adjustment	
			T/T vs. C/C	1.85 (1.18-2.90)	No adjustment	
			C/T vs. C/C	1.49 (1.21-1.83)	No adjustment	
Marzi (2007)[207]	German females and males	647/1632	T vs. C	1.36 (1.18-1.58)	Age, gender and BMI	Additive model
			T/T vs. C/C	1.92 (1.38-2.67)	Age, gender and BMI	
			C/T vs. C/C	1.33 (1.09-1.62)	Age, gender and BMI	
Melzer (2006)[208]	Italian females and males >=65 years	127/717	T vs. C	1.17 (0.80-1.72)	Age and gender	
			T/T vs. C/C	1.64 (0.93-2.87)	Age and gender	
			C/T vs. C/C	1.06 (0.70-1.60)	Age and gender	

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
Ng (2007)[234]	Hong Kong Chinese females and males	433/419	T vs. C	1.27 (0.71-2.29)	No adjustment	
Salonen (2007)[184]	Caucasian females and males	500/497	T vs. C	1.71 (1.41-2.08)	No adjustment	
Saxena (2006) [240]	Scandinavia, Poland and US females and males	8018	T vs. C	1.40(1.30-1.50)	No adjustment	Combined case-control and family-based samples
		6790	T vs. C	1.39 (1.29-1.50)	No adjustment	Combined case-control samples only
		.	T/T vs. C/C	1.86 (1.55-2.23)	No adjustment	Combined case-control samples only
		.	C/T vs. C/C	1.40 (1.27-1.55)	No adjustment	Combined case control samples only
	Scandinavian	946	T vs. C	1.27 (1.03–1.58)	No adjustment	Case-control group
	Swedish	966	T vs. C	1.45 (1.18–1.77)	No adjustment	Case-control group
	Polish	1,942	T vs. C	1.38 (1.20–1.59)	No adjustment	Case-control group
	US	2,246	T vs. C	1.45 (1.27–1.64)	No adjustment	Case-control group
	Botnia	430	T vs. C	1.47 (1.06–2.03)	No adjustment	Case-control group
	Swedish/Finnish	260	T vs. C	1.02 (0.69–1.51)	No adjustment	Case-control group
	All case-control groups	6,790	T vs. C	1.39 (1.29–1.50)	No adjustment	Case-control group
	Botnia sibs	260	T vs. C	1.83 (0.75–1.63)	No adjustment	Family-based group
	Swedish/Finnish sibs	212	T vs. C	1.56 (0.84–2.91)	No adjustment	Family-based

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
						group
	Scandinavian trios†	756	T vs. C	1.42 (1.09–1.86)	No adjustment	Family-based group
	All family-based groups	1,228	T vs. C	1.48 (1.17–1.87)	No adjustment	Family-based group
Scott (2006) [201]	Finnish females and males	1113/920	T vs. C	1.33 (1.14-1.56)	No adjustment	Additive model
Scott (2007)[177]	Finnish females and males	1161/1174	T vs. C	1.37 (1.31-1.43)	No adjustment	
Shaat (2007) [242]	Scandinavia pregnant women	644/1180	T vs. C	1.49 (1.28-1.75)	Age?	
		330/719	T/T vs. C/C	2.05 (1.41-2.99)	Age?	
		526/1042	C/T vs. C/C	1.56 (1.26-1.93)	Age?	
Sladek (2007) [186]	French females and males	.	T/T vs. C/C	2.77 (0.50)	.	GWAS
		.	C/T vs. C/C	1.65 (0.19)	.	GWAS
Vliet-Ostaptchouk (2006) [244]	Dutch Breda females and males	496/907	T vs. C	1.41 (1.19-1.66)	Age, sex and BMI	
		275/542	T/T vs. C/C	1.96 (1.37-2.80)	Age, sex and BMI	
		424/824	C/T vs. C/C	1.37 (1.08-1.73)	Age, sex and BMI	
Wang (2007)[246]	Finnish females and males	clinical trial (n=507)	T/T vs. C/C	1.14 (0.49-2.63)	Age, sex, BMI and FPG	
			C/T vs. C/C	1.29 (0.88-1.89)	Age, sex, BMI and FPG	
Weedon (2006) [247]	UK Caucasian females and males	2229/3538	T vs. C	1.48 (1.36-1.60)	.	
Nonobese T2DM						
Cauchi (2006) [212]	French females and males	2999/4998*	T vs. C	1.89 (1.72-2.09)	No adjustment	
		671/1439	T/T vs. C/C	3.63	No adjustment	
		1040/2268	C/T vs. C/C	1.85	No adjustment	
IFG						
Melzer (2006)[208]	Italian females and males >=65 years	114/830	T vs. C	1.25 (0.94-1.67)	Age and gender	
			T/T vs. C/C	1.42 (0.75-2.69)	Age and gender	
			C/T vs. C/C	1.45 (0.94-2.23)	Age and gender	
Munoz (2006)[250]	Non-diabetic European American females	13/125	T vs. C	Not reported	.	rs7903146 is not statistically

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
						associated IFG
	Non-diabetic African American females	11/107	T vs. C	Not reported	.	rs7903146 is not statistically associated IFG
Raitakari (2007)[237]	Finnish healthy children and adolescents(n=1663)	.	T/T vs. C/C	2.3 (1.0-5.3)	Age, gender, waist, physical activity, and insulin	
			C/T vs. C/C	1.4 (1.0-2.1)	Age, gender, waist, physical activity, and insulin	
IGT						
Damcott (2006)[200]	Amish females and males	139/342	T/T vs. C/C	1.55 (p=0.03)	Age, sex and pedigree structure	
T2DM & IFG						
Cauchi (2006) [213]	French females and males	1084/8868*	T vs. C	1.14 (1.00-1.31)	No adjustment	Baseline analysis
		920/5850*	T vs. C	1.20 (1.04-1.40)	No adjustment	Incident T2DM & IFG over 9 years
		2004/5850*	T vs. C	1.19 (1.07-1.38)	No adjustment	Incident and Prevalent T2DM & IFG
Melzer (2006)[208]	Italian females and males >=65 years	241/703	T vs. C	1.29 (1.04-1.60)	Age and gender	
			T/T vs. C/C	1.67 (1.05-2.65)	Age and gender	
			C/T vs. C/C	1.28 (0.93-1.76)	Age and gender	
T2DM & IGT						
Damcott (2006) [200]	Amish females and males	276/342	T/T vs. C/C	1.57 (p=0.008)	Age, sex and pedigree structure	
T1DM						
Field (2006) [219]	UK Caucasian females and males	11804/14530*	T vs. C	0.99 (0.94-1.05)	No adjustment	
		3480/4297	T/T vs. C/C	0.97 (0.85-1.10)	No adjustment	

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
		5413/6637	T/C vs. C/C	1.01 (0.93-1.08)	No adjustment	
Prediabetes/T2DM						
Saadi (2008)[239]	Emirati females and males	180/188	T vs. C	1.28 (0.89-1.84)	Age, gender, BMI, waist circumference	
Metabolic Syndrome						
Marzi (2007)[207]	German females and males	730/662	T vs. C	1.05 (0.88-1.25)	Age, gender and BMI	International Diabetes Federation definition
		370/1024	T vs. C	0.96 (0.79-1.16)	Age, gender and BMI	NCEP definition
Saadi (2008)[239]	Emirati females and males	180/188	T vs. C	No association		Data not shown
PCOS						
Barber (2007)[209]	UK females and males	358/2476	T vs. C	0.95 (0.81-1.17)	No adjustment	
	Finnish females	476/936	T vs. C	1.10 (0.90-1.34)	No adjustment	
Two or more metabolic syndrome features						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.37 (0.15-0.92)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.55 (0.30-0.99)	Age and gender	
High blood pressure or meds.						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.37 (0.16–0.90)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.65(0.35–1.20)	Age and gender	
Obesity						
Cauchi (2007)[214]	French Caucasian females and males	.	T vs. C	1.16 (0.96-1.40)	Age	Non-diabetics: BMI>=40 vs. <30
		.	T vs. C	1.13 (0.99-1.29)	Age	Non-diabetics: 30<=BMI<40 vs. <30
		.	T vs. C	1.69 (1.46-1.95)	Age	Diabetics: BMI>=40 vs. <30

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
		.	T vs. C	1.36 (1.20-1.55)	Age	Diabetics: 30<=BMI<40 vs. <30
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.53(0.20–1.35)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.67(0.36–1.22)	Age and gender	
High TG						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.68(0.32–1.43)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.58(0.33–0.99)	Age and gender	
Low HDL						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.83(0.37–1.86)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.67(0.37–1.21)	Age and gender	
Myocardial infarction						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	0.16(0.03–0.84)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	0.270.08–0.92)	Age and gender	
Poor renal function						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	3.15(1.27–7.81)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	1.74(0.96–3.17)	Age and gender	
Retinopathy						
Cauchi (2006)[251]	French males and females	.	T vs. C	Not reported	.	rs7903146 not associated with severe retinopathy
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	7.15(0.87–58.51)	Age and gender	
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	6.13(0.77–48.86)	Age and gender	
Dementia						
Melzer (2006)[208]	Italian diabetics >=65 years	.	CT/TT vs. CC	10.67(1.00–113.70)	Age and gender	

Table 5. Review of association studies examining the relationship between *TCF7L2* SNP rs7903146 and T2DM and related traits.

Author (Year)	Study Population	No. cases/controls	Genotype contrast	OR (95% CI)	Covariate adjustments	Notes
	Italian diabetics and IFG >=65 years	.	CT/TT vs. CC	11.62(1.38–97.57)	Age and gender	

Biological mechanism

Wnt signaling pathway

The exact mechanism by which the *TCF7L2* gene influences the susceptibility to T2DM is not clear. *TCF7L2*, also known as *TCF4*, is a nuclear receptor for cadherin-associated protein, beta 1 (β-catenin)[252]. As a consequence, *TCF7L2* may mediate the canonical Wingless and Int (Wnt) signaling pathway[252]. The Wnt signaling pathway is critical for normal embryogenesis, cell proliferation and motility. Mutations in different molecules involved in Wnt signaling have been identified in several cancers, e.g. colorectal, pancreatic, kidney, ovarian and uterine cancers [252-254]. Animal studies have suggested that the *TCF7L2*-null mice died shortly after birth due to the lack of epithelial stem-cell compartments in the small intestine[255]. The importance of Wnt signaling in glucose homeostasis is further highlighted by the recent finding that common variants in *HHEX* and *IDE* genes are associated with T2DM[177, 186, 187, 190]. *HHEX* is a target of Wnt signaling.

Impaired insulin secretion vs. insulin resistance

More studies have found associations with impaired insulin secretion than with increased insulin resistance[203, 229, 230]. **Table 6** reviewed associated studies on rs7903146 and diabetes-related continuous traits including measurements on insulin secretion and insulin resistance.

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
BMI (kg/m^2)	Bodhini (2007)[211]	Asian Indian females and males	23.7 ± 4.6	23.2 ± 4.4	24.3 ± 5.1	NS
	Cauchi (2006) [212]	French controls	22.75 ± 2.25	22.87 ± 2.31	22.83 ± 2.27	0.76
		French T2DM subjects	30.40 ± 6.30	30.01 ± 5.71	29.29 ± 5.75	$8.0*10^{-3}$
	Cauchi (2006) [213]	French controls at baseline (n=4434)	24.49 ± 3.67	24.51 ± 3.70	24.23 ± 3.52	0.39
		French controls at end of study (n=2925)	25.29 ± 3.89	25.27 ± 3.74	25.14 ± 3.76	0.84
	Guo (2007)[202]	Pima Indian females and males (n=3501)	37.4 ± 8.6	36.2 ± 8.1	34.2 ± 6.8	<0.05
	Kimber (2007) [224]	UK European controls (n=3291)	26.7 (4.7)	26.8 (4.6)	26.9 (4.5)	0.802
		UK European cases (n=3225)	31.6 (6.3)	31.3 (6.0)	30.4 (6.3)	0.002
	Kirchhoff (2008)[225]	German non-diabetic Caucasians (n=1065)	27.21±0.54	27.01±0.30	27.65 ± 0.30	
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	26.6 ± 0.4	26.8 ± 0.2	27.0 ± 0.2	0.24
	Lyssenko (2007)[230]	Swedish females and males	24.4± 3.3	24.5± 3.4	24.5± 3.5	NS
		Finnish females and males	24.2± 3.5	25.9± 4.0	25.6± 4.2	P<0.05
	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	27.37 ± 3.56	27.18 ± 4.16	27.75 ± 4.06	0.165
	Saadi (2008)[239]	Emirati females and males (n=368)	29.1 ± 6.2	28.5 ± 6.3	28.4 ± 6.7	0.22
	Saxena (2006) [240]	Scandinavia, Poland and US females and males (n=8258)	28.4 ± 5.4	28.4 ± 5.3	28.2 ± 5.2	P>0.05
	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	28.2 ± 1.0	28.9 ± 0.4	29.5 ± 0.4	0.51
Waist (cm)	Bodhini (2007)[211]	Asian Indian females and males	85.2 ± 10.9	82.9 ± 11.4	83.8 ± 11.8	NS
	Kimber (2007) [224]	UK European controls (n=3291)	92.9 (13.1)	92.5 (13.0)	91.7 (13.4)	0.549
		UK European cases (n=3225)	104.8 (14.3)	104.3 (13.6)	102.1 (14.5)	0.001
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	91.3 ± 0.9	92.5 ± 0.4	92.9 ± 0.4	0.21
	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	91.35 ± 10.24	91.93 ± 10.66	93.79 ± 9.88	0.009
	Saadi (2008)[239]	Emirati females and males (n=368)	90.6 ± 14.9	87.9 ± 13.2	84.8 ± 12.9	0.02
Fasting Plasma Glucose (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	4.7 ± 0.5	4.7 ± 0.4	4.6 ± 0.4	NS
	Cauchi (2006) [212]	French controls	5.11 ± 0.47	5.12 ± 0.48	5.09 ± 0.49	0.68
	Cauchi (2006) [213]	French controls at baseline (n=4434)	5.17 ± 0.47	5.18 ± 0.47	5.16 ± 0.49	0.61
		French controls at end of study (n=2925)	5.03 ± 0.43	5.03 ± 0.45	5.01 ± 0.44	0.87
	Damcott (2006) [200]	Amish nondiabetic subjects (n=664)	5.10 ± 0.04	5.13 ± 0.04	5.03 ± 0.07	0.92

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
	Guo (2007)[202]	Pima Indian females and males (n=3501)	5.01 ± 0.55	4.96 ± 0.57	5.36 ± 0.51	0.74
	Korner (2007)[226]	German Caucasian obese children (n=283)	4.83 ± 0.10	4.79 ± 0.04	4.68 ± 0.04	0.02
	Lyssenko (2007)[230]	Swedish females and males	4.9± 0.5	4.9± 0.5	4.9± 0.5	NS
		Finnish females and males	5.6± 0.5	5.6± 0.6	5.5± 0.6	P<0.05
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	5.61 ± 0.06	5.54 ± 0.03	5.49 ± 0.02	0.042
	Mayans (2007) [231]	Sweden non-diabetics (n=857)	5.4±0.7	5.3±1.0	5.2±0.7	0.16
		Sweden family-based non-diabetics (n=83)	5.4±0.1	4.7±0.7	5.0±0.6	0.17
	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	5.31 ± 1.27	5.19 ±1.23	5.08 ±1.22	0.028
	Saadi (2008)[239]	Emirati females and males (n=368)	5.9 ± 1.9	5.9 ± 1.8	6.3 ± 2.5	0.30
	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	5.2 ± 0.07	5.1 ± 0.02	5.1 ± 0.02	0.25
Glucose at 2h OGTT (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	6.0 ± 1.3	5.7 ± 1.1	5.6 ± 1.0	P<0.05 (TT vs. CC)
	Guo (2007)[202]	Pima Indian females and males (n=3501)	6.79 ± 1.69	7.13 ± 1.60	8.22	0.08
	Korner (2007)[226]	German Caucasian obese children (n=283)	6.28 ± 0.22	6.13 ± 0.09	5.89 ± 0.08	0.04
	Lyssenko (2007)[230]	Swedish females and males	5.9±1.6	5.8±1.5	5.8±1.5	NS
		Finnish females and males	6.5±1.6	6.4±1.5	6.1±1.5	P<0.05
	Mayans (2007) [231]	Sweden non-diabetics (n=857)	6.5±1.7	6.5±1.5	6.4±1.3	0.66
		Sweden family-based non-diabetics (n=83)	5.2±0.9	5.2±0.9	6.3±0.8	0.16
	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	6.5 ± 2.0	6.9 ± 2.4	6.6 ± 1.7	0.9
	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	6.7 ± 0.2	6.2 ± 0.07	6.1 ± 0.07	0.06
Fasting plasma insulin (pmol/l)	Cauchi (2006) [212]	French controls	39.17 ± 19.01	38.08 ± 25.79	35.56 ± 19.64	0.08
	Korner (2007)[226]	German Caucasian obese children (n=283)	88.6 ± 10.0	88.8 ± 5.3	80.5 ± 4.1	P>0.1
	Melzer (2006)[208]	Italian females and males >=65 years	70.48 ± 1.55	78.87 ± 1.70	81.74 ± 1.69	0.030

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
		(n=920)				
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	48.6 ± 2.1	48.9 ± 1.0	49.5 ± 0.9	0.60
	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	49.8 ± 3.1	53.4 ± 1.7	62.0 ± 2.1	0.004
Fasting plasma insulin (mU/l)	Saxena (2006) [240]	Scandinavia, Poland and US females and males (n=995)	7.27 ± 4.24	9.19 ± 6.66	8.94 ± 6.02	P>0.05
Fasting intact proinsulin (pmol/l)	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	4.46 ± 0.20	3.89 ± 0.08	3.56 ± 0.07	P<0.001
Fasting 32,33 split proinsulin (pmol/l)	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	4.69 ± 0.28	4.06 ± 0.11	3.85 ± 0.10	0.0028
Peak Insulin (pmol/l)	Korner (2007)[226]	German Caucasian obese children (n=283)	1080 ± 153	1029 ± 59	978 ± 58	P>0.1
Ln(Fasting plasma insulin) (pmol/l)	Cauchi (2006) [213]	French controls at baseline (n=4434)	3.68 ± 0.51	3.66 ± 0.51	3.61 ± 0.53	0.04
	Saadi (2008)[239]	Emirati females and males (n=368)	1.60 ± 0.29	1.64 ± 0.33	1.61 ± 0.31	0.8
Ln(Fasting plasma insulin) (mmol/l)	Damcott (2006) [200]	Amish females and males (n=664)	4.11 ± 0.03	4.09 ± 0.03	4.12 ± 0.06	0.92
		French controls at end of study (n=2925)	3.90 ± 0.57	3.89 ± 0.53	3.82 ± 0.55	0.09
Ln30min-plasma insulin (uU/ml)	Guo (2007)[202]	Pima Indian females and males (n=3501)	2.35 ± 0.27	2.34 ± 0.24	2.10	0.15
2h insulin (pmol/l)	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	372 ± 34	356 ± 17	442 ± 19	0.12
Proinsulin (pmol/l)	Dahlgren (2007)[216]	Swedish elderly males (n=1142)	6.8 ± 5.0	6.9 ± 4.1	8.3 ± 8.2	CT vs TT:0.02; CC vs.TT: 0.001
Log (2-h OGTT insulin) (pmol/L)	Saadi (2008)[239]	Emirati females and males (n=368)	2.30 ± 0.38	2.33 ± 0.40	2.35 ± 0.35	0.5
HbA1c (%)	Kimber (2007) [224]	UK European controls (n=3291)	5.63 (0.5)	5.60 (0.4)	5.56 (0.4)	0.003

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
		UK European cases (n=3225)	7.88 (1.4)	7.72 (1.5)	7.64 (1.5)	0.012
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	5.46 ± 0.04	5.38 ± 0.02	5.35 ± 0.02	0.012
	Saadi (2008)[239]	Emirati females and males (n=368)	5.9 ± 1.1	6.0 ± 1.3	6.3 ± 1.9	1.0
HOMA (mmol*mU/l^2)	Lyssenko (2007)[230]	Swedish females and males	2.2± 2.2	2.1± 2.9	1.9± 1.5	NS
		Finnish females and males	1.3± 1.0	1.4± 0.9	1.3± 1.0	NS
HOMA-B	Cauchi (2006) [212]	French controls	73.09 ± 54.07	69.69 ± 52.87	67.24 ± 42.76	0.18
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	77.7 ± 2.4	81.0 ± 1.2	83.3 ± 1.1	0.028
	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	95.75 ± 1.69	106.23 ± 1.61	113.19 ± 1.57	0.001
Ln(HOMA-B) (AU)	Cauchi (2006) [213]	French controls at baseline (n=4434)	4.41 ± 0.52	4.38 ± 0.52	4.35 ± 0.53	0.04
		French controls at end of study (n=2925)	4.73 ± 0.54	4.71 ± 0.52	4.66 ± 0.60	0.18
Log (HOMA2-%B) (%)	Saadi (2008)[239]	Emirati females and males (n=368)	1.81 ± 0.28	1.78 ± 0.34	1.80 ± 0.30	0.9
HOMA-IR	Cauchi (2006) [212]	French controls	1.26 ± 0.67	1.23 ± 0.88	1.15 ± 0.69	0.12
	Damcott (2006) [200]	Amish nondiabetic subjects (n=664)	2.81 ± 0.13	2.64 ± 0.12	2.78 ± 0.21	0.70
	Korner (2007)[226]	German Caucasian obese children (n=283)	2.75 ± 0.36	2.68 ± 0.17	2.31 ± 0.12	>0.1
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	92.9 ± 4.0	91.3 ± 1.8	90.1 ± 1.6	0.45
	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	82.80 ± 1.55	76.68 ± 1.66	74.06 ± 1.63	0.053
	Saxena (2006) (127)	Scandinavia, Poland and US females and males (n=995)	1.88 ± 1.29	2.30 ± 2.06	2.19 ± 1.63	p>0.05
Ln(HOMA-IR) (AU)	Cauchi (2006) [213]	French controls at baseline (n=4434)	0.41 ± 0.55	0.39 ± 0.55	0.34 ± 0.56	0.07
		French controls at end of study (n=2925)	0.61 ± 0.60	0.60 ± 0.56	0.52 ± 0.58	0.09
Log (HOMA2-IR) (mmol pmol/L^2)	Saadi (2008)[239]	Emirati females and males (n=368)	−0.12 ± 0.29	−0.07 ± 0.32	−0.11 ± 0.31	0.8
Glycated hemoglobin (%)	Bodhini (2007)[211]	Asian Indian females and males	5.7 ± 0.5	5.6 ± 0.4	5.5 ± 0.4	NS
	Cauchi (2006) [212]	French controls	5.21 ± 0.40	5.22 ± 0.38	5.19 ± 0.35	0.62
GLP-1 (pmol/l) at	Schafer (2007)[241]	German non-diabetic females and males	17.3 ± 2.0	17.3 ± 1.3	16.1 ± 0.9	0.91

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
0 min		(n=155)				
GLP-1 (pmol/l) at 30 min	Schafer (2007)[241]	German non-diabetic females and males (n=155)	38.1 ± 3.5	38.8 ± 4.0	34.1 ± 2.1	0.45
GLP-1 (pmol/l) at 120 min	Schafer (2007)[241]	German non-diabetic females and males (n=155)	28.9 ± 2.4	29.0 ± 1.7	28.9 ±1.5	0.87
Cholesterol (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	4.67 ± 0.91	4.65 ± 0.89	4.54 ± 0.95	NS
	Cauchi (2006) [212]	French controls	5.67 ± 0.95	5.64 ± 0.93	5.67 ± 0.95	0.65
	Cauchi (2006) [213]	French controls at baseline (n=4434)	5.72 ± 1.01	5.69 ± 0.99	5.72 ± 0.94	0.69
		French controls at end of study (n=2925)	5.72 ± 0.90	5.73 ± 0.91	5.69 ± 0.93	0.77
	Kimber (2007) [224]	UK European controls (n=3291)	5.32 (1.1)	5.35 (1.1)	5.25 (1.0)	0.375
		UK European cases (n=3225)	4.48 (0.9)	4.45 (0.9)	4.46 (0.9)	0.757
Cholesterol (mg/dl)	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	208.83 ± 1.21	214.35 ± 1.20	212.92 ± 1.21	0.680
HDL (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	1.12 ± 0.24	1.12 ± 0.26	1.08 ± 0.24	NS
	Cauchi (2006) [212]	French controls	1.72 ± 0.42	1.73 ± 0.44	1.74 ± 0.46	0.86
	Cauchi (2006) [213]	French controls at baseline (n=4434)	1.64 ± 0.42	1.64 ± 0.43	1.66 ± 0.44	0.74
		French controls at end of study (n=2925)	1.54 ± 0.36	1.53 ± 0.36	1.55 ± 0.39	0.67
	Kimber (2007) [224]	UK European controls (n=3291)	1.65 (0.5)	1.64 (0.5)	1.66 (0.5)	0.563
		UK European cases (n=3225)	1.37 (0.4)	1.37 (0.4)	1.39 (0.4)	0.306
HDL (mg/dl)	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	56.32 ± 1.30	54.13 ± 1.31	52.55 ± 1.29	0.008
LDL (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	2.94 ± 0.70	2.90 ± 0.78	2.85 ± 0.79	NS
	Cauchi (2006) [212]	French controls	3.51 ± 0.90	3.47 ± 0.86	3.51 ± 0.88	0.67
	Cauchi (2006) [213]	French controls at baseline (n=4434)	3.56 ± 0.93	3.55 ± 0.90	3.57 ± 0.90	0.84
		French controls at end of study (n=2925)	3.66 ± 0.79	3.67 ± 0.77	3.63 ± 0.77	0.73
	Kimber (2007) [224]	UK European controls (n=3291)	2.98 (1.0)	2.98 (1.0)	2.92 (0.9)	0.632
		UK European cases (n=3225)	2.17 (0.8)	2.12 (0.8)	2.14 (0.8)	0.393
LDL (mg/dl)	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	126.73 ± 1.33	132.27 ± 1.29	131.38 ± 1.31	0.495
TG (mmol/l)	Bodhini (2007)[211]	Asian Indian females and males	1.17 ± 0.01	1.19 ± 0.02	1.32 ± 0.02	NS
	Cauchi (2006) [212]	French controls	0.99 ± 0.64	0.97 ± 0.58	0.96 ± 0.54	0.61
	Cauchi (2006) [213]	French controls at baseline (n=4434)	1.14 ± 0.76	1.12 ± 0.79	1.10 ± 0.66	0.59
		French controls at end of study (n=2925)	1.14 ± 0.59	1.16 ± 0.61	1.10 ± 0.61	0.25

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
	Kimber (2007) [224]	UK European controls (n=3291)	1.56 (1.0)	1.64 (1.3)	1.56 (1.0)	0.326
		UK European cases (n=3225)	2.21 (1.5)	2.25 (1.4)	2.21 (1.4)	0.756
TG (mg/dl)	Melzer (2006)[208]	Italian females and males >=65 years (n=920)	102.65 ± 1.56	113.5 ± 1.58	118.24 ± 1.57	0.006
Serum creatinine (μmol/l)	Kimber (2007) [224]	UK European controls (n=3291)	94.5 (19.4)	95.2 (18.4)	93.3 (19.5)	0.419
		UK European cases (n=3225)	98.8 (24.1)	99.8 (27.0)	97.5 (23.4)	0.252
GAUC	Damcott (2006) [200]	Amish nondiabetic subjects (n=664)	18.81 ± 0.32	18.99 ± 0.30	18.96 ± 0.51	0.28
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	15.8 ± 0.3	15.3 ± 0.1	15.0 ± 0.1	0.013
	Saxena (2006) (127)	Scandinavia, Poland and US females and males (n=721)	339.8 ± 262.8	271.4 ± 214.5	270.0 ± 195.3	p>0.05
$AUC_{glucose}$ (mmol/l*min)	Elbein (2007) [205]	Europid non-diabetics	834 (809–859)	867 (838–898)	875 (804–952)	0.16
		African American non-diabetics	794 (758–833)	821 (777–869)	863 (731–1020)	NS
IAUC	Damcott (2006) [200]	Amish nondiabetic subjects (n=664)	665.2 ± 41.0	637.7 ± 39.3	630.3 ± 66.4	0.54
	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	621 ± 26	594 ± 12	616 ± 11	0.54
	Saxena (2006) [240]	Scandinavia, Poland and US females and males (n=721)	3,911 ± 3,658	4,971 ± 3,176	5,229 ± 3,248	P<<0.05
$AUC_{insulin}$ (pmol/l × min)	Elbein (2007) [205]	Europid non-diabetics	34930 (31687–38504)	41361 (36948–46301)	48460 (36562–64230)	0.016
		African American non-diabetics	50277 (43594–57983)	38899 (33007–45842)	45851 (26597–79041)	0.06
AUC C-peptide: AUC glucose (pmol:mmol)	Kirchhoff (2008)[225]	German non-diabetic Caucasians (n=1065)	316±5	298±5	278±11	0.0002
AUC proinsulin: AUC glucose (pmol:mmol)	Kirchhoff (2008)[225]	German non-diabetic Caucasians (n=1065)	0.065±0.006	0.054±0.002	0.053±0.002	0.019
Insulin	Schafer (2007)[241]	German non-diabetic females and males	17.8 ± 1.2	18.2 ± 0.5	16.8 ± 0.5	0.02

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
sensitivity$_{OGTT}$ (arbitrary units)		(n=1110)				
Insulin sensitivity (pmol/liter per min)	Palmer (2008)[235]	Hispanic American non-diabetic females and males (n=1268)	2.11 ± 1.81	2.20 ± 1.83	2.01 ± 1.70	0.2317
		African American non-diabetic females and males (n=581)	1.81 ± 1.22	1.81 ± 1.34	1.42 ± 0.75	0.6079
Insulin sensitivity (AU)	Kirchhoff (2008)[225]	German non-diabetic Caucasians (n=1065)	17.02±1.15	18.10±0.53	16.93±0.48	0.011
Insulin secretion$_{OGTT}$ (pmol/mmol)	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	292 ± 10	301 ± 5	319 ± 5	0.003
Insulin/glucose ratio (pmol/mmol)	Schafer (2007)[241]	German non-diabetic females and males (n=1110)	124 ± 13	127 ± 5	143 ± 5	0.003
Insulin-to-glucose ratio at 30min	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	88.4 ± 5.9	86.1 ± 2.7	88.6 ± 2.5	0.70
Insulin-to-glucose ratio at 60min	Loos (2007)[229]	Europid non-diabetic females and males (n=1697)	108.5 ± 8.6	120.2 ± 4.5	134.0 ± 4.5	0.0035
$\sqrt{IS}$	Damcott (2006) [200]	Amish nondiabetic subjects (n=664)	0.82 ± 0.04	0.77 ± 0.04	0.78 ± 0.09	0.39
S_i (10^{-5} (min*[pmol/l]$^{-1}$)	Damcott (2006) [200]	Non-Amish nondiabetic Caucasians (n=48)	5.62 ± 0.44	3.77 ± 0.71	2.67 ± 1.18	0.03
S_i (10^{-4} min^{-1} [uU/ml]$^{-1}$)	Elbein (2007) [205]	Europid non-diabetics	3.17 (2.79-3.60)	2.94 (2.54-3.40)	1.74 (1.24-2.44)	0.004
		African American non-diabetics	2.70 (2.35-3.10)	2.91 (2.46-3.43)	2.34 (1.45-3.77)	NS
S_g (min^{-1})[b]	Elbein (2007) [205]	Europid non-diabetics	0.0158 (0.0147–0.0169)	0.0167 (0.0153–0.0181)	0.0141 (0.0116–0.017)	0.21
		African American non-diabetics	0.0175 (0.0155–0.0197)	0.0170 (0.0147–0.0195)	0.0135 (0.009–0.0203)	NS
AIRg (pmol/l)	Damcott (2006) [200]	Non-Amish nondiabetic Caucasians (n=48)	510.9 ± 44.9	496.3 ± 77.6	244.6 ± 123.1	0.05
AIR_g(pmol/l×min)	Elbein (2007) [205]	Europid non-diabetics	2183 (1925–2476)	2074 (1796–2394)	2501 (1793–3488)	0.56

Table 6. Review of association studies examining the relationship between *TCF7L2* rs7903146 and continuous traits by variants.

Outcome	Author (Year)	Study Population	Genotype CC	Genotype CT	Genotype TT	*P* value
		African American non-diabetics	3456 (2820–4230)	3018 (23270–3846)	3498 (1740–7026)	NS
AIR (pmol/liter)	Palmer (2008)[235]	Hispanic American non-diabetic females and males (n=1268)	806.98 ± 664.31	730.62 ± 642.30	687.81 ± 748.99	0.0319
		African American non-diabetic females and males (n=581)	963.97 ± 841.56	793.63 ± 703.28	754.01 ± 524.66	0.2591
LnAIR (uU/ml)	Guo (2007)[202]	Pima Indian females and males (n=3501)	2.36 ± 0.28	2.32 ± 0.27	2.01	0.06
Insulinogenic index (mU/mmol)	Saxena (2006) [240]	Scandinavia, Poland and US females and males (n=995)	10.9 ± 12.7	16.5 ± 50.5	18.1 ± 33.1	P<<0.05
	Lyssenko (2007)[230]	Swedish females and males	9.5± 4.9	9.2± 5.5	10.2± 5.3	P<0.05
		Finnish females and males	4.6± 3.3	5.1± 4.0	5.4± 4.3	NS
Insulinogenic index (pmol/mmol)	Elbein (2007) [205]	Europid non-diabetics	91.5 (81.9–102.1)	97.8 (86.1–111.1)	102.0 (74.3–140.0)	0.64
		African American non-diabetics	170 (137–212)	110 (85–141)	127 (55–291)	0.033
DI (S_i x AIRg)	Damcott (2006) [200]	Non-Amish nondiabetic Caucasians (n=48)	2,674 ± 249	1,941 ± 422	824 ± 670	0.02
	Elbein (2007) [205]	Europid non-diabetics	1152 (1001–1326)	1061 (903–1248)	726 (501–1052)	0.067
		African American non-diabetics	1596 (1278–1993)	1450 (1112–1890)	1361 (634–2922)	NS
DI (mU^2/l^2)	Lyssenko (2007)[230]	Swedish females and males	7.1± 6.2	7.2± 5.4	8.1± 6.2	P<0.05
		Finnish females and males	4.3± 3.7	4.5± 3.6	4.8± 3.8	NS
	Saxena (2006) [240]	Scandinavia, Poland and US females and males (n=995)	22.5 ± 28.9	35.8 ± 112.9	42.6 ± 79.9	P<<0.05
DI (min^{-1})	Palmer (2008)[235]	Hispanic American non-diabetic females and males (n=1268)	1348.80 ± 1208.10	1307.78 ± 1245.86	1231.26 ± 1297.01	0.0725
		African American non-diabetic females and males (n=581)	1541.38 ± 1386.08	1242.28 ± 1096.36	1032.92 ± 932.11	0.1547

GAUC, glucose area under the OGTT curve; HOMA-IR, homeostatis model assessment of insulin resistance; IAUC, insulin area under the OGTT curve; IS, insulin secretion; WHR = Waist –to-Hip ratio

The finding that the variants within *TCF7L2* are associated with a decreased insulin secretion has been supported by subsequent association studies[200, 201, 203, 213, 215, 229, 230, 240, 241]. For example, in the Diabetes Prevention Study, carriers of the T risk allele at rs7903146 had significantly lower levels of insulin secretion than did CC homozygotes ($P < 0.001$ for corrected insulin response)[203]. A study by Saxena *et al.* suggested that the insulinogenic index ($P = 0.003$) and insulin disposition index ($P = 0.004$) for the rs7903146 risk allele was reduced ~50% in homozygous individuals [240]. However, Munoz et al. noticed that, in non-diabetic women, rs12255372 was associated with reduced insulin secretion but not rs7903146[233].

A study by Cauchi et al.[212] demonstrated that the *TCF7L2* gene is highly expressed in the pancreas which apparently contradicts the murine models [256]. The significant expression in human pancreatic β-cells suggests that *TCF7L2* may be involved in β-cell development and/or function, and differentiation from the precursor cells[212]. Damcott et al. (2006) found that variants within *TCF7L2* were associated with insulin resistance in the Amish [200]. Authors put forth the hypothesis that variants with *TCF7L2* disrupt adipogenesis and/or adipocyte function by altering the transcriptional regulation of CEBPA and PPARG, two important regulators of adipogenesis for β-catenin/TCF complex, leading to deposition of triglycerides in peripheral tissues and resulting in insulin resistance[200]. Moreover, Chandak et al. found an association of the rs12255372 risk allele in non-diabetic Indian controls with higher glycaemia and higher HOMA-insulin resistance, suggesting defects in insulin secretion and an increase in insulin resistance[215]. A study by Elbein et al. suggested that *TCF7L2* was associated with reduced insulin sensitivity, but not insulin secretion in US participants of European descent [205].

Additional evidence for a role of *TCF7L2* in the regulation of insulin secretion comes from a birthweight study. Freathy *et al*. [257]genotyped the rs7903146 variant in 15,709 individuals from six studies, and in 8344 mothers from three studies. Each fetal copy of the T2DM risk allele was associated with an 18-g increase in birthweight ($P = 0.001$), and each maternal copy with a 30-g increase in offspring birthweight ($P = 2.8 \times 10^{-5}$). The association still holds (31 g, corrected $P = 0.003$) when stratified by fetal genotype. This suggests that the association was primarily driven by maternal genotype. They also analyzed diabetes-related traits in 10 314 non-diabetic individuals. From these analyses, they suggested the most likely mechanism for the birthweight effect is that the risk allele reduces maternal insulin secretion [the disposition index was reduced by 0.15 standard deviations ($P = 1 \times 10^{-4}$). This would result in elevated maternal blood glucose levels in pregnancy and hence increased offspring birthweight.

Why do *TCF7L2* mutations impair insulin secretion? The exact mechanism is still unclear. It has been suggested that variants of *TCF7L2* gene influence the susceptibility to T2DM through altered transcriptional regulation of insulinotropic hormone glucagon-like peptide 1 (GLP-1), a peptide secreted by the intestinal endocrine L-cells[2, 256]. Dominant-negative *TCF7L2* was shown to repress proglucagon gene mRNA expression and GLP-1 synthesis. GLP-1 can lower blood glucose levels through the stimulation of insulin secretion and biosynthesis, the inhibition of glucagon release and gastric emptying and the enhancement of peripheral insulin sensitivity[256]. GLP-1-based therapies for T2DM are currently marketed such as Byetta, an injectable GLP-1 analogue. Alternatively, as *TCF7L2* is part of the WNT signaling[252], a pathway critical for normal embryogenesis, cell

proliferation and motility, an effect on beta-cell mass, pancreatic beta-cell development and/or beta-cell function implicates itself.

TCF7L2 and BMI-related traits

It is worth noting that results on the association between BMI and *TCF7L2* were inconsistent[2, 201, 203, 224, 237, 244]. Several studies reported a negative association between BMI and *TCF7L2*[2, 203, 224, 244], however, Kimber et al.[224] found that this inverse association was only noted in diabetic patients, not in controls. Another two studies did not observe any association with BMI[201, 237]. It has been suggested that a chronic reduction in the anabolic effect of insulin may explain the association of *TCF7L2* variants with BMI in diabetic patients, but not in controls[249].

No studies to date have demonstrated an additive interaction between body mass traits and *TCF7L2* variants. The study by Duan et al., which evaluated the interaction between obesity and SNP rs12255372 among French patients with established coronary heart disease, found no evidence for effect modification on the multiplicative scale ($p>0.34$) [218]. Another study by Wang et al. did not find an interaction between rs12255372 and BMI or lean body mass in Finnish men aged 50 to 70 years, either[246]. In contrast, a multiplicative interaction was noted in a European Caucasian (rs7903146: $p=0.001$; rs12255372: $p=0.04$) and a Japanese population (rs7903146: $p=0.031$) [204, 223]. Both studies found that the risk of T2DM increased in lean individuals whereas the risk decreased in obese/over-weight individuals. For individuals with a lower BMI, the risk of T2DM increased as BMI decreased[204]. Watanabe et al. reported an interaction between SNP rs12255372 of *TCF7L2* and percent body fat ($p=0.016$) on 30-minute plasma insulin concentrations in families of a proband with

previous gestational diabetes mellitus in Mexican Americans [245]. Watanabe et al. further proposed that *TCF7L2* variants may have dual effects, limiting β-cell compensation through acute effects in lean people, but minimizing the insulin secretion defects related to adiposity [245]. The mechanism of action of *TCF7L2* variants in the context of obesity and/or other metabolic impairments is an important area for further research.

B. Diabetic retinopathy

Diabetic retinopathy, one of the common and severe complications of T2DM, is a leading cause of blindness in people 20 to 74 years of age[258-260]. Diabetic retinopathy remains an important problem with the rapid increase of prevalence of diabetes worldwide.

1. Clinical manifestation of diabetic retinopathy

The earliest clinical signs of diabetic retinopathy are microaneurysms, small outpouchings from retinal capillaries, and dot intraretinal hemorrhages[261]. These signs are present in nearly 80 percent of those with T2DM for 20 years[262]. As the disease progresses, patients with preproliferative retinopathy have an increase in the number and size of intraretinal hemorrhages. This may be accompanied by cotton-wool spots; both of these signs indicate regional failure of the retinal microvascular circulation, which results in ischemia[261].

Proliferative diabetic retinopathy involves the formation of new blood vessels that develop from the retinal circulation. New vessels can extend into the vitreous cavity of the eye and can hemorrhage into the vitreous, resulting in visual loss. Late in the course of the disease, in the presence of severe retinal hypoxia, new blood vessels may form within the stroma of the iris and may extend, with accompanying fibrosis, into the structures that drain the anterior chamber angle of the eye[261].

Another important change is diabetic macular edema, which involves the breakdown of the blood–retinal barrier, with leakage of plasma from small blood vessels in the macula, the central portion of the retina that is responsible for the major part of visual function. This causes swelling of the central retina. Resorption of the fluid elements from plasma leads to the deposition of its lipid and lipoprotein components and the formation of hard exudates. Although diabetic macular edema does not cause total blindness, it frequently leads to severe loss of central vision and is often difficult to successfully treat with laser photocoagulation[261].

2. Epidemiology of diabetic retinopathy

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) is the first epidemiological study to estimate the prevalence and incidence of diabetic retinopathy in the US[263]. This study was designed to examine all persons receiving care for diabetes in south central Wisconsin[264]. The WESDR found that prevalence of diabetic retinopathy (1979-80) varied from 17% to 98% in persons with younger onset diabetes with duration of diabetes fewer than 5 years and 15 or more years, respectively; for those with older onset diabetes the prevalence were 29% and 78%, respectively[263].

Subsequent to the WESDR, many epidemiological studies on retinopathy were performed and the Eye Disease Prevalence Research Group summarized study results from these studies[265]. The estimated crude prevalence of diabetic retinopathy in the diabetic US population 40 years and older was 3.4% (95% CI, 3.2%-3.6%) and the estimated crude prevalence of vision-threatening diabetic retinopathy in the same population was 0.75% (95% CI, 0.66%-0.85%)[265]. The prevalence of diabetic retinopathy increases as age increases

among Caucasian (OR per step in age category = 1.47, P <.001), African American (OR = 1.30, P <.001), and Hispanic (OR = 1.58, P <.001) persons[265].

In ARIC, the overall three-year incidence and cumulative prevalence of any retinopathy, regardless of diabetes status, were 3.8% and 7.7%, respectively; for Caucasians were 3.6% and 5.5%; for African Americans were 4.5% and 13.0%[266]. African American participants have significantly higher cumulative prevalence than Caucasian participants (P <0.001), but not incidence. When stratified by diabetes status, diabetic participants had a substantially higher 3-year incidence (10.1% vs. 2.9%, P <0.001) and cumulative prevalence (27.2% vs. 4.3%, P <0.001) than non-diabetic participants.

3. Risk factors for diabetic retinopathy

Duration and degree of glycemia are major predictors for the development of diabetic retinopathy[258-260].

Duration of disease

Duration of disease is probably the strongest risk factor for diabetic retinopathy[267]. Among younger-onset patients with age at diagnosis less than 30 years old in the WESDR, the prevalence of any retinopathy was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years[268]. The prevalence of proliferative diabetic retinopathy was 0% at 3 years and increased to 25% at 15 years[268]. The incidence of retinopathy and proliferative diabetic retinopathy also increased with increasing duration among younger-onset patients with diabetes[268]. Still in the WESDR, among diabetic patients with age at diagnosis ≥ 30 years old, the prevalence of diabetic retinopathy varied from 28.8% in diabetic patients for less

than five years to 77.8% for 15 or more years[262]. The rate of proliferative diabetic retinopathy varied from 2.0% in diabetic patients for less than five years to 15.5% for 15 or more years.

Hyperglycemia/Glycemic control

The Diabetes Control and Complications Trial (DCCT) found that intensive diabetes management with three or more daily insulin injections or a continuous subcutaneous insulin infusion reduced the mean risk of retinopathy by 76% (95% CI 62–85) among type 1 diabetes patients without retinopathy, and by 36 months, reduced the risk of progression by 54% (95% CI 39–66) among type 1 diabetes patients with minimal-to-moderate non-proliferative diabetic retinopathy[267].

The protective effect of intensive glycemic control has also been for confirmed in another randomized clinical trail in patients with T2DM. The U.K. Prospective Diabetes Study (UKPDS) reported that the overall rate of microvascular complications was decreased by 25% in patients receiving intensive therapy versus conventional therapy, and for every percentage point decrease in HbA_{1c} (e.g., from 8 to 7%), there was a 35% reduction in the risk of microvascular complications[267].

The ARIC study identified hyperglycemia as a risk factor for retinopathy[266]. One standard deviation (42 mg/dl) increase in fasting serum glucose was associated with increased incidence of any retinopathy (OR 1.6, 95% CI 1.3 to 2.1) and retinopathy among those without diabetes (OR 1.5, 95% CI 1.0 to 2.3) after adjusting for age, gender, race, study center, current smoking, mean arterial blood pressure, total cholesterol and plasma fibrinogen.

Hypertension/Blood pressure control

The UKPDS reported that, with a median follow-up of 8.4 years, hypertensive patients with T2DM with tight blood pressure control (<150/85 mmHg) had a 34% reduction in progression of retinopathy and a 47% reduced risk of deterioration in visual acuity of three lines in association with a 10/5 mmHg reduction in blood pressure[267].

The study results from ARIC suggested hypertension as a risk factor for retinopathy[266]. After adjusting for age, gender, race, and study center, increased risk of any retinopathy (OR 1.5, 95% CI 1.0 to 2.3, per standard deviation increase in risk factor levels) and non-diabetic retinopathy (OR 1.4, 95% CI 0.9 to 2.3) with higher levels of mean arterial blood pressure was observed.

4. Retinopathy in diabetes development

Retinopathy is found in people with prediabetes [260] which suggests that microvascular disease may contribute to the development of T2DM [269, 270]. Studies showed that microvascular abnormalities such as arteriolar narrowing and impaired microvascular blood flow in the skin and skeletal muscles have been noted in persons with T2DM and in persons at high risk of developing diabetes, such as those with prediabetes and first-degree relatives of persons with diabetes[271-274]. Previous ARIC studies suggested that the retinal arteriolar narrowing is independently associated with risk of diabetes[274] and that retinopathy predicts subsequent risk of clinical diabetes in individuals with a family history of diabetes[275], supporting a microvascular role in the development of diabetes. Therefore, early identification of individuals with increased risk for retinopathy among diabetics and non-diabetics may be important for effective intervention.

Retinal microvascular signs (e.g., retinopathy, arteriolar narrowing, arterio-venous nicking) are potential markers of systemic arteriolar disease. Previous ARIC studies have demonstrated that narrower retinal arteriolar diameters are related to elevated blood pressure[276], incident T2DM [274, 277] and incident hypertension[278]. Larger venular calibers have been further shown to predict the progression of retinopathy, independent of severity of retinopathy[279]. In this study, the severity level of retinopathy was derived by concatenating the levels for the two eyes, giving the eye with the higher level greater weight[280]. This scheme provided a 15-step severity scale. The progression to proliferative retinopathy was estimated from all persons who were free of this complication at the baseline examination; for persons with no or only nonproliferative retinopathy, progression was defined as the first instance of an increase in the severity of retinopathy by two steps or more from the baseline level at any of the follow-up examinations[279, 280]. The 14-year rate of progression to retinopathy was 86%, and rate of progression to proliferative retinopathy was 37%[280].

5. Genetics of diabetic retinopathy

Evidence is accumulating that not only is there underlying genetic susceptibility to diabetes, but genetic variation also plays a role in the development of diabetic retinopathy[281].

Familial aggregation

A study of 322 families from south India reported an approximate threefold increased risk for retinopathy in siblings of probands with retinopathy relative to siblings of those without[282].

In follow-up studies from the Diabetes Control and Complications Trial (DCCT) with patients who had type 1 diabetes[283], there was evidence for aggregation of more severe

retinopathy: Correlations for the severity of retinopathy were 0.187 (all family members), 0.327 (parent-offspring), 0.249 (father-child), 0.391 (mother-child), and 0.060 (sib-sib). These results are consistent with the familial study in 656 patients with T2DM from 282 Mexican-American sibships from Starr County, Texas[284].

Candidate gene studies

Although a large number of candidate genes have been examined in subjects with diabetes, no definitive major predisposing genes or functional consequences of genetic variants have been identified for retinopathy[285, 286]. One of the well-studied genes is *VEGF* (vascular endothelial growth factor). *VEGF* is produced in many cell types in the retina and is known to be a mediator of ischemia-induced vascularization and neovascularization[287]. Three SNPs in the promoter and 5´UTR regions of the gene were studied in different populations, but the studies are inconclusive[286]. Another popular susceptibility gene is *eNOS* (endothelial nitric oxide synthase) which plays an active role in vascular relaxation and upregulates vascular growth protein expression. Results for an association between SNPs within the *eNOS* gene and DR are conflicting across different ethnic populations[286].

Regarding the association between *TCF7L2* rs7903146 and retinopathy, an earlier case-control study in a French population reported the lack of an association with severe retinopathy (effect estimates not reported)[251]. The InCHIANTI study of elderly Europeans reported an association with diabetic retinopathy (OR=7.15, 95%CI=0.87-58.51, *P*=0.067) in 127 persons with diabetes. However the number of participants with diabetic retinopathy was very small (*n*=12) and results were not statistically significant[288].

The ARIC study evaluated whether the Apolipoprotein E (*APOE*) gene is associated with retinal microvascular signs[289, 290]. After adjusting for age, sex, systolic blood pressure, total serum cholesterol, triglycerides, and other covariates, *APOE* ε4 was associated with retinopathy in non-diabetic Caucasian (OR, 1.3; 95% CI, 1.0-1.6) and African American (1.4; 1.0-2.1) individuals[290], however, APOE gene polymorphisms are not associated with diabetic retinopathy in either Caucasians (1.04; 0.66–1.65) or African-Americans (0.95; 0.57–1.56) with T2DM[289]. In addition, no strong association with other retinal microvascular signs including retinal arteriolar and venular diameter were noted[290].

C. Public health significance

Although the *TCF7L2* gene effect is consistently observed across ethnically diverse populations [2, 4], studies conducted in African Americans have been of small sample size and have demonstrated inconsistent results[4, 199, 203-205]. Moreover, literature on *TCF7L2* gene–environment interaction assessment is limited, particularly on biologic interaction viewed as a departure from expected additivity[291]. Gene-environment interaction has been strongly implicated in the pathogenesis of T2DM[292] and an understanding of a given genetic variant in its metabolic context is critical to determining the health implications of a given variant and the priority it should receive for identifying interventions to reduce its associated risk. Thus, the proposed study will contribute to the extant knowledge in several ways. It will quantify the effect of the *TCF7L2* gene on incident prediabetes, as well as its association with retinal microvascular signs (retinopathy, focal narrowing, AV nicking, CRAE, CRVE). The detailed phenotypic characterization available on the ARIC cohort members will permit adjustment for a range of potential confounders and evaluation on gene-environment interactions. The evaluation of gene-environment interactions will be another important strength of this study.

This study will potentially contribute significant knowledge about the etiology of prediabetes, T2DM and retinopathy, and may aid in the development of screening strategies and treatment regimes utilizing genetic information.

CHAPTER IV

PRELIMANARY STUDIES

A. ARIC study design

ARIC is a prospective investigation of the etiology and natural history of subclinical and clinically manifest atherosclerosis funded by the National Heart, Lung, and Blood Institute (NHLBI). It includes a cohort of 15,792 middle-aged men and women, ages 45 to 64 years old at recruitment (1987-1989), which was selected as a probability sample from four U.S. communities. The cohort was re-examined every three years through January 1999 (**Table 7**). The study also conducts an on-going epidemiologic surveillance of cardiovascular and cerebrovascular disease hospital admissions and mortality of all residents 35 to 74 years of age in the study communities from which the cohort was recruited. Recruitment of the cohort occurred during 1987-89 in four U.S. locations: Forsyth CO, NC; Jackson, MS; seven

Table 7. Sample Size in the ARIC Cohort Clinical Examination Visits by Ethnicity and Gender.

	Visit 1 1987-89	Visit 2 1990-92	Visit 3 1993-95	Visit 4 1996-98
Study Center				
Forsyth County, NC	4035	3679	3340	2851
Jackson, MS	3728	3148	2622	2368
Minneapolis, MN	4009	3827	3497	3252
Washington County, MD	4020	3694	3426	3185
Total (all ethnic groups)	15,792	14,348	12,885	11,656
Ethnicity/Gender				
African American Men	1631	1331	1097	963
African American Women	2639	2246	1900	1701
European American Men	5429	5054	4601	4169
European American Women	6049	5675	5248	4792
Total (excludes other ethnic groups)	15,748	14,306	12,846	11,625

northwestern suburbs of Minneapolis, MN; and Washington CO, MD. Approximately 4,000 participants were recruited from each community. The overall recruitment response rates varied from 42% in African American men to 68% in white women[122]. Women constituted slightly more than 50% of the baseline ARIC cohort, permitting analyses by gender. African-Americans were over-sampled in Forsyth CO and were exclusively sampled in Jackson and comprised 27% of the baseline cohort. This provides sufficient power to investigate findings by ethnicity in the aggregate, and as often as possible, in the two different geographic locations. The very small sample sizes for the two other ethnicities recorded at baseline (Asian, n=34; American Indian, n=14) preclude interpretation of stratified analyses and are therefore not included in this study.

After a home interview which established a baseline socio-demographic and cardiovascular disease profile of all enumerated residents in each study community who were willing to participate, age-eligible residents were invited to participate in a baseline, and three subsequent clinical examinations, scheduled at three year intervals. The baseline examination (Visit 1) was conducted between 1987 and 1989; Visit 2 was held between 1990 and 1992; Visit 3 between 1993 and 1995; and the last clinic visit (Visit 4) was conducted between 1996 and 1998.

ARIC study personnel also continue to contact cohort members annually by telephone to establish vital status and assess indices of cardiovascular disease, including hospitalizations. Annual follow-up interviews have continued after the last clinic exam (Visit 4), and those data will be available to the investigators on a continuing basis. The follow-up of the ARIC cohort has been quite successful, with completeness of follow-up at high levels

through the present, namely the 12th contact of individuals examined during 1986-1989. Responses to cohort contact year 09 - the latest complete contact cycle - based on 14,881 eligible individuals contacted during 1995 -1997 – are as follows: 96% contacted and alive; 1% deceased (during the contact year); 1% refused; 1% could not be reached, but were reported alive by next of kin/contact persons; and 1% were not contacted during this cycle. The responses to follow-up contact year 13 (calendar years 1998-2000), as tracked by the ARIC Coordinating Center, suggests that these patterns are unchanged and that completeness of follow-up remains between 97 and 98%.

B. Extant ARIC data resources and their quality

Access to the ARIC data and approval of their use for the study proposed here has been granted by the ARIC Steering Committee, and the IRB application has been approved (please see **Appendix 1**). All procedures and interviews in the ARIC study were conducted under quality assurance programs. These are described in the data collection protocols for each study area (ARIC protocol manuals 1-18). For each cohort examination, the quality assurance procedures were assembled into a manual (ARIC protocol No. 12: Quality Assurance and Quality Control). Briefly, written manuals of operations were developed for each clinical examination and the community surveillance component. Data collection instruments were provided with on-entry range, and consistency checks, and with question by question instructions for their administration. Instruments were pilot tested before implementation. Central and continuous on-site training was conducted for all staff. Annual (for interviewers) or bi-annual (for technicians) recertification was conducted and documented at the Coordinating Center. Annual field center and central laboratory/reading center monitoring visits were made by Coordinating Center staff.

Specific data quality analyses were conducted periodically by the ARIC Quality Control Committee, with reports to the Steering Committee. Overall data quality and completeness were monitored by means of quarterly data management reports, reviewed by the study's relevant administrative and procedural over-sight committees (the Steering and its Executive Committee; Cohort Operations, Community Surveillance, Laboratory, Quality Control, Sampling/Recruitment, and Ultrasound Committees) and annually reviewed by the Policy Board. A system of phantom IDs was maintained throughout the study to routinely monitor blinded repeat measurements by the same and different technicians. Laboratory and reading center results were monitored by the Coordinating Center and the Quality Control Committee for completeness, blinded repeatability, and also for trends over time. Equipment calibration and maintenance protocols were followed for all field center and reading center equipment; results were documented and monitored by the Coordinating Center or relevant Reading Center or Laboratory, and supported by on-site monitoring visits by trained staff and contracted maintenance personnel. In addition, external standardization where appropriate and rigorous internal quality control measures were conducted by the central laboratories and reading centers, specific to the laboratory, imaging, or processing technology.

C. DNA extraction and storage

Genomic DNA has been isolated from all ARIC participants by the ARIC DNA laboratory under the direction of Dr. Eric Boerwinkle. DNA was extracted from frozen buffy coat, which was thawed, washed, recovered by centrifugation, and submitted to overnight digestion at 37°C with cell lysis buffer. Phenol/chloroform methods were used to recover precipitated DNA, which was then solubilized in 0.1x TE buffer by incubation at 37 °C for 1-3 days. Buffy coat from 10 ml of human blood yields approximately 250 - 400 ug of

genomic DNA. A portion of each primary aliquot for the entire ARIC cohort has been removed from storage and transferred to 96-well microtiter plates in a constant volume/constant concentration format. Working plates for PCR and routine genotyping (10 ng per reaction) have then been replicated from these master plates, using a Biomek FX workstation. The DNA has been used in many previous ARIC studies.

D. SNP genotyping

Genomic DNA from the ARIC cohort was genotyped by the ARIC Central Laboratory for *TCF7L2* rs7903146 using Taqman® (Applied Biosystems, Foster City, CA) methods. The TaqMan assay uses fluorogenic probes in a 5' nuclease assay to identify differences in DNA sequence. For high through-put processing, we employed the Applied Biosystems 7900HT Sequence Detection System. Briefly, allele-specific probes approximately 13-30 bp in length are labeled at the 5' end with a fluorescent reporter dye and one of the following two quencher dyes at the 3' end: TAMRA (fluorescent dye) or MGB (a nonfluorescent dye that binds in the minor groove). These probes are blocked at the 3' end to prevent extension during PCR. The proximity of the reporter dye molecule to the quencher dye molecule masks the fluorescent activity of the reporter dye as long as the probe remains intact. During the annealing and extension phase of the PCR reaction, primers and probes bind to the DNA strand in a site-specific manner. As the *Taq* DNA polymerase extends the DNA strand from the primer, its 5' nuclease activity degrades the bound probe and releases the reporter dye, causing an increase in the fluorescence intensity of the reporter dye. Each allele-specific probe is labeled with a different reporter dye, usually FAM (6-carboxy-fluorescein) and VIC (Applied Biosystems proprietary reagent). Genotypes are determined by analysis of the FAM and VIC fluorescent signals. An increase in only one of the

fluorescent signals indicates that the sample is homozygous for either the FAM- or VIC-specific allele while an increase in both signals is indicative of heterozygosity at the locus. All PCR reagents are included in the TaqMan Universal PCR Master Mix (Applied Biosystems). The AB 7900HT system includes software for optimizing probe and primer design and PCR conditions, thereby reducing the occurrence of non-specific probe binding (Primer Express™).

Laboratory-designed probes and primers were obtained from Applied Bioystems (Foster City, CA) and IDT (Coralville, IA), respectively. Assay-on-Demand (AoD) and Assay-by-Design (AbD) are ready-to-use genotyping products supplied by Applied Biosystems. The AoD product consists of validated, pre-designed assays and the AbD are custom-designed. The AoD and AbD products consist of a concentrated reaction mix that contains both primers and probes. The total reaction volume of 5 µL will include 3 ng of human genomic DNA, 4 mM $MgCl_2$, 200 µM each dCTP, dATP, and dGTP, 400 µM dUTP, and 0.35 units of AmpliTaq Gold DNA polymerase. All PCR reactions took place in optical 384-well reaction plates (Applied Biosystems). Thermal cycling of PCR reactions were carried out using the Dual 384-Well GeneAmp® PCR System 9700 (Applied Biosystems), and the DNA Engine Tetrad (MJ Research). Within two hours after completion of PCR, the fluorescent activity for each plate was determined using the ABI 7900HT. Quantification of fluorescence was made by comparing each sample's fluorescent activity to that of a background dye present in the reaction buffer, and a blank standard containing no DNA. These comparisons were made to normalize the samples for variation in pipetting as well as to normalize the results for reactivity of the PCR. The ABI 7900HT Sequence Detection Software makes these comparison calculations and uses the results to automatically assign

and store genotypes in an Oracle Database that was exported in computerized format.

DNA laboratory quality assurance

ARIC Central Laboratory maintains a strict adherence to quality control procedures; the major components include standard protocols, laboratory safety standards, cross-training, computerized freezer inventory, sample retrieval lists, separate pre- and post- PCR areas, bar-coded labels, standardized DNA concentrations, robotic liquid handling, redundancy, data validity checks, data fire walls, negative controls, blind duplicate program, Hardy-Weinberg test and missing data rate. In brief, this proposed study implemented a sophisticated blind duplicate program in which 5% of samples were re-genotyped. In total, 726 ARIC participants were genotyped in duplicate. The percentage of agreement ranged from 98% and simple Kappa coefficients ranged from 0.97 indicating a good genotyping quality. Moreover, No Hardy-Weinberg deviation was detected ($P > 0.05$ in both African American and Caucasian participants).

E. Preliminary data on T2DM in the ARIC study

ARIC investigators have a longstanding interest in understanding the influence of genetic factors in the etiology and pathogenesis of diabetes and hyperglycemia. The ARIC study contains a rich set of measurements of diabetes and hyperglycemia and its risk factors in which to study the association of variants of *TCF7L2* and diabetes/hyperglycemia.

1. Descriptive statistics of the ARIC cohort at baseline

An overview of characteristics of the participants in the baseline ARIC cohort by gender and race are presented in **Table 8.** The average age at first examination for the entire study population was approximately 54 years. Mean BMI in all groups approached or

exceeded the cutoff for obesity (BMI $\geq$ 30.0), and was highest in African American women. African American participants were more likely to be current smokers than Caucasians. Physical activity indices in both races were moderate (2-3). The prevalence of hypertension was approximately 28% overall, with higher prevalences in African Americans. Among non-diabetic participants, the mean HOMA-IR was highest in African American women followed by African American men and lowest in Caucasian women. The average systolic and diastolic blood pressure was higher in men of both races. Mean HDL was higher in women than men in both ethnicities.

Table 8. Distribution of Selected Diabetes-, Obesity-, and CVD-Related Phenotypes in the ARIC study. Data are presented as mean (standard deviation) unless otherwise indicated.

	African American		Caucasian	
	Males	Females	Males	Females
Sample Size, N (%)	1631 (10)	2635 (17)	5428 (34)	6050 (38)
Ever Smokers, N (%)	1170 (72)	1115 (42)	3914 (72)	2987 (49)
Current Smokers, N (%)	622 (38)	651 (25)	1337 (25)	1507 (25)
Leisure Time Physical Activity (score 1-5)	2.06 (0.58)	2.07 (0.58)	2.42 (0.52)	2.50 (0.54)
IFG*, N (%)	227 (14)	289 (11)	817 (15)	475 (8)
HOMA-IR (uU/ml*mmol/l) [§]	2.85 (2.29)	3.63 (2.86)	2.81 (2.17)	2.30 (2.01)
Glucose (mmol/l) [§]	5.53 (0.60)	5.46 (0.58)	5.60 (0.50)	5.37 (0.49)
Insulin (μU/ml) [§]	11.29 (8.34)	14.56 (10.36)	11.07 (7.95)	9.37 (7.31)
Diabetes[+], N (%)	293 (18)	528 (21)	553 (10)	493 (8)
Hypertension[‡], N (%)	887 (55)	1487 (57)	1541 (29)	1580 (26)
Family Diabetes History, N (%)	400 (25)	774 (29)	1226 (23)	1483 (25)
Age (years)	54 (6)	53 (6)	55 (6)	54 (7)
BMI (kg/m^2)	28 (5)	31 (7)	27 (4)	27 (6)
Waist (cm)	97 (13)	101 (16)	100 (10)	93 (15)
Triglycerides (mg/dl)	120 (94)	110 (70)	148 (100)	129 (86)
HDL (mg/dl)	50 (17)	58 (17)	43 (12)	57 (17)
LDL (mg/dl)	137 (42)	138 (44)	140 (36)	136 (40)
SBP (mm Hg)	130 (22)	128 (21)	120 (16)	117 (18)
DBP (mm Hg)	82 (13)	78 (12)	73 (10)	70 (10)

*IFG is defined as the FPG falls between 6.1 (100 mg/dL) and 6.9mmol/l (126 mg/dL); [+]Diabetes defined as FPG levels of at least 7.0 mmol/L (126 mg/dL), nonfasting glucose levels of at least 11.1 mmol/L (200 mg/dL), current use of medications prescribed to treat diabetes (eg, insulin or sulfonylureas), or a positive response to the question "Has a doctor ever told you that you had diabetes (sugar in the blood)?"; [‡]Hypertension defined as SBP >=140mmHg or DBP>=90mmHg or self-reported medication use; [§]Excluding cases of prevalent T2DM.

2. Diabetes prevalence and incidence in the ARIC study

Eighteen hundred individuals were diagnosed with T2DM at the baseline exam. One thousand forty six of these individuals were Caucasian and 821 were African American, with a greater proportion of female diabetic participants in comparison to males.

Among 12,845 adults without T2DM at baseline, 766 female and 755 male incident T2DM events were noted during 9 years of follow-up (**Table 9**). The incidence of T2DM was highest in African American females and lowest in Caucasian females [the unadjusted relative risk of incident T2DM in African American females was 2.16 times (95% CI: 1.89-2.47) that in Caucasian females].

Table 9. Incident T2DM in 12,845 Adults without Diabetes at Baseline, by Sex and Race

	Females		Males	
	African-American	Caucasian	African-American	Caucasian
No. of persons at risk	1828	5297	1114	4606
Incident cases of T2DM	327	439	186	569
Risk (95% CI)	0.18 (0.16-0.20)	0.08 (0.07-0.09)	0.17 (0.15-0.19)	0.12 (0.11-0.13)

3. Preliminary data on *TCF7L2*-T2DM associations in ARIC

The preliminary data on the association between *TCF7L2* and incident T2DM in the ARIC Study has been published in *Diabetes*[293], and is summarized as follows.

Objectives

In this study, we investigated whether the rs7903146 SNP of the *TCF7L2* gene is associated with T2DM in a large community-based cohort of African-American and Caucasian middle-aged adults participating in the Atherosclerosis Risk in Communities

(ARIC) Study. A second objective was to evaluate whether the risk of T2DM was associated with the rs7903146 SNP in the context of metabolic impairments.

Study subjects

A total of 12,029 baseline examination participants (2,727 African-Americans and 9,302 Caucasians) were included in the current analysis, after applying the exclusion criteria. The institutional review boards at all participating institutions approved the procedures and all participants included in the analysis gave informed consent.

Outcome assessment

Individuals were classified as having diabetes if any of the following conditions were met: fasting serum glucose levels of at least 7.0 mmol/L (126 mg/dl), nonfasting glucose levels of at least 11.1 mmol/L (200 mg/dl), current use of hypoglycemic medications (e.g., insulin or sulfonlyureas), or a self-reported physician diagnosis of diabetes[294]. In this study, individuals with diabetes at baseline were excluded. Individuals without diabetes at baseline who subsequently met any of these criteria at visit 2, 3, or 4 were considered to have incident T2DM.

Exposure assessment

SNP7903146 has three different genotypes: CC, CT and TT. We compared heterozygous CT-genotype and homozygous TT-genotype individuals to CC-genotype individuals, using the rs7903146 CC-genotype as the referent group, and the T allele as the risk variant. A variable taking on the values 0 for genotype CC, 1 for genotype CT, and 2 for genotype TT was used to test for additive genetic effects.

Covariate assessment

Demographic information including race, gender, cigarette smoking was self-reported. Individuals with a BMI ≥30 kg/m^2 were classified as obese[295]. Hypertension was defined as systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg or anti-hypertension medication use[296]. Low HDL was defined as less than 40 mg/dl in males and 50 mg/dl in females. Impaired fasting glucose was defined by a fasting glucose level between 100 and 125 mg/dl[297].

Statistical analyses

All analyses were stratified by race to crudely account for population stratification. We estimated the predicted cumulative incidence/risk of T2DM over a 9-year follow-up using the Kaplan Meier approach. We used Cox proportional hazards to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of incident diabetes. Covariates, including ever smoking, BMI, obesity, hypertension, HDL, LDL, and work, sport, leisure time physical activity level, were assessed as potential confounders and dismissed from all further analyses.

Variables were considered as potential effect measure modifiers if either of the following criteria were met: departures from additivity of effect as assessed by the ICR[291], or an indication of context specific effects in the previous *TCF7L2* literature. ICRs were quantified as follows: ICR= HR_AB – HR_A – HR_B + 1, where HR_AB represents the joint effect of metabolic exposure and the SNP, and HR_A and HR_B represent the main effects of metabolic exposure and the SNP, respectively[291]. Departures from zero suggest that the exposure of interest and the SNP interact to cause T2DM. The HR and the variance covariance matrix were used to calculate ICR values and their 95% confidence intervals[298].

As our interaction analyses indicated obesity and low HDL as possible effect modifiers, we further divided the ARIC population into three mutually exclusive subgroups according to the presence of none, one (obesity only, or low HDL only), or both of these two metabolic risk factors.

Results

A total of 485 (17.8%) and 923 (9.9%) incident T2DM cases were identified among African American and Caucasian ARIC participants, respectively (**Table 10**)[293]. The rs7903146 T allele was observed with similar frequency in African-American and Caucasian individuals, but was more common among incident T2DM cases compared with non-cases in both races (**Table 10**)[293]. The risk of T2DM was highest among TT individuals, followed by CT individuals, and lowest among CC individuals in both races. As previously documented, the risk of T2DM was higher in African Americans compared to Caucasians with the same genotype.

Table 10. Genotypic frequency of *TCF7L2* rs7903146 by race and incident type 2 diabetes status, cumulative incidence of type 2 diabetes by race and genotype over 9 years of follow-up, and estimated hazard ratio of rs7903146 on type 2 diabetes by race: The ARIC Study (Adapted from Yan[293])*

	African American				Caucasian			
	Controls/Cases	Cumulative Incidence (%) (95%CI)	HR (95% CI) †	*P* value‡	Controls/Cases	Cumulative Incidence (%) (95%CI)	HR (95% CI) †	*P* value‡
N	2242/485	20.6 (18.7, 22.5)			8379/923	10.7 (10.0, 11.4)		
Genotype, N (%)								
CC	1156 (52)/225 (46)	11.3 (10.2, 12.4)	1.00		4295 (51)/430 (47)	9.7 (8.8, 10.6)	1.00	
CT	921 (41)/212 (44)	21.1 (20.8, 21.4)	1.17 (1.02, 1.34)	0.03	3391 (40)/392 (42)	11.3 (10.2, 12.4)	1.18 (1.07, 1.30)	<0.01
TT	165 (7)/48 (10)	27.9 (19.3, 36.5)	1.36 (1.03, 1.79)	0.03	693 (8)/101(11)	13.6 (11.1, 16.1)	1.38 (1.14, 1.68)	<0.01
T allele	28%/32%				29%/32%			

Abbreviation: CI, confidence interval; HR, hazard ratio.

*The genotypic distributions were in agreement with Hardy-Weinberg equilibrium in African-Americans and Caucasians.

†Adjusted for age at baseline, study center and gender.

‡*P* value for HR.

We identified obesity (Caucasians: ICR=0.69; 95% CI (0.10, 1.27); P =0.02) and low HDL (African Americans: ICR=0.57; 95% CI (0.18, 0.96); P =0.004) as important effect measure modifiers[293]. Individuals with one T allele or two T alleles had the highest hazards of developing T2DM if they were obese and had low HDL, followed by individuals with any one of these two risk factors compared to those with none of the traits (**Table 11**). Homozygous individuals (TT) with two metabolic risk factors had the highest HR of T2DM of 6.04 (95% CI: 3.70, 9.87) in African Americans and 9.35 (6.72, 13.00) in Caucasians compared to CC individuals with none of these two. A similar trend was observed for risk differences (RDs) and risks of T2DM. When studied separately, we observed a larger ICR for obesity (*P*=0.02) in Caucasians and low HDL (*P*=0.004) in African Americans, but testing by bootstrapping [299] did not support significant racial differences.

Table 11. Association of *TCF7L2* rs7903146 with T2DM [HR (95% CI)]† modified by the number of metabolic risk factors (obesity and low HDL) in ARIC(Adapted from Yan[293]).

	African American			Caucasian		
# of risk factors	CC genotype	CT genotype	TT genotype	CC genotype	CT genotype	TT genotype
None	1	1.14 (0.88, 1.48)	1.30 (0.77, 2.20)	1	1.19 (0.98, 1.44)	1.42 (0.97, 2.09)
One	2.31 (1.71, 3.12)	2.70 (2.04, 3.58)	3.16 (2.15, 4.65)	2.46 (1.96, 3.08)	3.09 (2.50, 3.82)	3.88 (2.93, 5.16)
Two	3.49 (2.46, 4.95)	4.59 (3.33, 6.33)	6.04 (3.70, 9.87)	6.77 (5.33, 8.62)	7.96 (6.34, 9.98)	9.35 (6.72, 13.00)

Abbreviation: CI, confidence interval; HDL, high density lipoprotein cholesterol; HR, hazard ratio.

*Abnormal metabolic traits included obesity and low HDL.

†Adjusted for age at baseline, study center and gender.

Discussion and Conclusion

TCF7L2 has been implicated as an important T2DM susceptibility gene in different populations. Our study replicates the association between the T allele at rs7903146 and T2DM risk in Caucasians and provides the first significant evidence of association in a large, population-based African-American population[4, 199, 203-205]. The rs7903146 was significantly associated with T2DM risk in another two African ancestry studies[4, 199], but none of these two studies were population-based. Our study also contributes new evidence for additive interaction between *TCF7L2* variants and obesity (P=0.02) in Caucasians, and HDL cholesterol (P=0.004) in African Americans (Table 3). Indeed, we demonstrate that the risk of developing T2DM associated with this *TCF7L2* variant is substantially increased in the context of some of these well known metabolic risk factors for T2DM.

The majority of current literature suggests that *TCF7L2* is associated with impaired insulin secretion, but not with increased insulin resistance[203, 229, 230]. We found a slightly lower fasting insulin and HOMA-IR concentration among individuals with the T risk allele, suggestive of impaired insulin secretion. A possible explanation of our study findings is that *TCF7L2* may impair beta cell function, which when combined with insulin resistance caused by other factors provides a "double hit" that disproportionately increases the risk for T2DM. Although our study has implicated, for the first time, interesting relationships between these metabolic risk factors, the *TCF7L2* variants and T2DM, the mechanism of action of *TCF7L2* variants on T2DM remains to be determined.

In conclusion, this prior published study research provided important new evidence for an association between *TCF7L2* and T2DM in a large African American population. It

also provided estimates of the predicted cumulative incidence of T2DM over 9 years of follow-up associated with this genetic variant, in the context of metabolic impairments that usually precede and coexist with T2DM. The study findings need to be replicated in other population-based studies and further study is needed on the mechanisms by which the *TCF7L2* gene acts in the context of metabolic traits in the pathogenesis of T2DM.

CHAPTER V

RESEARCH DESIGN AND METHODS

A. Overview

The present study utilized data collected from the ARIC study, a community-based prospective cohort study examining cardiovascular and pulmonary disease, and disease variation over time. The ARIC study includes a cohort of 15,792 middle-aged men and women, aged 45 to 64 years old at recruitment (1987-1989), which was selected as a probability sample from four U.S. communities, and followed-up every three years through January 1999.

For Manuscript 1, we estimated the association between SNP rs7903146 in *TCF7L2* and prediabetes as quantified by incident impaired fasting glucose (IFG), and the extent to which metabolic risk factors modified the association using the proportional hazard regression modeling.

For Manuscript 2, we characterized the associations between SNP rs7903146 in *TCF7L2* and retinal phenotypes, and how hypertension and diabetes modified the association. Retinal phenotypes included retinopathy, arteriovenous (AV) nicking, focal arteriolar narrowing, central retinal artery equivalent (CRAE) and the central retinal venular equivalent (CRVE). Logistic regression models were fit to estimate the association between SNP rs7903146 in *TCF7L2* and the odds of prevalent retinal microvascular signs (retinopathy, AV nicking,

focal arteriolar narrowing); generalized linear models were fit to estimate adjusted mean retinal vascular calibers (CRAE, CRVE) for each genotype of rs7903146.

B. Exposure assessment

The *TCF7L2* rs7903146 SNP was genotyped by the ARIC Central Laboratory using Taqman® assays (Applied Biosystems, Foster City, CA). Laboratory-designed probes were obtained from Applied Bioystems and primers from IDT (Coralville, IA). All PCR reactions took place in optical 384-well reaction plates (Applied Biosystems). Five percent of samples were re-genotyped for quality control as blind duplicates. The percent agreement between blind duplicates was 98% and the simple Kappa coefficient was 0.97 indicating good genotyping quality. Details on SNP genotyping were described in Chapter IV: D. SNP Genotyping above.

Following published literature[4] and our previous findings[3], we assumed an additive mode of inheritance and compared heterozygous CT-genotype and homozygous TT-genotype individuals to CC-genotype individuals, using the rs7903146 CC-genotype as the referent group.

C. Outcome assessment

1. Impaired fasting glucose (IFG)

As a measure of prediabetes, individuals with fasting serum glucose levels of 100–125 mg/dl (5.6–6.9 mmol/l)[294] were classified as having IFG. Individuals without IFG at baseline who subsequently met this criterion for incident IFG at visit 2, 3, or 4 were considered to be incident cases. The 2-h glucose value from OGTT at visit 4 was not considered in the diagnosis of IFG.

2. Retinal phenotypes

Retinal phenotypes included retinopathy, focal retinal arteriolar narrowing, arterio-venous (A/V) nicking, central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) at visit 3. Following is a summary of the ARIC Study methods for taking retinal photographs and evaluating them for retinal abnormalities/characteristics[300].

Retinal photography

Technicians at the ARIC examination centers took one 45°nonstereoscopic color retinal photograph of one eye of each participant using a fundus camera that does not require pharmacologic dilation of the pupil (Canon CR-45UAF; Canon USA, Inc., Lake Success, NY). Centered between the optic disc and the macula, the photograph documented the optic disc, the macula, substantial portions of the temporal vascular arcades, and approximately 2 disc diameters of retina nasal to the optic disc. Photography was performed in a darkened room (to a degree that would barely allow one to read a newspaper), allowing the pupil to dilate. Typically, dilation to at least 4 mm was necessary to obtain an optimal image, although sometimes a partially usable image could be obtained through a 3-mm pupil. The eye to be photographed was systematically chosen to achieve balance (i.e., right eye for even identification numbers, left eye for odd identification numbers). If photography was not feasible in the eye selected by algorithm because of poor dilation or ocular media opacities (e.g., cataract), the technician was allowed to switch eyes. Completion of the entire photography session typically took less than 15 minutes. Photographs were mounted in plastic sheets and sent to a central reading center.

Evaluation of retinal vascular abnormalities

The ARIC Study photographs were assessed by the Retinal Reading Center for retinal vascular abnormalities using two different technologies: semiquantitative manual grading on a light box and measurement of retinal vessel caliber on an image processor. To establish the correspondence between measurements on film and dimensions in the eye, the diameter of the average optic nervehead was assumed to be approximately 3.4 mm on film and 1850 μm in the eye.

The method used to evaluate focal vascular abnormalities was adapted principally from the Modified Airlie House Classification of Diabetic Retinopathy, which includes some lesions that are not necessarily diabetic. The grader examined the retinal photograph with a monocular 8× stand viewer on a "daylight" (i.e., 6200° K color rating) fluorescent light box. The grader compared possible abnormalities with standard and example photographs to help determine their presence and severity.

Retinopathy

Retinopathy was defined if any characteristic lesion as defined by the Early Treatment Diabetic Retinopathy Study severity scale was present: retinal hemorrhages (blot or flame shaped), microaneurysms, soft or hard exudates, macular edema, intraretinal microvascular abnormalities, venous beading, swelling, or laser photocoagulation scars.

Focal retinal arteriolar narrowing & A/V nicking

Focal narrowing was considered definite if an arteriole estimated to be 50-μm diameter or greater (approximately 1/3 of the diameter of a major vein at the disc margin) had a constricted area of 2/3 or less the width of proximal and distal vessel segments. AV nicking was considered definite if the venous blood column was tapered on both sides of its

crossing under an arteriole (rare crossings of venules over arterioles were ignored). Focal arteriolar narrowing and AV nicking were defined as present if graded as definite or probable and as absent if not.

CRAE & CRVE

Measurements were based on retinal vessels located 0.5–1 disc diameter from the optic disc using computer designed software that summarized diameters as central retinal arteriolar equivalent (CRAE) and venular equivalent (CRVE), which represented the average arteriolar and venular diameter, respectively, to detect and quantify generalized retinal arteriolar narrowing.

D. Other Covariates

Demographic information was self-reported. A positive family history of diabetes was defined by participant report of diabetes in either biological parent. Self-reported cigarette smoking exposure was defined as ever smoking versus never smoking obtained by a personal interview. Body mass index (BMI) was calculated as measured weight (kg) divided by the square of measured height (m^2). Individuals with a BMI $\geq$30 kg/m^2 were classified as obese[301]. Elevated waist circumference (WC) was defined as WC$\geq$102cm in males or WC$\geq$88cm in females[302]. Blood pressure was measured three times using a random zero sphygmomanometer and the average of the last two measurements was used for this analysis. Hypertension was defined as systolic blood pressure $\geq$ 140mmHg or diastolic blood pressure $\geq$ 90mmHg or a history of anti-hypertension medication use[296]. Glucose was assessed by a modified hexokinase/glucose-6-phosphate dehydrogenase procedure[303]. Plasma total cholesterol levels, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were

measured by enzymatic methods. Low HDL-C was defined as less than 40 mg/dl in males and 50 mg/dl in females. High triglyceride was defined as triglyceride levels higher than 200 mg/dl[304]. Insulin was measured by radioimmunoassay (^{125}Insulin kit; Cambridge Medical Diagnosis, Bilerica, MA). Physical activity was quantified using a slightly modified version of the Baecke physical activity questionnaire[305], that classified work, sport and leisure activities into categories ranging from 1 (low) to 5 (high). For example, leisure time physical activity was derived from four questions regarding the frequency of television watching, walking, bicycling during the leisure time, and walking and/or bicycling to/from work, and was measured on a 5-point scale, with 1 indicating the lowest level of activity and 5 the highest.

E. Statistical analysis

1. Assessment of population substructure

Hardy-Weinberg equilibrium (HWE) was examined for SNP rs7903146, by race. For a biallelic locus in a randomly mating population, where the frequency of alleles are represented by 'p' and 'q', the distribution of genotypes in the referent population should be $p^2 + 2pq + q^2$. Deviations from HWE are assessed using a chi-square test with degrees of freedom equal to the number of alleles (n) – 1. Significant deviations from HWE may be indicative of laboratory error[306] or a violation of the factors necessary to maintain HWE in a population, such as population admixture. While the power of HWE to detect population admixture is small, assessing HWE before analysis can generally reduce false positive findings of genes underlying complex traits[307].

2. Association analyses

All association analyses were examined within each ethnic (African American or Caucasian) group. Manuscript 1 used proportional hazard regression to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of incident IFG associated with SNP 7903146 T risk allele. The hazard function was formulated on the age scale and date of onset of IFG was interpolated using blood glucose levels at the visits at each end of the triennial intervals[308]. Plots of the log (-log) survival curves and the Cox test were utilized to assess violations of proportional hazard assumptions. Manuscript 2 used logistic regression to estimate odds ratios (ORs) and 95% CIs of prevalent focal retinal lesions (retinopathy, focal arteriolar narrowing, AV nicking) associated with the *TCF7L2* rs7903146, and employed generalized linear models to estimate adjusted mean retinal vascular calibers (CRAE, CRVE) for each genotype of rs7903146.

Genetic models

Following published literature[4] and our previous findings[3], we assumed an additive mode of inheritance and compared heterozygous CT-genotype and homozygous TT-genotype individuals to CC-genotype individuals, using the rs7903146 CC-genotype as the referent group. A variable taking on the values 0 for genotype CC, 1 for genotype CT, and 2 for genotype TT was used to test for additive genetic effects.

3. Assessment of confounding

In Manuscript 1, covariates including age, gender, and ARIC field center were always included in the models for minimal confounding adjustment. Otherwise, a change-in-estimate approach with a criterion of 0.10 was used to adjust for potential confounders including ever

smoking (yes/no), obesity, high LDL, low HDL, hypertension, high triglyceride, physical activity, and elevated waist circumference.

In Manuscript 2, following published literature[290, 309], all models were adjusted for age, study center, sex, current smoking (yes/no), obesity (yes/no), total serum cholesterol, total serum triglycerides, mean arterial blood pressure, and antihypertensive medication. As hypertension is an important risk factor for retinal microvascular signs, hypertension was also included in the model when it was not assessed as an effect measure modifier.

4. Assessment of modification

An important aspect of this project is the evaluation of gene-environment interactions which was assessed on the multiplicative and additive scales between genotypes and different metabolic risk factors including obesity, elevated waist circumference, hypertension, high triglycerides, and low HDL-C. In Manuscript 2, we only evaluated hypertension and diabetes as modifiers. A Wald χ^2 test for significance of the estimated β-coefficient for the interaction term and the interaction contrast ratio (ICR) were employed to assess the departure from multiplicativity and additivity, respectively [291, 298]. A *p* value <0.05 was considered to indicate an important modifier, despite the multiple tests as interaction tests tend to be underpowered[310].

A multiplicative interaction was determined by a Wald χ^2 test for significance of the estimated β coefficient, $\hat{\beta}$, for the interaction term. If $\hat{\beta}$, is significantly different from the null value, which corresponds to β=0, and a hazard ratio or odds ratio (e^{β})=1, we concluded a multiplicative interaction existed.

An additive interaction was assessed by testing ICR. In terms of proportional hazard regression, ICRs were quantified as follows: ICR= HR_AB – HR_A – HR_B + 1, where HR_AB represents the joint effect of metabolic exposure and the SNP, and HR_A and HR_B represent the main effects of metabolic exposure and the SNP, respectively[291]. For logistic regression, odds ratios replace the hazards ratios in the above ICR equation. Thus, ICR refers to the increased risk due to an additive interaction between the metabolic risk factors and the T risk allele adjusted for confounders. Assuming an additive mode of inheritance, the ICR comparing TT to CT is equal to the ICR comparing CT to CC when the metabolic exposure of interest is constant, thus only one ICR was reported. Departures from zero suggest that the exposure of interest and the SNP interact to cause the outcome of interest. The HR and the variance covariance matrix were used to calculate ICR values and their 95% confidence intervals[298].

5. Multiple comparisons

Association mapping often involves estimating single-locus models separately for each candidate marker and then evaluating statistical significance. As expected, a large number of dependent tests are performed, necessitating a correction for multiple comparisons. To minimize the impact of the multiple tests, we applied a crude Bonferroni correction, noting that such an approach is an over-correction because many of the analytic runs assessed the same dependent variable.

CHAPTER VI

RESULTS

A. Manuscript 1: Transcription Factor 7-Like 2 (*TCF7L2*) Polymorphism and Context-Specific Risk of Impaired Fasting Glucose in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study

ABSTRACT

AIMS/HYPOTHESIS: Although variants in the transcription factor 7-like 2 (*TCF7L2*) gene are consistently associated with impaired fasting glucose (IFG) in Caucasians, data from large population-based studies of African Americans are lacking. Moreover, few studies have investigated the effects of *TCF7L2* on IFG in the context of other metabolic risk factors for diabetes.

METHODS: We investigated the association between the *TCF7L2* rs7903146 polymorphism and incident IFG defined as fasting serum glucose levels of 100–125 mg/dl (5.6–6.9 mmol/l) in 1,377 African American and 5,152 Caucasian participants without diabetes and IFG at intake who participated in the Atherosclerosis Risk in Communities (ARIC) Study in 1987-1989 and were followed for 9 years.

RESULTS: Incident IFG was identified in 810 (58.8%) and 2,652 (51.5%) African-Americans and Caucasians, respectively. Compared to homozygous CC Caucasian individuals, heterozygous CT [hazard ratio=1.09 (95% CI=1.03-1.15)] and homozygous TT

[1.18 (1.05-1.33)] individuals had significantly higher risk of developing IFG over 9 years of follow-up. The association between the rs7903146 genotype and IFG risk was stronger in Caucasians with obesity or high triglycerides. No association of the *TCF7L2* rs7903146 polymorphism and incident IFG was noted in African Americans.

CONCLUSIONS/INTERPRETATION: Our study replicates the association between the T allele at rs7903146 and IFG risk in Caucasians but not in African Americans. Our study also provides new evidence for interactions between *TCF7L2* and metabolic risk factors on the occurrence of IFG in Caucasians.

1. Introduction

Impaired fasting glucose (IFG), an intermediate stage between normoglycemia and diabetes, is characterized by defects in insulin sensitivity and early-phase insulin secretion [1, 2]. The transcription factor 7-like 2 (*TCF7L2*) gene, a Wingless and Int (Wnt) signaling-associated transcription factor located on chromosome 10q25, has emerged as a consistently replicated susceptibility gene for type 2 diabetes and IFG [3-7]. In our previous work, we demonstrated a significant association between the T allele at single nucleotide polymorphism (SNP) rs7903146 and the risk of incident type 2 diabetes in middle-aged African American and Caucasian participants of the Atherosclerosis Risk in Communities (ARIC) Study[8]. The rs7903146 T allele has been described either as the causal risk variant or the closest correlate to an unidentified functional variant [9], possibly impairing the glucagon-like peptide-1-induced insulin secretion[10], but the exact mechanism is still under investigation.

Although an effect of *TCF7L2* on IFG has been observed in Caucasians [6, 7], no studies of *TCF7L2* and prediabetes as quantified by incident IFG have been conducted in African Americans. Moreover, potential *TCF7L2* gene–metabolic risk factors interactions on IFG have been largely unexplored.

Our previous work focused on the association between the rs7903146 SNP and type 2 diabetes[8]. In this study, we investigated whether the rs7903146 SNP of the *TCF7L2* gene is associated with incident IFG in a large community-based cohort of African-American and Caucasian middle-aged adults in the ARIC Study. A second objective is to evaluate whether the effect of the rs7903146 SNP on IFG varies by obesity and triglyceride levels.

2. Methods

a. Study subjects and phenotype definitions

The ARIC Study is an ongoing, longitudinal cohort study of cardiovascular and other major diseases among 15,792 men and women, aged 45 to 64 years old at baseline (1987-1989), selected from 4 US communities: Forsyth County, NC; Jackson, MS; the northwestern suburbs of Minneapolis, MN; and Washington County, MD. By design, African-Americans were over-sampled at the Forsyth County site and were exclusively sampled in Jackson and thus constituted 27% of the baseline cohort. The sampling procedures and methods used in ARIC have been described in detail elsewhere[11].

We excluded ARIC participants who were not African-American or Caucasian (*n*=48), African-Americans from Minnesota and Maryland field centers (*n*=55), participants with prevalent diabetes at baseline or incident diabetes during follow-up (*n*=3,379), participants with prevalent IFG at baseline (*n*=4,472), participants with missing genotype

data or who did not provide consent for the use of their DNA (*n*=525), and participants with missing information on incident IFG (*n*=784). Diabetes was defined as fasting serum glucose levels of at least 7.0 mmol/L (126 mg/dl), nonfasting glucose levels of at least 11.1 mmol/L (200 mg/dl), current use of hypoglycemic medications (e.g., insulin or sulfonlyureas), or a self-reported physician diagnosis of diabetes[1]. After these exclusions, 6,529 baseline examination participants (1,377 African American and 5,152 Caucasians) were available for analysis. The institutional review boards at all participating institutions approved the procedures and all participants included in the analysis gave informed consent.

All covariates were measured at the baseline exam (visit 1). As a measure of prediabetes, individuals with fasting serum glucose levels of 100–125 mg/dl (5.6–6.9 mmol/l)[1] were classified as having IFG. Individuals without IFG at baseline who subsequently met this criterion for incident IFG at visit 2, 3, or 4 were considered to be incident cases in the analysis.

Self-reported cigarette smoking exposure was defined as ever smoking versus never smoking obtained by a personal interview. Body mass index (BMI) was calculated as measured weight (kg) divided by the square of measured height (m^2). Individuals with a BMI $\geq$30 kg/m^2 were classified as obese[12]. Elevated waist circumference (WC) was defined as WC$\geq$102cm in males or WC$\geq$88cm in females[13]. Blood pressure was measured three times using a random zero sphygmomanometer and the average of the last two measurements was used for this analysis. Hypertension was defined as systolic blood pressure $\geq$ 140mmHg or diastolic blood pressure $\geq$ 90mmHg or a history of anti-hypertension medication use[14]. Glucose was assessed by a modified hexokinase/glucose-6-phosphate

dehydrogenase procedure[15]. Plasma total cholesterol levels, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured by enzymatic methods. Low HDL-C was defined as less than 40 mg/dl in males and 50 mg/dl in females. High triglyceride was defined as triglyceride levels higher than 200 mg/dl[16]. Insulin was measured by radioimmunoassay (^{125}Insulin kit; Cambridge Medical Diagnosis, Bilerica, MA). Physical activity was quantified using a slightly modified version of the Baecke physical activity questionnaire[17], that classified work, sport and leisure activities into categories ranging from 1 (low) to 5 (high).

b. SNP genotyping

The *TCF7L2* rs7903146 SNP was genotyped by the ARIC Central Laboratory using Taqman® assays (Applied Biosystems, Foster City, CA). Laboratory-designed probes were obtained from Applied Bioystems and primers from IDT (Coralville, IA). All PCR reactions took place in optical 384-well reaction plates (Applied Biosystems). Five percent of samples were re-genotyped for quality control and 726 ARIC participants were genotyped in duplicate. The percent agreement between blind duplicates was 98% and the simple Kappa coefficient was 0.97 indicating good genotyping quality.

c. Statistical analysis

All analyses were stratified by race to crudely account for population stratification. To assess whether genotype distribution within each race departed from Hardy-Weinberg equilibrium, a χ^2 goodness-of-fit test was used. We estimated the predicted cumulative incidence/risk of IFG over a 9-year follow-up under a semiparametric regression model. We used Cox proportional hazards to estimate the hazard ratios (HRs) and 95% confidence

intervals (CIs) of incident IFG. The hazard function was formulated on the age scale and date of onset of IFG was interpolated using blood glucose levels at the visits at each end of the triennial intervals[18]. To interrogate the consistency of our findings, we investigated the association between *TCF7L2* and persistent IFG incidence defined as at least two IFG diagnoses at visit 2, 3 or 4, and the first occurrence of incident IFG was used to calculate the time-to-event. In addition, we assessed the association between IFG and *TCF7L2* using a more stringent definition of IFG, categorizing individuals with a fasting glucose value of 110 - 125 mg/dl as affected. Lastly, we evaluated the association between rs7903146 and repeated fasting glucose values over 9 years of follow-up (visit 1-4) in the ARIC study population using Generalized Estimating Equation models.

Covariates including history of ever smoking, BMI, obesity, hypertension, plasma HDL-C, and history of work, sport, leisure time physical activity level were assessed as potential confounders and were removed from all further analyses as the adjustment for these covariates made no difference in the association between *TCF7L2* and incident IFG. Following the published literature [4] and our findings from previous research [8], we compared heterozygous CT-genotype and homozygous TT-genotype individuals to CC-genotype individuals, using the rs7903146 CC-genotype as the referent group, and the T allele as the risk variant. A variable taking on the values 0 for genotype CC, 1 for genotype CT, and 2 for genotype TT was used to test for additive genetic effects.

Gene–environment interaction testing was assessed on the multiplicative and additive scales between genotypes and different metabolic risk factors including obesity, elevated waist circumference, hypertension, high triglycerides, and low HDL-C. A Wald χ^2 test for

significance of the estimated β-coefficient for the interaction term and the interaction contrast ratio (ICR) were employed to assess the departure from multiplicativity and additivity, respectively [19, 20]. Variables were considered as potential effect measure modifiers if they departed from multiplicativity and additivity of effect as assessed by the Wald χ^2 test and the ICR, respectively [19]. A *p* value <0.05 was considered to indicate an important modifier, despite the multiple tests as interaction tests tend to be underpowered [21]. ICRs were quantified as follows: ICR= HR_AB – HR_A – HR_B + 1, where HR_AB represents the joint effect of metabolic exposure and the SNP, and HR_A and HR_B represent the main effects of metabolic exposure and the SNP, respectively[19]. Thus, ICR refers to the increased risk due to an additive interaction between the metabolic risk factors and the T risk allele adjusted for age, gender, and study center. Assuming an additive mode of inheritance, the ICR comparing TT to CT is equal to the ICR comparing CT to CC when the metabolic exposure of interest is constant, thus only one ICR was reported. Departures from zero suggest that the exposure of interest and the SNP interact to cause IFG. The HR and the variance covariance matrix were used to calculate ICR values and their 95% confidence intervals[20].

3. Results

The allele frequencies for rs7903146 in both races were in Hardy–Weinberg equilibrium (p>0.05). Selected baseline characteristics of the ARIC Study participants by race and genotype status are presented in Table 1. At the baseline exam, no significant differences in demographic or behavioral characteristics (age, gender, leisure physical activity level, and smoking) were noted by genotype status in Caucasian and African

American ARIC participants. Moreover, no significant differences in hypertension, glucose, insulin, obesity relate traits, triglycerides, and HDL-C were noted.

Over the course of 9 years of follow-up, incident IFG was identified in 810 (58.8%) and 2,652 (51.5%) African American and Caucasian ARIC participants, respectively (Table 2). The rs7903146 T allele was observed with similar frequency in African-American and Caucasian individuals, but was more common among incident IFG cases compared with non-cases in Caucasians (Table 2). The rs7903146 T allele was significantly associated with incident IFG in Caucasian participants [$HR_{CT\ vs.\ CC}$ (95% CIs)=1.09 (1.03, 1.15); $HR_{TT\ vs.\ CC}$ (95% CIs)=1.18 (1.05, 1.33)], but not in African American participants [$HR_{CT\ vs.\ CC}$ (95% CIs)=0.99 (0.89, 1.10); $HR_{TT\ vs.\ CC}$ (95% CIs)=0.98 (0.79, 1.22)] (Table 2).

To interrogate the consistency of our findings, we investigated the association between *TCF7L2* and persistent IFG incidence defined as at least two IFG diagnoses at visit 2, 3 or 4 and obtained similar effect estimates in Caucasians; however, in African Americans the effect estimates improved but were still not statistically significant (Online Appendix Table 1). In addition, we assessed the association between IFG and *TCF7L2* using a more stringent definition of IFG (110 - 125 mg/dl) and similar results were obtained (data not shown). Lastly, a significant association between the rs7903146 T allele and repeated fasting glucose across visit 1-4 was noted in Caucasians (β=0.2480 with p=0.0389) but not in African Americans (β=0.3002 with p=0.2826), which is consistent with the IFG findings.

We identified obesity and high triglyceride as important effect measure modifiers in Caucasians, but no important modifiers were noted in African Americans (Table 3; Figure 1; Online Appendix Table 2). Specifically, among non-obese Caucasians, heterozygous CT

[HR=1.07 (95% CI=1.00, 1.14)] and homozygous TT [1.14 (1.00, 1.30)] individuals had slightly higher HRs (95%CI) of IFG over 9 years of follow-up compared to homozygous CC individuals, whereas among obese Caucasians, heterozygous CT [1.28 (1.12, 1.47)] and homozygous TT [1.65 (1.25, 2.17)] individuals had significantly higher HRs (95%CI) of IFG compared to CC individuals (multiplicative interaction *p* value=0.01). Similar results were obtained for high triglycerides. When each effect measure modifier was studied separately, we observed a slightly larger ICR for obesity in Caucasians (Table 3), but testing by bootstrapping did not find significant differences between ICRs for obesity and high triglycerides[22].

Table 12. (MS1: Table 1) Selected characteristics of the Atherosclerosis Risk in Communities Study participants at baseline, by race and genotype status.

	African American				Caucasian			
	CC	CT	TT	*p*	CC	CT	TT	*p*
n	695	569	113		2679	2084	389	
Age (years)	52 ± 6	52 ± 6	53 ± 6	0.76	54 ± 6	54 ± 6	53 ± 6	0.06
Sex (male)	241 (34.68)	210 (36.91)	36 (31.86)	0.52	1010 (37.70)	807 (38.72)	146 (37.53)	0.75
Ever Smoked	344 (49.50)	292 (51.32)	53 (46.90)	0.64	1499 (56.00)	1163 (55.83)	213 (54.76)	0.90
Leisure-time Physical Activity [a]	2.12 ± 0.59	2.16 ± 0.59	2.11 ± 0.56	0.42	2.5 ± 0.54	2.5 ± 0.53	2.53 ± 0.52	0.53
Obese[b]	214 (30.79)	155 (27.29)	31 (27.43)	0.37	412 (15.38)	273 (13.12)	49 (12.6)	0.06
BMI (kg/m^2)	28.37 ± 5.89	27.99 ± 5.23	27.70 ± 5.02	0.32	25.83 ± 4.29	25.55 ± 4.16	25.64 ± 4.23	0.07
Elevated WC[c]	366 (52.66)	276 (48.59)	56 (49.56)	0.35	1142 (42.63)	842 (40.40)	161 (41.39)	0.30
WC (cm)	95.13 ± 14.70	94.05 ± 13.10	93.12 ± 13.03	0.21	92.55 ± 12.41	91.76 ± 11.87	92.03 ± 11.91	0.08
Hypertension[d]	302 (43.64)	238 (42.05)	47 (41.59)	0.82	529 (19.86)	376 (18.13)	61 (15.72)	0.08
Glucose (mg/dl)[e]	91.33 ± 5.77	91.58 ± 5.34	91.60 ± 5.39	0.70	92.40 ± 4.88	92.46 ± 4.82	92.13 ± 4.90	0.47
Insulin (μU/ml)[e]	11.12 ± 7.75	10.45 ± 6.82	10.19 ± 6.51	0.18	8.23 ± 5.40	7.88 ± 5.13	7.89 ± 4.87	0.06
High triglyceride[f]	24 (3.55)	20 (3.60)	3 (2.70)	0.97	245 (9.16)	198 (9.52)	36 (9.28)	0.91
Triglycerides (mg/dl)	95.32 ± 52.14	95.25 ± 52.75	91.05 ± 50.34	0.71	118.64 ± 67.09	117.94 ± 72.40	121.85 ± 84.62	0.61
Low HDL-C[g]	174 (25.74)	139 (25.05)	22 (19.82)	0.42	883 (33.02)	651 (31.30)	132 (34.02)	0.35
HDL-C (mg/dl)	58.85 ± 18.26	58.88 ± 18.61	61.18 ± 19.00	0.45	54.26 ± 17.21	54.83 ± 17.44	53.91 ± 17.17	0.43

Data are means ± SE or *n* (%) unless otherwise indicated. Abbreviations: BMI, body mass index; HDL-C, high density lipoprotein cholesterol; WC, waist circumference. [a]Leisure time physical activity was derived from four questions regarding the frequency of television watching, walking, bicycling during the leisure time, and walking and/or bicycling to/from work, and was measured on a 5-point scale, with 1 indicating the lowest level of activity and 5 the highest[26]; [b]obesity was defined as BMI ≥30 kg/m^2; [c]elevated WC was defined as WC≥102cm in males or WC≥88cm in females; [d]hypertension was defined as systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg or a history of anti-hypertension medication use; [e]prevalent diabetes and IFG cases were excluded; [f]high triglyceride was defined as triglyceride levels higher

than 200 mg/dl; [g]low HDL-C was defined as less than 40 mg/dl in males and 50 mg/dl in females.

Table 13. (MS1: Table 2) Genotypic frequency of *TCF7L2* rs7903146 by race and incident IFG status, cumulative incidence of IFG by race and genotype over 9 years of follow-up, and estimated hazard ratio of rs7903146 on IFG by race: The ARIC Study.

	African American				Caucasian			
	Non-Cases/Cases	Cumulative Incidence (%) (95%CI)	HR (95% CI)[a]	*p*[b]	Non-Cases/Cases	Cumulative Incidence (%) (95%CI)	HR (95% CI)[a]	*p*[b]
n	567/810	63.73 (60.64, 66.58)			2500/2652	53.87 (52.37, 55.31)		
CC	291(51)/404(50)	63.78 (59.84, 67.34)	1.00		1354(54)/1325(50)	52.19 (50.26, 54.04)	1.00	
CT	221(39)/348(43)	63.64 (60.05, 66.91)	0.99 (0.89, 1.10)	0.86	966(39)/1118(42)	55.19 (53.42, 56.89)	1.09 (1.03, 1.15)	0.01
TT	55(10)/58(7)	63.50 (56.49, 69.38)	0.98 (0.79, 1.22)		180(7)/209(8)	58.24 (54.66, 61.53)	1.18 (1.05, 1.33)	
T allele (%)	29/29				27/29			

Abbreviation: CI, confidence interval; HR, hazard ratio; IFG, impaired fasting glucose.
[a]Adjusted for age at baseline, study center and gender; [b]*p* value for HR.

Table 14. (MS1: Table 3) Association of *TCF7L2* rs7903146 with IFG [HR (95% CI)] modified by obesity and high triglycerides, respectively, over 9 years of follow-up in ARIC

Characteristics	CC genotype		CT genotype		TT genotype		Multiplicative Interaction	Additive Interaction
	N	HR (95% CI)[a]	N	HR (95% CI)[a]	N	HR (95% CI)[a]	p[b]	ICR (p[c])
African-American								
Obesity[d]								
No	481	1	413	1.03 (0.90, 1.17)	82	1.06 (0.82, 1.38)	0.40	-0.12 (0.42)
Yes	214	1.40 (1.15, 1.71)	155	1.31 (1.08, 1.59)	31	1.22 (0.87, 1.70)		
High triglycerides[e]								
No	652	1	535	1.00 (0.90, 1.12)	108	1.01 (0.80, 1.26)	0.32	0.44 (0.30)
Yes	24	1.18 (0.71, 1.97)	20	1.63 (1.05, 2.51)	3	2.23 (0.90, 5.56)		
Caucasian								
Obesity[d]								
No	2267	1	1808	1.07 (1.00, 1.14)	340	1.14 (1.00, 1.30)	0.01	0.38 (0.007)
Yes	412	1.52 (1.33, 1.73)	273	1.96 (1.74, 2.21)	49	2.53 (2.03, 3.17)		
High triglycerides[e]								
No	2429	1	1882	1.07 (1.00, 1.14)	352	1.14 (1.00, 1.29)	0.02	0.36 (0.002)
Yes	245	1.31 (1.11, 1.54)	198	1.73 (1.51, 1.99)	36	2.30 (1.76, 3.00)		

Abbreviation: ICR, interaction contrast ratio; IFG, impaired fasting glucose; CI, confidence interval; HR, hazard ratio.

[a]Adjusted for age at baseline, study center and gender; [b]p value for the Wald χ^2 test; [c]p value for ICR; [d]obesity was defined as BMI $\geq$30 kg/m^2; [e]high triglyceride was defined as triglyceride levels higher than 200 mg/dl.

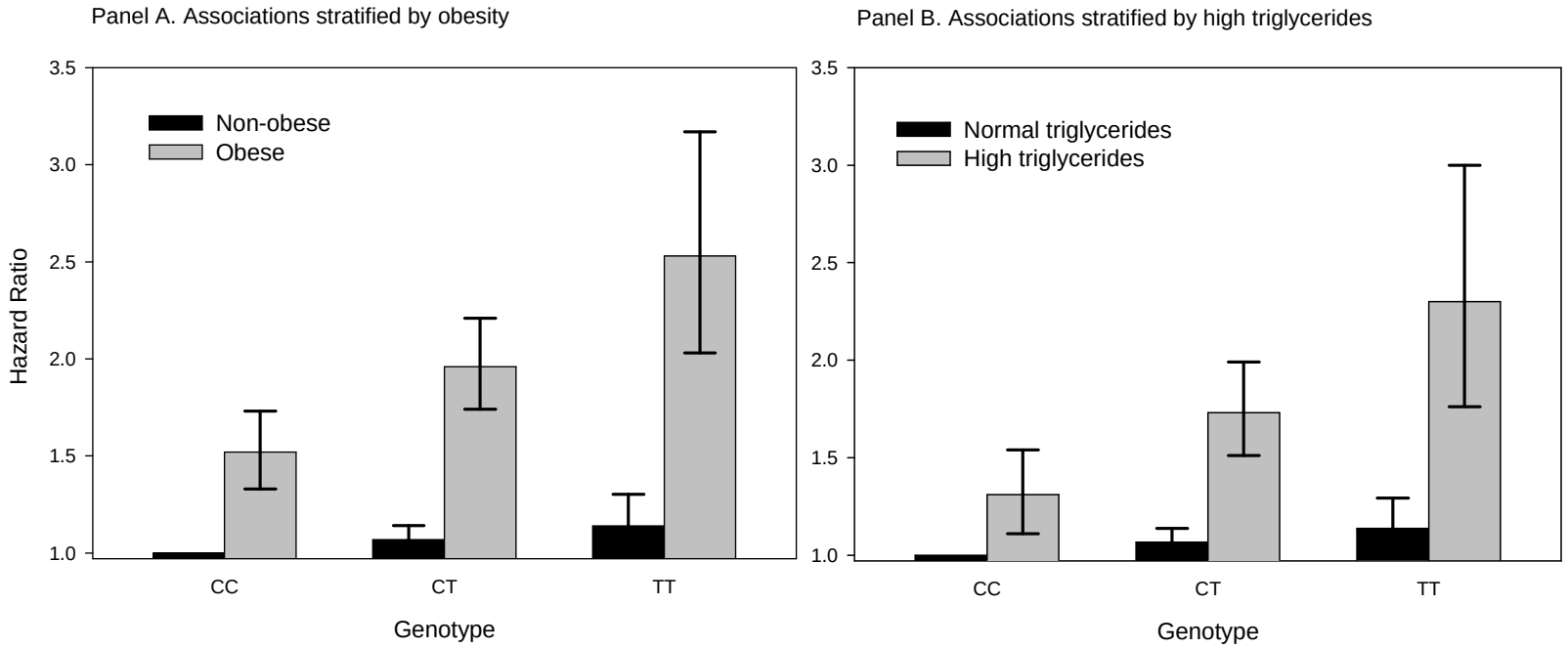

Figure 1 (MS1: Figure 1) Association of *TCF7L2* rs7903146 with incident IFG in Caucasian participants stratified by obesity[a] (Panel A, left) or high triglycerides[b] (Panel B, right) in the ARIC Study.

[a]Obesity was defined as BMI ≥30 kg/m^2; [b]high triglyceride was defined as triglyceride levels higher than 200 mg/dl.

Table 15. (MS1: Supplemental Table 1) Genotypic frequency of *TCF7L2* rs7903146 by race and persistent IFG incidence[a], and estimated hazard ratio of rs7903146 on IFG by race: The ARIC Study[b]

	African American			Caucasian		
	Non-Cases/Cases	HR (95% CI) [c]	*p*[d]	Non-Cases/Cases	HR (95% CI) [c]	*p*[d]
n	1005/372			3937/1179		
CC	519(52)/176(47)	1		2091(53)/588(50)	1	
CT	407(41)/162(44)	1.11 (0.95, 1.30)	0.1802	1589(40)/495(42)	1.09 (0.99, 1.19)	0.0675
TT	79(8)/34(9)	1.24 (0.91, 1.70)		293(7)/96(8)	1.18 (0.99, 1.41)	
T allele (%)	28/31			27/29		

Abbreviation: CI, confidence interval; HR, hazard ratio; IFG, impaired fasting glucose.

[a]Persistent IFG incidence was defined as at least two IFG occasions for visit 2, 3, or 4; [b]the genotypic distributions were in agreement with Hardy-Weinberg equilibrium in African-Americans and Caucasians; [c]adjusted for age at baseline, study center and gender; [d]*p* value for HR.

Table 16. (MS1: Supplemental Table 2) Association of *TCF7L2* rs7903146 with IFG [HR (95% CI)] modified by low HDL, hypertension and elevated WC, respectively, over 9 years of follow-up in ARIC[a]

Characteristics		HR (95% CI)[b]			Multiplicative Interaction	Additive Interaction
		CC genotype	CT genotype	TT genotype	p[c]	ICR (p[d])
African-American						
Low HDL[e]	No	1	1.02 (0.90, 1.16)	1.04 (0.81, 1.35)	0.84	-0.03(p =0.86)
	Yes	1.35 (1.09, 1.66)	1.34 (1.10, 1.63)	1.33 (0.93, 1.90)		
Hypertension[f]	No	1	0.92 (0.80, 1.06)	0.84 (0.63, 1.12)	0.11	0.18(p =0.09)
	Yes	0.94 (0.78, 1.14)	1.04 (0.87, 1.24)	1.14 (0.86, 1.53)		
Elevated WC[g]	No	1	1.05 (0.89, 1.22)	1.09 (0.80, 1.50)	0.43	-0.11(p =0.45)
	Yes	1.47 (1.20, 1.79)	1.41 (1.16, 1.71)	1.35 (1.01, 1.79)		
Caucasian						
Low HDL[e]	No	1	1.09 (1.01, 1.18)	1.19 (1.03, 1.39)	0.97	0.03(p =0.69)
	Yes	1.38 (1.24, 1.53)	1.50 (1.36, 1.66)	1.64 (1.39, 1.93)		
Hypertension[f]	No	1	1.09 (1.02, 1.17)	1.20 (1.05, 1.37)	0.93	0.04(p =0.71)
	Yes	1.29 (1.14, 1.46)	1.42 (1.26, 1.60)	1.56 (1.25, 1.95)		
Elevated WC[g]	No	1	1.05 (0.97, 1.14)	1.11 (0.94, 1.31)	0.14	0.16(p =0.04)
	Yes	1.42 (1.28, 1.57)	1.63 (1.48, 1.80)	1.88 (1.61, 2.20)		

Abbreviation: ICR, interaction contrast ratio; IFG, impaired fasting glucose; CI, confidence interval; HR, hazard ratio; WC, waist circumference.

[a]All subgroups had sample sizes of 26 or greater and 70 or greater in African Americans and Caucasians, respectively; [b]adjusted for age at baseline, study center and gender; [c]p value for the Wald χ^2 test; [d]p value for ICR; [e]low HDL-C was defined as less than 40 mg/dl in males and 50 mg/dl in females; [f]hypertension was defined as systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg or a history of anti-hypertension medication use; [g]elevated WC was defined as WC≥102cm in males or WC≥88cm in females.

4. Discussion

TCF7L2 has been implicated as an important IFG susceptibility gene in different Caucasian populations [6, 7]. To our knowledge, our study is the first population-based study on the *TCF7L2* rs7903146 and prediabetes as measured by incident IFG in African Americans and no association was noted. An earlier study in non-diabetic African American women (*n*=118 with 11 prevalent IFG cases) reported the lack of an association with prevalent IFG (effect estimates not reported) [23], which is consistent with our findings. Our study replicates the association between the T allele at rs7903146 and IFG risk in Caucasians, and contributes new evidence for interactions between *TCF7L2* variants and obesity and high triglycerides in Caucasians. Indeed, we demonstrate that the risk of developing IFG associated with this *TCF7L2* variant is substantially increased in the context of well known metabolic risk factors for type 2 diabetes.

We and other investigators have previously demonstrated an association between the *TCF7L2* rs7903146 and type 2 diabetes in both races [3, 4, 8]. In contrast, in this study, no association with IFG was noted in African Americans. Further investigation of the association between *TCF7L2* rs7903146 and persistent, incident IFG (2 or more occasions) demonstrated similar effect estimates in Caucasians. Similarly, in African Americans the effect estimates remained below thresholds of nominal statistical significance (Online Appendix Table 1). The lack of association between rs7903146 and IFG within the African American could reflect confounding by unmeasured covariates that are differentially distributed in African American and Caucasian participants, which warrants further investigation. Second, the limited power to detect such a modest effect in the African

American sample (calculated as 20% for a relative risk of 1.10) may also explain our findings.

Our data identified obesity and high triglycerides as significant effect measure modifiers in Caucasians. When studied separately, the most prominent interaction with genotype was for obesity (Table 3, Figure 1). Although we are unable to elucidate the pathogenesis underlying the observed statistical interactions, strong evidence indicates that abnormal metabolic traits including obesity and dyslipidemia aggregate in diabetic patients and their relatives [24, 25]. Genetic factors interacting with shared and unique environmental factors may cause this aggregation of metabolic traits [24]. Although our study has implicated, for the first time, interesting relationships between these metabolic risk factors, the *TCF7L2* variants and IFG in Caucasians, the role of *TCF7L2* variants in pathogenesis of IFG in the context of metabolic risk factors remains to be determined.

Our study findings have public health significance of potential importance since they suggest that having one or two rs7903146 T risk alleles only partially informs one's risk for prediabetes, as quantified by IFG. In the Caucasian population, the risk of IFG conferred by the T risk allele of rs7903146, even in the context of metabolic risk factors, only demonstrated a modest risk. In the African American population, no association between the T risk allele and IFG was noted. Thus, the cumulative risk of IFG likely depends on multiple susceptibility variants, the gene-gene interactions, and most importantly, "established" risk factors for type 2 diabetes such as BMI and other lifestyle habits.

In conclusion, our study replicates the association between the T allele at rs7903146 and IFG risk in Caucasians, whereas no associations were observed in African Americans.

Our study provides new evidence for interactions between *TCF7L2* and metabolic risk factors on the risk of IFG in Caucasians, as was previously demonstrated for type 2 diabetes. The reported differences between African American and Caucasian subpopulations require replication in larger epidemiological studies, as we were underpowered to detect the very modest effects that were observed in the Caucasians.

ACKNOWLEDGEMENTS

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute (NHLBI) contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

We are indebted to the staff and participants in the Atherosclerosis Risk in Communities Study for their important contributions.

5. References

[1] ADA (2007) American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 30 Suppl 1: S42-47

[2] Nathan DM, Davidson MB, DeFronzo RA, et al. (2007) Impaired Fasting Glucose and Impaired Glucose Tolerance: Implications for care. Diabetes Care 30: 753-759

[3] Grant SF, Thorleifsson G, Reynisdottir I, et al. (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. NatGenet 38: 320-323

[4] Cauchi S, El Achhab Y, Choquet H, et al. (2007) *TCF7L2* is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. Journal of molecular medicine (Berlin, Germany)

[5] Florez JC, Jablonski KA, Bayley N, et al. (2006) *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. NEnglJMed 355: 241-250

[6] Melzer D, Murray A, Hurst A, et al. (2006) Effects of the diabetes linked *TCF7L2* polymorphism in a representative older population. BMC Medicine 4: 34

[7] Raitakari OT, Ronnemaa T, Huupponen R, et al. (2007) Variation of the transcription factor 7-like 2 (*TCF7L2*) gene predicts impaired fasting glucose in healthy young adults: the Cardiovascular Risk in Young Finns Study. Diabetes Care 30: 2299-2301

[8] Yan Y, North KE, Ballantyne CM, et al. (2009) Transcription factor 7-like 2 (*TCF7L2*) polymorphism and context-specific risk of type 2 diabetes in African American and Caucasian adults: the Atherosclerosis Risk in Communities study. Diabetes 58: 285-289

[9] Helgason A, Palsson S, Thorleifsson G, et al. (2007) Refining the impact of *TCF7L2* gene variants on type 2 diabetes and adaptive evolution. Nat Genet 39: 218-225

[10] Cauchi S, Froguel P (2008) *TCF7L2* genetic defect and type 2 diabetes. Current diabetes reports 8: 149-155

[11] The ARIC Investigators (1989) The Atherosclerosis Risk in Communnities (ARIC) Study: design and objectives. Am J Epidemiol 129: 687-702

[12] US Department of Health and Human Service (2000) The practical guide—identification, evaluation, and treatment of overweight and obesity in adults. NIH publication no. 004084. National Institutes of Health, Bethesda, MD

[13] Grundy SM, Cleeman JI, Daniels SR, et al. (2005) Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement: Executive Summary. Circulation 112: e285-290

[14] Chobanian AV, Bakris GL, Black HR, et al. (2003) Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 42: 1206-1252

[15] Eckfeldt JH, Chambless LE, Shen YL (1994) Short-term, within-person variability in clinical chemistry test results. Experience from the Atherosclerosis Risk in Communities Study. ArchPatholLab Med 118: 496-500

[16] Expert Panel on Detection EaToHBCiA (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285: 2486-2497

[17] Baecke JA, Burema J, Frijters JE (1982) A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J ClinNutr 36: 936-942

[18] Pencina MJ, Larson MG, D'Agostino RB (2007) Choice of time scale and its effect on significance of predictors in longitudinal studies. Statistics in medicine 26: 1343-1359

[19] Rothman KJ, Greenland S (1998) Modern Epidemiology. Lippincott-Raven, Philadelphia, PA

[20] Li R, Chambless L (2007) Test for additive interaction in proportional hazards models. Annals of epidemiology 17: 227-236

[21] Greenland S (1983) Tests for interaction in epidemiologic studies: a review and a study of power. Statistics in medicine 2: 243-251

[22] Carpenter J, Bithell J (2000) Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. Statistics in medicine 19: 1141-1164

[23] Munoz J, Lok KH, Gower BA, et al. (2006) Polymorphism in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with reduced insulin secretion in nondiabetic women. Diabetes 55: 3630-3634

[24] Li JK, Ng MC, So WY, et al. (2006) Phenotypic and genetic clustering of diabetes and metabolic syndrome in Chinese families with type 2 diabetes mellitus. Diabetes/metabolism research and reviews 22: 46-52

[25] Shaw JT, Purdie DM, Neil HA, Levy JC, Turner RC (1999) The relative risks of hyperglycaemia, obesity and dyslipidaemia in the relatives of patients with Type II diabetes mellitus. Diabetologia 42: 24-27

[26] Richardson MT, Ainsworth BE, Wu HC, Jacobs DR, Jr., Leon AS (1995) Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity. Int J Epidemiol 24: 685-693

B. Manuscript 2: Transcription Factor 7-Like 2 (*TCF7L2*) Polymorphism and Retinal Vascular Signs in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study

ABSTRACT

Purpose: To investigate the association between the transcription factor 7-like 2 (*TCF7L2*) rs7903146 polymorphism and retinal microvascular phenotypes in the Atherosclerosis Risk in Communities (ARIC) Study (1993-1995).

Design: Population-based, cross-sectional study.

Methods: A total of 10,320 middle-aged African American (n=2,199) and Caucasian (n=8,121) men and women were selected from four United States communities to examine the association between *TCF7L2* rs7903146 polymorphism and retinal microvascular signs (retinopathy, focal arteriolar narrowing, arteriovenous nicking, arteriolar and venular calibers). Photographs on one randomly selected eye were graded for presence of retinal microvascular signs and used to measure retinal vessel calibers.

Results: After adjusting for age, sex, study center, mean arterial blood pressure, total serum cholesterol, triglycerides, and other covariates, few associations of *TCF7L2* rs7903146 and retinal microvascular signs were noted. *TCF7L2* rs7903146 T risk allele was significantly associated with focal arteriolar narrowing in Caucasians with hypertension [odds ratio $(OR)_{CT\ vs.\ CC}$ (95% CI) = 1.25 (1.09-1.44); $OR_{TT\ vs.\ CC}$= 1.56 (1.18-2.06); P = 0.002] and in Caucasians without diabetes [$OR_{CT\ vs.\ CC}$ = 1.18 (1.06-1.32); $OR_{TT\ vs.\ CC}$ = 1.40 (1.12, 1.75); P

= 0.003]. No significant association of the *TCF7L2* rs7903146 polymorphism and retinal vascular signs was noted among African American individuals.

Conclusions: *TCF7L2* rs7903146 is not consistently associated with retinal microvascular signs. However, we report an association between the *TCF7L2* rs7903146 polymorphism and focal arteriolar narrowing in Caucasians with hypertension or without diabetes. Further research in other large, population-based studies is needed to replicate these findings.

1. Introduction

Retinal microvascular signs (e.g. retinopathy) and changes in retinal vessel caliber are common fundus findings in adults aged 40 years and older[1]. Narrowing in retinal vascular caliber has been shown to predict the risk of diabetes[2] and to be related to retinopathy in people with diabetes[3], hypertension, or cardiovascular disease in the general population[1]. In addition to risk factors such as diabetes and hypertension, genetic factors may also play a role in the development of these retinal microvascular signs[4, 5].

Transcription factor 7-like 2 (*TCF7L2*), a Wingless and Int (Wnt) signaling-associated transcription factor located on chromosome 10q25, has emerged as a consistently replicated susceptibility gene for type 2 diabetes[6-8], possibly through the impairment of glucagon-like peptide-1-induced insulin secretion[9]. In our previous work, we confirmed that the T allele at single nucleotide polymorphism (SNP) rs7903146 located in intron 3 of *TCF7L2* confers risk for incident type 2 diabetes in middle-aged African Americans and Caucasians[7]. However, whether the *TCF7L2* gene also has similar effects on the retinal microvasculature is less clear. To our knowledge, no studies examining the association of the

TCF7L2 gene to retinal microvascular signs have been conducted but two studies evaluated retinopathy, which relies on less precise global assessments or self-report.

A study in a French population reported no evidence of an association with prevalent, severe diabetic retinopathy[10], whereas the InCHIANTI study indicated an association of the *TCF7L2* gene with reported diabetic retinopathy (odds ratio=7.15, 95%CI=0.87-58.51)[11], although the estimates were notably imprecise. Moreover, potential effects of hypertension on the association of *TCF7L2* gene and retinopathy have been largely unexplored.

In this study, we investigated whether the *TCF7L2* rs7903146 polymorphism is associated with retinal microvascular signs and retinal vessel caliber in a large community-based cohort of African-American and Caucasian middle-aged adults. A second objective is to evaluate whether the effect of the rs7903146 SNP varies by hypertension or diabetes status.

2. Methods

a. Study population

The ARIC Study is an ongoing, longitudinal cohort study of cardiovascular and other major diseases among 15,792 men and women, aged 45 to 64 years old at baseline (1987-1989), selected from 4 US communities: Forsyth County, NC; Jackson, MS; the northwestern suburbs of Minneapolis, MN; and Washington County, MD[12]. By design, African-Americans were over-sampled at the Forsyth County site and were exclusively sampled in Jackson and thus constituted 27% of the baseline cohort. Of the 15,792 participants at baseline, 12,887 (86%) returned for the third examination when retinal photography was first performed in 1993-1995.

We excluded ARIC participants who were not African-American or Caucasian (*n*=38), African-Americans from Minnesota and Maryland field centers (*n*=42), participants with missing genotype data or who did not provide consent for the use of their DNA (*n*=803), participants who did not have retinal photographs (*n*=224), participants who had ungradeable photographs (*n*=1458), and participants who had diabetes diagnosed before 20 years old (*n*=2). After these exclusions, 10,320 participants (2,199 African American and 8,121 Caucasians) were available for analysis. Characteristics of participants with and without gradable retinal photographs have been previously described[13, 14].

b. Assessment of Retinal Microvascular Signs

The retinal photography procedures and grading of retinal microvascular signs have been published in detail elsewhere[13]. In brief, one eye was randomly selected from each participant and a 45° retinal photograph, centered on the region of the optic disc and the macula, was taken using an autofocus film camera after a five-minute dark adaptation. If the selected eye was considered too difficult or not possible to photograph with adequate quality, the other eye was photographed instead.

These retinal photographs were evaluated at the Fundus Photograph Reading Center at the University of Wisconsin, Madison, by trained graders who were masked to participant characteristics. We measured and defined the presence of focal retinal microvascular abnormalities, including retinopathy, arteriovenous (AV) nicking, and focal arteriolar narrowing. Retinopathy was defined based on the presence of any of the following lesions: retinal hemorrhages (blot or flame shaped), microaneurysms, soft or hard exudates, macular edema, intraretinal microvascular abnormalities, venous beading, swelling, or laser

photocoagulation scars. AV nicking and focal arteriolar narrowing were defined as present if graded as definite or probable and as absent if not. Retinal arteriolar and venular calibers were measured using a computer-assisted approach. The fundus photographs were digitized and the diameters of all arterioles and venules in an area half to one disc diameters from the optic disc were measured. These diameters were summarized as the central retinal artery equivalent (CRAE) and the central retinal venular equivalent (CRVE)[13]. Quality control procedures have been previously reported[13].

c. *TCF7L2* Genotyping

The *TCF7L2* rs7903146 SNP was genotyped by the ARIC Central Laboratory using Taqman® assays (Applied Biosystems, Foster City, CA). Laboratory-designed probes were obtained from Applied Bioystems and primers from IDT (Coralville, IA). All PCR reactions took place in optical 384-well reaction plates (Applied Biosystems). Five percent of samples were re-genotyped for quality control as blind duplicates. The percent agreement between blind duplicates was 98% and the simple Kappa coefficient was 0.97 indicating good genotyping quality.

d. Measurement of Covariates

Self-reported race, sex, and study center were ascertained at baseline (1987-1989). Other covariates including age, current smoking, obesity, total serum cholesterol, total serum triglycerides, mean arterial blood pressure, and antihypertensive medication were obtained at visit 3 (1993-1995). At each visit, blood pressure was measured three times using a random zero sphygmomanometer and the average of the last two measurements was used for analyses. Hypertension was defined as systolic blood pressure $\geq$ 140mmHg or diastolic blood

pressure ≥ 90mmHg or current use of anti-hypertension medication use at visit 1, 2, or 3[15]. Mean arterial blood pressure was defined as one-third of systolic blood pressure plus two-thirds of diastolic blood pressure at visit 3[16]. Diabetes was defined as fasting serum glucose levels of at least 7.0 mmol/L (126 mg/dl), nonfasting glucose levels of at least 11.1 mmol/L (200 mg/dl), current use of hypoglycemic medications, or a self-reported physician diagnosis of diabetes at visit 1, 2 or 3[17]. Plasma total cholesterol and triglyceride levels were measured by enzymatic methods; high-density lipoprotein cholesterol (HDL-C) was measured after dextran-magnesium precipitation of the non-HDL-C; and glucose was assessed by a modified hexokinase/glucose-6-phosphate dehydrogenase procedure[18]. Self-reported cigarette smoking exposure was defined as current smoking versus non-smoking obtained by a personal interview. Body mass index (BMI) was calculated as measured weight (kg) divided by the square of measured height (m^2). Individuals with a BMI ≥30 kg/m^2 were classified as obese[19].

e. Statistical Analysis

All analyses were stratified by race to crudely account for population stratification. To assess whether genotype distribution within each race departed from Hardy-Weinberg equilibrium, a χ^2 goodness-of-fit test was used. Logistic regression was used to model the association of focal retinal lesions (retinopathy, focal arteriolar narrowing, AV nicking) with the *TCF7L2* rs7903146 polymorphism, and odds ratios (ORs) and 95% confidence intervals (CIs) were obtained. Following published literature[8] and our previous findings[7], we assumed an additive mode of inheritance and compared heterozygous CT-genotype and homozygous TT-genotype individuals to CC-genotype individuals, using the rs7903146 CC-genotype as the referent group. A variable taking on the values 0 for genotype CC, 1 for genotype CT,

and 2 for genotype TT was used to test for log additive genetic effects in logistic regression models. Generalized linear models were used to obtain adjusted mean retinal vascular calibers (CRAE, CRVE) for each genotype of rs7903146. All models were adjusted for age, study center, sex, current smoking (yes/no), obesity (yes/no), total serum cholesterol, total serum triglycerides, mean arterial blood pressure, and antihypertensive medication. Hypertension was also included in the model when it was not assessed as an effect measure modifier.

As hypertension and diabetes are strongly associated with retinal microvascular signs and *TCF7L2* is a diabetes-related gene, we assessed the potential interactions between genotype and hypertension and interactions between genotype and diabetes on retinal microvascular phenotypes, respectively, and performed sub-group analyses with and without hypertension/diabetes. A Wald χ^2 test for significance of the estimated β-coefficient for the interaction term (SNP × hypertension or SNP × diabetes) and the interaction contrast ratio (ICR) with *P* value were used to assess the departure from multiplicativity and additivity, respectively[20, 21]. A *P* value <0.05 was considered to indicate an important modifier, despite the multiple tests as interaction tests tend to be underpowered[22]. ICRs were quantified as follows: ICR= OR_AB – OR_A – OR_B + 1, where OR_AB represents the joint effect of hypertension/diabetes and the SNP, and OR_A and OR_B represent the main effects of hypertension/diabetes and the SNP, respectively[20]. Thus, ICR refers to the increased odds due to an additive interaction between hypertension/diabetes and the T risk allele adjusted for aforementioned covariates. Assuming an additive mode of inheritance, the ICR comparing TT to CT is equal to the ICR comparing CT to CC, thus only one ICR was reported. Departures from zero suggest that hypertension/diabetes and the SNP interact to cause retinal

microvascular signs. The OR and the variance covariance matrix were used to calculate ICR values and *P* values[21]. For retinal vascular calibers (CRAE, CRVE), only the *P* value from multiplicative interaction test was estimated.

3. Results

The rs7903146 T allele was observed with same frequency (29%) in African-American and Caucasian individuals, and the genotype frequencies for rs7903146 in both races were consistent with Hardy–Weinberg equilibrium ($P>0.05$). Selected characteristics of the ARIC Study participants by race and genotype status are presented in **Table 1**. No statistically significant differences in demographic or behavioral characteristics (sex, and current smoking) were noted by genotype status except for age in Caucasian participants (Table 1). Moreover, no statistically significant differences in hypertension, mean arterial blood pressure, obesity, triglycerides, HDL-C, LDL-C, and total cholesterol by genotype were noted except for individuals with T allele who had significantly higher fasting glucose and were more likely to be diabetic in Caucasians (Table 1).

The associations between retinal lesions and rs7903146 are presented in **Table 2**. The heterozygous CT-genotype and homozygous TT-genotype individuals had a slightly higher prevalence of retinal lesions when compared with CC-genotype individuals in both races except for AV nicking in Caucasians. Assuming an additive mode of inheritance, the rs7903146 T allele was marginally significantly associated with prevalent focal arteriolar narrowing in Caucasians [$OR_{CT\ vs.\ CC}$ (95% CIs) = 1.11 (1.00, 1.23); $OR_{TT\ vs.\ CC}$ (95% CIs) = 1.23 (1.00, 1.51); $P = 0.05$], but not in African American participants [$OR_{CT\ vs.\ CC}$ (95% CIs) = 1.10 (0.88, 1.36); $OR_{TT\ vs.\ CC}$ (95% CIs) = 1.20 (0.78, 1.85); $P = 0.40$] (Table 2). No

significant associations were noted for AV nicking, retinopathy, or retinal arteriolar or venular diameters (CRAE, CRVE) with rs7903146 (**Table 3**).

Hypertension and diabetes were important effect measure modifiers for focal arteriolar narrowing in Caucasians [multiplicative $P = 0.03$ (hypertension), $P = 0.04$ (diabetes); additive ICR = 0.41 and P=0.006 (hypertension), ICR = -0.29 and P=0.04 (diabetes)], but not in African American participants (P>0.05). When stratified by hypertension or diabetes status, *TCF7L2* rs7903146 was significantly associated with an increased odds of focal arteriolar narrowing in Caucasian individuals, however only among those with hypertension or without diabetes (**Table 4**); no associations were noted in African American participants (data not shown). Our analysis in Caucasian individuals with hypertension AND without diabetes indicated that *TCF7L2* rs7903146 was associated with focal arteriolar narrowing [$OR_{CT\ vs.\ CC}$ (95% CIs) = 1.40 (1.19, 1.64); $OR_{TT\ vs.\ CC}$ (95% CIs) = 1.96 (1.43, 2.68); $P < 0.0001$], which is consistent with our interaction analyses. No significant interactions with hypertension or diabetes were observed for other retinal lesions and retinal vessel calibers (CRAE, CRVE).

Table 17. (MS2: Table 1) Distribution of selected characteristics by race and rs7903146 genotype status: the Atherosclerosis Risk in Communities Study (1993-1995)

	African American				Caucasian			
	CC	CT	TT	*P* value[a]	CC	CT	TT	*P* value[a]
n	1099	923	177		4105	3321	695	
Age, years	58.4 ± 5.6	58.3 ± 5.4	58.9 ± 5.6	0.36	60.1 ± 5.6	59.9 ± 5.6	59.6 ± 5.6	0.03
Male sex	399 (36.31)	353 (38.24)	65 (36.72)	0.66	1894 (46.14)	1528 (46.01)	339 (48.78)	0.39
Current smoker	232 (21.28)	194 (21.20)	34 (19.32)	0.86	672 (16.38)	570 (17.17)	117 (16.83)	0.66
Obesity Present[b]	516 (46.95)	418 (45.29)	86 (48.86)	0.60	1223 (29.81)	926 (27.92)	200 (28.78)	0.20
Hypertension Present[c]	720 (65.51)	619 (67.06)	116 (65.54)	0.75	1612 (39.28)	1311 (39.48)	262 (37.70)	0.68
Mean arterial blood pressure, mm Hg[d]	94.06 ± 12.87	94.28 ± 12.61	92.98 ± 12.76	0.46	87.83 ± 11.08	87.55 ± 11.38	87.24 ± 11.05	0.32
Diabetes Present[e]	283 (25.75)	258 (27.95)	58 (32.77)	0.12	534 (13.01)	536 (16.14)	136 (19.57)	<0.01
Glucose, mg/dL	119.74 ± 54.11	121.25 ± 57.38	128.02 ± 61.89	0.19	105.80 ± 31.34	108.5 ± 35.47	111.18 ± 36.60	<0.01
Triglycerides, mg/dL	115.99 ± 72.35	113.05 ± 60.12	113.64 ± 59.79	0.60	150.57 ± 91.84	149.22 ± 91.66	151.24 ± 116.54	0.78
HDL-C, mg/dL	55.73 ± 18.84	54.78 ± 17.82	53.90 ± 18.83	0.33	51.08 ± 17.70	51.82 ± 18.54	50.20 ± 17.11	0.05
LDL-C, mg/dL	127.88 ± 36.20	129.15 ± 37.24	130.26 ± 37.15	0.61	126.99 ± 33.10	126.56 ± 34.90	127.03 ± 33.21	0.85
Total Cholesterol, mg/dL	206.43 ± 39.13	206.45 ± 38.71	206.89 ± 39.96	0.99	207.90 ± 36.98	207.94 ± 37.95	206.36 ± 35.65	0.57

Data are means ± SE or *n* (%) unless otherwise indicated. Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. [a]*P* value is based on ANOVA (continuous) and χ^2 (categorical), comparing differences for individual characteristic across genotypes; [b]obesity was defined as body mass index ≥30 kg/m^2; [c]hypertension was defined as systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg or a history of anti-hypertension medication use; [d]mean arterial blood pressure was defined as one-third of systolic blood pressure plus two-thirds of diastolic blood pressure; [e]diabetes was defined as fasting serum glucose levels of at least 7.0 mmol/L (126 mg/dl), nonfasting glucose levels of at least 11.1 mmol/L (200 mg/dl), current use of hypoglycemic medications, or a self-reported physician diagnosis of diabetes.

Table 18. (MS2: Table 2) Retinal lesions by *TCF7L2* rs7903146 genotype, by race: the Atherosclerosis Risk in Communities Study (1993-1995)

		African American				Caucasian			
Retinal Lesion	Genotype	*n*	No. with Lesion (%)	OR (95% CI)[a]	*P* value[b]	*n*	No. with Lesion (%)	OR (95% CI)[a]	*P* value[b]
AV nicking	CC	1083	179 (16.53)	1.00	0.24	4058	585 (14.42)	1.00	0.58
	CT	915	156 (17.05)	1.12 (0.93, 1.35)		3286	433 (13.18)	1.03 (0.93, 1.14)	
	TT	174	33 (18.97)	1.26 (0.86, 1.83)		689	106 (15.38)	1.06 (0.86, 1.30)	
Focal arteriolar narrowing	CC	1076	136 (12.64)	1.00	0.40	4041	598 (14.80)	1.00	0.05
	CT	912	120 (13.16)	1.10 (0.88, 1.36)		3268	543 (16.62)	1.11 (1.00, 1.23)	
	TT	173	26 (15.03)	1.20 (0.78, 1.85)		688	104 (15.12)	1.23 (1.00, 1.51)	
Retinopathy	CC	1099	138 (12.56)	1.00	0.36	4105	236 (5.75)	1.00	0.27
	CT	923	128 (13.87)	1.10 (0.90, 1.35)		3321	205 (6.17)	1.09 (0.94, 1.26)	
	TT	177	24 (13.56)	1.21 (0.81, 1.81)		695	45 (6.47)	1.18 (0.88, 1.58)	

Abbreviations: AV, arteriovenous; CI, confidence interval; OR, odds ratio.

[a]Adjusted for age, study center, gender, current smoking (yes/no), obesity, total serum cholesterol, total serum triglycerides, mean arterial blood pressure, hypertension, and antihypertensive medication; [b]*P* value for OR in the log additive genetic model.

Table 19. (MS2: Table 3) Mean retinal vessel calibers (CRAE/CRVE) by *TCF7L2* rs7903146 genotype, by race: the Atherosclerosis Risk in Communities Study (1993-1995)

		African American			Caucasian		
Retinal Vessel Index	Genotype	*n*	Multivariate Adjusted[a]	*P* value[b]	*n*	Multivariate Adjusted[a]	*P* value[b]
Mean retinal arteriolar diameter (95% CI), µm	CC	1090	163.44 (161.97, 164.90)	0.14	4096	161.03 (160.25, 161.82)	0.29
	CT	916	164.54 (162.95, 166.13)		3312	160.53 (159.71, 161.35)	
	TT	177	162.92 (160.47, 165.36)		694	160.79 (159.56, 162.02)	
Mean retinal venular diameter (95% CI), µm	CC	1090	202.43 (200.88, 203.97)	0.72	4096	194.65 (193.87, 195.44)	0.72
	CT	916	201.60 (199.93, 203.28)		3312	194.85 (194.03, 195.67)	
	TT	177	201.92 (199.33, 204.51)		694	194.44 (193.21, 195.67)	

Abbreviations: CI, confidence interval; CRAE, central retinal artery equivalent; CRVE, central retinal venular equivalent.

[a]Adjusted for age, study center, gender, current smoking (yes/no), obesity, total serum cholesterol, total serum triglycerides, mean arterial blood pressure, hypertension, antihypertensive medication, and CRAE (when the outcome is CRVE)/CRVE (when the outcome is CRAE); [b]*P* value for 1 degree freedom test of association between vessel calibers and rs7903146 under the log additive genetic model.

Table 20. (MS2: Table 4) Retinal Lesions and *TCF7L2* rs7903146 genotype by hypertension or diabetes status in Caucasians: the Atherosclerosis Risk in Communities Study (1993-1995)

Retinal Lesion	Geno-type	*n*	No. with Lesion (%)	OR (95% CI)[a]	*n*	No. with Lesion (%)	OR (95% CI)[a]
		With Hypertension			Without Hypertension		
AV nicking	CC	1595	280 (17.55)	1.00	2462	305 (12.39)	1.00
	CT	1297	222 (17.12)	1.18 (1.02, 1.36)	1989	211 (10.61)	0.91 (0.78, 1.05)
	TT	259	60 (23.17)	1.39 (1.04, 1.86)	430	46 (10.70)	0.83 (0.61, 1.11)
P value[b]				0.03			0.21
Focal arteriolar narrowing	CC	1584	330 (20.83)	1.00	2456	268 (10.91)	1.00
	CT	1290	320 (24.81)	1.25 (1.09, 1.44)	1978	223 (11.27)	0.96 (0.82, 1.12)
	TT	259	67 (25.87)	1.56 (1.18, 2.06)	429	37 (8.62)	0.92 (0.68, 1.25)
P value[b]				**0.002**			0.59
Retinopathy	CC	1612	136 (8.44)	1.00	2492	100 (4.01)	1.00
	CT	1311	112 (8.54)	1.04 (0.85, 1.26)	2010	93 (4.63)	1.12 (0.90, 1.39)
	TT	262	23 (8.78)	1.08 (0.73, 1.60)	433	22 (5.08)	1.26 (0.81, 1.94)
P value[b]				0.71			0.31
		With Diabetes			Without Diabetes		
AV nicking	CC	525	89 (16.95)	1.00	3533	496 (14.04)	1.00
	CT	525	88 (16.76)	0.99 (0.78, 1.26)	2761	345 (12.50)	1.04 (0.92, 1.16)
	TT	134	21 (15.67)	0.98 (0.61, 1.58)	555	85 (15.32)	1.07 (0.85, 1.35)
P value[b]				0.94			0.56
Focal arteriolar narrowing	CC	519	88 (16.96)	1.00	3522	510 (14.48)	1.00
	CT	526	91 (17.30)	0.85 (0.65, 1.11)	2742	452 (16.48)	1.18 (1.06, 1.32)
	TT	136	15 (11.03)	0.73 (0.42, 1.24)	552	89 (16.12)	1.40 (1.12, 1.75)
P value[b]				0.24			**0.003**
Retinopathy	CC	534	78 (14.61)	1.00	3571	158 (4.42)	1.00
	CT	536	76 (14.18)	1.05 (0.82, 1.34)	2785	129 (4.63)	1.01 (0.84, 1.22)
	TT	136	23 (16.91)	1.10 (0.67, 1.8)	559	22 (3.94)	1.02 (0.70, 1.48)
P value[b]				0.71			0.91

Abbreviations: AV, arteriovenous; CI, confidence interval; OR, odds ratio.
[a]Adjusted for age, study center, gender, current smoking (yes/no), obesity, total serum cholesterol, total serum triglycerides, mean arterial blood pressure, hypertension (when stratified by diabetes), and antihypertensive medication; [b]*P* value for OR in the log additive genetic model.

4. Discussion

Our study reports on the association between the *TCF7L2* rs7903146 polymorphism and retinal microvascular lesions and retinal vascular caliber in a middle-aged biracial population. No associations were noted except for focal arteriolar narrowing in Caucasians. The *TCF7L2* rs7903146 was significantly associated with a greater frequency of focal arteriolar narrowing among Caucasians with hypertension or without diabetes, but not among

those without hypertension or with diabetes, suggesting an interaction between *TCF7L2* variants and hypertension and diabetes status in Caucasians.

To our knowledge, there are few studies for direct comparison. An earlier case-control study in a French population reported the lack of an association with severe retinopathy (effect estimates not reported)[10], which is consistent with our findings on retinopathy in Caucasians. The InCHIANTI study of elderly Europeans reported an association with diabetic retinopathy (OR=7.15, 95%CI=0.87-58.51, *P*=0.067) in 127 persons with diabetes. However the number of participants with diabetic retinopathy was very small (*n*=12) and results were not statistically significant[11]. Notably, these two studies did not report the definition for retinopathy used, which may differ from ours.

We observed an association between *TCF7L2* rs7903146 and focal arteriolar narrowing in Caucasians, but not in African Americans. The lack of association in the African American examinees could reflect confounding by unmeasured covariates that are differentially distributed in African American and Caucasian participants, which warrants further investigation. More likely however, the limited power to detect such a modest effect in the African American sample (calculated as 26% for a relative risk of 1.15) may explain our findings. The latter is supported by the observation of very similar effect size estimates between African American and Caucasian participants, and therefore warrants further study in additional African American populations.

It is not known why *TCF7L2* rs7903146 was associated with retinal focal arteriolar narrowing. To determine whether the effect of *TCF7L2* rs7903146 on focal arteriolar narrowing was due to hyperglycemia, we further adjusted for fasting glucose values in the

models, but no attenuation of genetic effects were noted. It is possible that the *TCF7L2* rs7903146 variant may be related to focal arteriolar narrowing not through its effect on diabetes but through other, retinal-specific mechanisms (i.e. pleiotropic effects). The Wnt/β-catenin/T-cell factor (TCF) (canonical) signaling pathway may inhibit the adipogenic differentiation of pericytes (a contractile cell in small retinal arterioles), which may have a later effect in regulating retinal microvascular function. This pathway also regulates vascular smooth muscle cell proliferation, suggesting that it may be involved in intimal thickening[23]. Prolonged exposure to elevated blood pressure may lead to retinal vessel vasospasm, intimal thickening, medial hyperplasia and arteriosclerosis manifesting as either generalized or focal arteriolar narrowing[24]. However, we found only a relation with focal and not generalized arteriolar narrowing as measured by CRAE and biological mechanisms remain speculative.

An alternate explanation of our positive findings could be chance considering the large number of comparisons made in assessing association in the context of possible effect modification. To minimize the impact of the multiple tests we could apply a crude Bonferroni correction (five phenotypes in the context of multiple strata defined by diabetes, hypertension, combined diabetes and hypertension grouping, and the full sample N=30), noting that such an approach is an over-correction because many of the analytic runs assessed the same dependent variable. If such a correction were applied, most of the results reported in this paper would not be statistically significant except in the subgroup with hypertension AND without diabetes.

Our study has notable strengths, including a large, biracial, population-based cohort, standardized assessment of retinal photographs, and detailed information on a variety of risk

factors. To our knowledge, this is the first population-based study that systematically examines the association between *TCF7L2* rs7903146 and retinal microvascular lesions and caliber in middle-aged African Americans and Caucasians.

Several important limitations also deserve mention. First, grading was performed from a single 45° fundus photograph that was taken through a nonpharmacologically dilated pupil. This can underestimate the prevalence of retinal microvascular lesions, which could have biased the results toward the null. Second, we found that the *TCF7L2* rs7903146 is related to higher risk of retinal AV nicking only in Caucasians who had hypertension (P=0.03). This association could have arisen by chance; the pathophysiology underlying any relationship between AV nicking and rs7903146 has not been established. Third, as diabetes and fasting glucose values are plausibly intermediate variables between *TCF7L2* and retinal phenotypes, our analyses conditional on diabetes/fasting glucose values need to be interpreted with caution as this method may introduce confounding[25]. Finally, our samples of African American and diabetic Caucasians are limited to 2,199 and 1,206 examinees, respectively, thus true associations between retinal lesions and the *TCF7L2* variant could have been missed in these subpopulations. Replication of our findings in other large, population-based studies could help better elucidate these relationships.

In summary, *TCF7L2* rs7903146 is not consistently associated with retinal microvascular signs. However, our study is the first to report an association between the *TCF7L2* rs7903146 polymorphism and focal arteriolar narrowing in Caucasians with hypertension or without diabetes. No significant associations were noted for other retinal microvascular signs in either race group. Other large, population-based studies are needed to

confirm our findings.

ACKNOWLEDGEMENTS / DISCLOSURE

A. Funding / Support: The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute (NHLBI) contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

B. Financial Disclosure:

Employee and shareholder of Merck & Co., Inc. - C.J.G.

No financial disclosures - Y.Y., K.E.N., R.K., G.H., E.M.L., B.E.K., K.M.R., S.L.W, E.B., J.S.P., F.B., A.K.

C. Contributions to Authors:

Design and conduct (Y.Y., K.E.N., R.K, G.H., C.J.G., B.E.K., K.M.R., S.L.W, F.B.);

Collection of data (K.E.N., R.K., G.H., B.E.K., E.B.);

Analysis of data (Y.Y., K.E.N., C.J.G.,);

Interpretation of data (Y.Y., K.E.N., R.K., C.J.G., E.M.L., A.K.);

Preparation of the manuscript (Y.Y., K.E.N.,);

Review and approval of the manuscript (Y.Y., K.E.N., R.K., G.H., C.J.G., E.M.L., B.E.K., K.M.R., S.L.W, E.B., J.S.P., F.B., A.K.)

D. Statement about Conformity with Author Information: The institutional review boards at all participating institutions and at the Fundus Photograph Reading Center at the University of Wisconsin approved the procedures and all participants included in the analysis gave informed consent.

E. Other Acknowledgments: We are indebted to the staff and participants in the Atherosclerosis Risk in Communities Study for their important contributions.

.

5. References

1. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. Surv Ophthalmol 2001;46:59-80.

2. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. Surv Ophthalmol 2009;54:74-95.

3. Nguyen TT, Wang JJ, Sharrett AR, et al. Relationship of retinal vascular caliber with diabetes and retinopathy: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care 2008;31:544-9.

4. Wang JJ, Wong TY. Genetic determinants of retinal vascular caliber: additional insights into hypertension pathogenesis. Hypertension 2006;47:644-5.

5. Xing C, Klein BE, Klein R, Jun G, Lee KE, Iyengar SK. Genome-wide linkage study of retinal vessel diameters in the Beaver Dam Eye Study. Hypertension 2006;47:797-802.

6. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat.Genet. 2006;38:320-323.

7. Yan Y, North KE, Ballantyne CM, et al. Transcription Factor 7-Like 2 (TCF7L2) Polymorphism and Context-Specific Risk of Type 2 Diabetes in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study. Diabetes 2008.

8. Cauchi S, El Achhab Y, Choquet H, et al. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J Mol Med 2007.

9. Cauchi S, Froguel P. TCF7L2 genetic defect and type 2 diabetes. Curr Diab Rep 2008;8:149-55.

10. Cauchi S, Meyre D, Dina C, et al. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes 2006;55:2903-8.

11. Melzer D, Murray A, Hurst AJ, et al. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. BMC Med 2006;4:34.

12. The ARIC Investigators. The Atherosclerosis Risk in Communnities (ARIC) Study: design and objectives. Am. J. Epidemiol. 1989;129:687-702.

13. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology 1999;106:2269-2280.

14. Klein R, Clegg L, Cooper LS, et al. Prevalence of age-related maculopathy in the Atherosclerosis Risk in Communities Study. Arch Ophthalmol 1999;117:1203-10.

15. Chobanian AV, Bakris GL, Black HR, et al. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-1252.

16. Sharrett AR, Hubbard LD, Cooper LS, et al. Retinal Arteriolar Diameters and Elevated Blood Pressure: The Atherosclerosis Risk in Communities Study. Am. J. Epidemiol. 1999;150:263-270.

17. ADA. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2007;30 Suppl 1:S42-7.

18. Eckfeldt JH, Chambless LE, Shen YL. Short-term, within-person variability in clinical chemistry test results. Experience from the Atherosclerosis Risk in Communities Study. Arch.Pathol.Lab Med. 1994;118:496-500.

19. US Department of Health and Human Service. The practical guide—identification, evaluation, and treatment of overweight and obesity in adults. NIH publication no. 004084. Bethesda, MD: National Institutes of Health, 2000.

20. Rothman KJ, Greenland S. Modern Epidemiology, 2nd ed. Philadelphia, PA: Lippincott-Raven, 1998.

21. Li R, Chambless L. Test for additive interaction in proportional hazards models. Ann Epidemiol 2007;17:227-36.

22. Greenland S. Tests for interaction in epidemiologic studies: a review and a study of power. Stat Med 1983;2:243-51.

23. Kirton JP, Crofts NJ, George SJ, Brennan K, Canfield AE. Wnt/beta-catenin signaling stimulates chondrogenic and inhibits adipogenic differentiation of pericytes: potential relevance to vascular disease? Circ Res 2007;101:581-9.

24. Tso MO, Jampol LM. Pathophysiology of hypertensive retinopathy. Ophthalmology 1982;89:1132-45.

25. Rothman KJ, Greenland S, Lash TL. Modern Epidemiology, 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2008.

CHAPTER VII

CONCLUSIONS

A. Recapitulation of overall study aims and results

1. Overall study aims

The goal of this project was to measure the associations between *TCF7L2* and prediabetes/retinal phenotypes using the Atherosclerosis Risk in Communities (ARIC) data. The ARIC study is an ongoing, bi-racial population-based longitudinal study of cardiovascular-related diseases in 15,792 males and females. Manuscript 1 addressed Aims 1 and 2, and Manuscript 2 addressed Aims 3 and 4.

AIM 1: To estimate the association between SNP rs7903146 in *TCF7L2* and prediabetes as quantified by incident impaired fasting glucose (IFG).
Research question: Is SNP rs7903146 in *TCF7L2* associated with incident IFG?

AIM 2: To estimate the extent to which metabolic risk factors including obesity, elevated waist circumference, hypertension, low HDL, high LDL, high triglyceride modify the association between SNP rs7903146 in *TCF7L2* and incident IFG.
Research question: To what extent do metabolic risk factors modify the association between SNP rs7903146 in *TCF7L2* and incident IFG?

AIM 3: To estimate the association between SNP rs7903146 in *TCF7L2* and retinal phenotypes including retinopathy, arteriovenous (AV) nicking, focal arteriolar narrowing, central retinal artery equivalent (CRAE) and the central retinal venular equivalent (CRVE). Research question: Is SNP rs7903146 in *TCF7L2* associated with prevalent retinal phenotypes?

AIM 4: To estimate the extent to which hypertension and diabetes modify the association between SNP rs7903146 in *TCF7L2* and retinal phenotypes. Research question: To what extent do hypertension and diabetes modify the association between SNP rs7903146 in *TCF7L2* and prevalent retinal phenotypes?

2. Results

Results from Manuscript 1 suggested that SNP rs7903146 in *TCF7L2* is associated with incident IFG in Caucasians, but not in African Americans. Obesity and high triglycerides were associated with increases in the estimated effect of SNP rs7903146 on incident IFG in Caucasians. Results from Manuscript 2 suggested that SNP rs7903146 in *TCF7L2* is associated with prevalent focal arteriolar narrowing in Caucasians with hypertension and in Caucasians without diabetes. Other focal retinal lesions and vessel calibers were not significantly associated with the rs7903146 polymorphism among Caucasian individuals. No significant association of the *TCF7L2* rs7903146 polymorphism and retinal vascular signs was noted among African American individuals.

Evaluating two related, yet distinct phenotypes allowed us to consider different stages in the natural history of T2DM. We extended our study of incident T2DM events by evaluating prediabetes as measured by IFG (Manuscript 1) and retinal phenotypes

(Manuscript 2), as they can provide information not captured by studies of incident T2DM events. For example, the association between *TCF7L2* and prevalent focal arteriolar narrowing among non-diabetic Caucasians suggested that this candidate gene may be related to focal arteriolar narrowing not through its effect on diabetes but through other, retinal-specific mechanisms (i.e. pleiotropic effects), which clearly needs further investigation.

Our study results also highlight the advantage of considering gene-by-environment interaction. The *TCF7L2* rs7903146 main effect is marginally significant for focal arteriolar narrowing ($P = 0.05$). If we correct for multiple testing, this main effect is basically null; thus an analysis limited to examine the main effect of *TCF7L2* rs7903146 would conclude that *TCF7L2* is not associated with focal arteriolar narrowing. However, by incorporating the two important modifiers, hypertension and diabetes, we found that *TCF7L2* rs7903146 was significantly associated with increased prevalence of focal arteriolar narrowing in Caucasians with hypertension AND without diabetes, even after adjusting for multiple testing.

B. Strengths

This dissertation work has notable strengths, including a large, biracial, population-based cohort, standardized assessment of retinal photographs, and detailed information on a variety of risk factors. To our knowledge, our study is the first population-based study on the *TCF7L2* rs7903146 and prediabetes as measured by incident IFG in African Americans, and also the first the population-based study that systematically examines the association between *TCF7L2* rs7903146 and retinal microvascular lesions and caliber in middle-aged African Americans and Caucasians. This work underscores the necessity of considering gene-environment interactions in genetic epidemiology research, as described above.

Results from this study may also have significant public health implications. Investigating the gene-environment interaction is critical to determining the health implications of a given variant and the priority it should receive for identifying interventions to reduce its associated risk. Given the recent rise in the prevalence of diabetes, the information on the association between *TCF7L2*, metabolic risk factors and incident IFG in Caucasians presented in this study may be important for public health initiatives to encourage lifestyle changes (e.g. diet, physical activity) in such patients at risk.

C. Limitations

While the study sample is sufficient for the estimation of the main effects of SNP rs7903146 in Caucasians, power to assess the main effects in African American, and also gene-environment interactions, especially within the African American stratum, was limited. Thus, it is possible that true associations between IFG/retinal lesions and the *TCF7L2* variant could have been missed in this study. However, the study is adequately powered to address the main aims in Caucasians, and thus makes an important contribution to the understanding of this major disease in this population.

As the ARIC population is a biracial, middle-aged population sample from four US communities, the study results may not be generalizable to other ethnicities, other age groups, and other cultures around the world with different lifestyle/environmental factors and different hereditary patterns. In addition, the retinal fundus grading was performed from a single 45° fundus photograph that was taken through a nonpharmacologically dilated pupil. This can underestimate the prevalence of retinal microvascular lesions, which could have biased the results toward the null.

D. Future Directions

As a continued line of research stemming from this dissertation work, the associations between 19± well replicated susceptibility genes thus far for T2DM (**Table 2**) and retinal phenotypes in the ARIC Study will be assessed. To my knowledge, this will be the first population-based study that systematically examines the association between 19 diabetes susceptibility genes and retinal miscrovascular lesions and calibre in middle-aged African Americans and Caucasians.

Of 19± diabetes susceptibility genes, 10 SNPs (one SNP from each gene) have been genotyped in the entire ARIC cohort. Genotypic information of the rest 9 genes will be obtained from the ARIC GWAS data, either genotyped or imputed, once genotyping data are released from the full ARIC cohort. The allelic and genotypic frequencies of these 10 genotyped SNPs as well as the assessment of HWE are presented in **Table 21**. Of these 10 SNPs, one is out of HWE (*PPARG* rs1801282; P<0.0001) and thus excluded from further analysis.

The preliminary analysis on each individual SNP (a total of 9 SNPs) and retinal phenotypes including retinopathy, focal narrowing, AV nicking, CRAE and CRVE were conducted in African Americans and Caucasians, respectively (**Table 22, Table 23**). The same analytic strategy as Manuscript 2 applies. Table 22 presents the statistical significance (P value) for each individual association analysis, and Table 23 provides more detailed information on effect estimates (OR with 95% CI) and P values. All analyses were adjusted for age, sex, center and other covariates same as Manuscript 2. A few positive associations are noted such as the previous findings on *TCF7L2* and focal narrowing in Caucasians,

however, after applying for a Bonferroni correction (N = 5 participants group * 5 phenotypes * 9 SNPs = 225), no association retains significant. Besides the individual SNP-outcome association, a risk score which is comprised of all evaluated SNPs, not just those that display significant results with retinal phenotypes, will be constructed in order to increase the generalizeability of the results. While individual SNPs may show marginal or null effects, an aggregate score may show stronger effects and help increase our understanding and potentially help elucidate possible pathways of disease.

As it is unknown how the diabetes susceptibility genes influence the risk of retinal miscrovascular phenotypes, this extension of my dissertation work should be informative. While the limited power in our African American subpopulation is recognized, the study is adequately powered to conduct the main association analysis in Caucasians, and thus may make a contribution to the etiology of retinal miscrovascular diseases.

Table 21. Allelic and genotypic frequencies of 10 diabetes susceptibility polymorphisms and HWE assessment in the ARIC Study.

		African Americans						Caucasians					
Gene	SNP	N	Allele	Allelic freq	Geno-type	Genotypic freq	HWE P value	N	Allele	Allelic freq	Geno-type	Genotypic freq	HWE P value
CDKN2A/2B	rs10811661	2210	C	0.0663	C/C	0.0032	0.3514	8300	C	0.1740	C/C	00310	0.6687
			T	0.9337	C/T	0.1262			T	0.8260	C/T	0.2861	
					T/T	0.8706					T/T	0.6829	
IGF2BP2	rs4402960	2159	G	0.4912	G/G	0.2362	0.3472	8227	T	0.3126	T/T	0.0969	0.7283
			T	0.5088	T/T	0.2538			G	0.6874	G/T	0.4314	
					G/T	0.5100					G/G	0.4717	
CDKAL1	rs7754840	2210	G	0.4215	G/G	0.1706	0.1730	8239	C	0.3119	C/C	0.0981	0.7329
			C	0.5785	C/C	0.3276			G	0.6881	C/G	0.4276	
					C/G	0.5018					G/G	0.4743	
HHEX	rs1111875	2193	T	0.2193	T/T	0.0502	0.5746	8248	T	0.4056	T/T	0.1649	0.8903
			C	0.7807	C/T	0.3383			C	0.5944	C/C	0.3537	
					C/C	0.6115					C/T	0.4815	
SLC30A8	rs13266634	2144	T	0.0793	T/T	0.0089	0.1024	8229	T	0.3105	T/T	0.0979	0.5130
			C	0.9207	C/T	0.1409			C	0.6895	C/T	0.4251	
					C/C	0.8503					C/C	0.4770	
TCF7L2	rs7903146	2199	T	0.2904	T/T	0.0805	0.3850	8121	T	0.2901	T/T	00856	0.5253
			C	0.7096	C/T	0.4197			C	0.7099	C/T	0.4089	
					C/C	0.4998					C/C	0.5055	
FTO	rs12255372	2182	T	0.3116	T/T	0.0949	0.6237	8094	T	0.2856	T/T	0.0830	0.5278
			G	0.6884	G/T	0.4335			G	0.7144	G/T	0.4052	
					G/G	0.4716					G/G	0.5117	
PPARG	rs1801282	2245	C	0.2274	C/G	0.0494	**0.0000**	8110	C	0.2606	C/C	01551	**0.0000**
			G	0.7726	C/C	0.2027			G	0.7394	C/G	0.2110	
					G/G	0.7479					G/G	0.6339	
KCNJ1	rs5219	2154	A	0.0692	A/A	0.0051	0.8166	8181	A	0.3719	A/A	0.1403	0.4348
			G	0.9308	A/G	0.1281			G	0.6281	G/G	0.3965	
					G/G	0.8668					A/G	0.4631	
Intergenic	rs9300039	2191	A	0.1155	A/A	0.0137	0.8695	8250	A	0.0899	A/A	0.0095	0.1297
			C	0.8845	A/C	0.2036			C	0.9101	A/C	0.1610	
					C/C	0.7827					C/C	0.8296	

\

Table 22. Summary of Associations between diabetes susceptibility polymorphisms and retinal vascular signs in the ARIC study, stratified by hypertension and DM.

		P value (NS indicates non-statistical significance defined by P > 0.05)																	
	Participant Group	*TCF7L2* rs7903146		*CDKN2A/2B* rs10811661		*IGF2BP2* rs4402960		*CDKAL1* rs7754840		*HHEX* rs1111875		*SLC30A8* rs13266634		*FTO* rs12255372		*KCNJ11* rs5219		Chrom 11rs9300039	
		W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B
Retino-pathy	All	NS	NS	NS	NS	NS	**0.040**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Hyt Only	NS	NS	NS	NS	NS	**0.013**	**0.051**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-Hyt Only	NS	NS	NS	NS	NS	NS	**0.018**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.027**	NS
	DM Only	NS	NS	NS	NS	NS	**0.002**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-DM Only	NS	NS	**0.036**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AV nicking	All	NS	NS	NS	NS	NS	NS	NS	NS	**0.021**	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Hyt Only	**0.027**	NS	NS	NS	NS	NS	NS	NS	**0.003**	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	DM Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.034**	NS	NS
	Non-DM Only	NS	NS	NS	NS	NS	NS	NS	NS	**0.045**	NS	NS	NS	NS	NS	NS	NS	NS	NS
FN	All	**0.050**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.004**	NS	NS	NS	NS	NS	NS	NS
	Hyt Only	**0.002**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.004**	NS	NS	NS	NS	NS
	Non-Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.006**	NS	NS	NS	NS	NS	NS	NS
	DM Only	NS	NS	NS	NS	NS	NS	**0.011**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-DM Only	**0.003**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.005**	NS	**0.014**	NS	NS	NS	NS	NS
CRAE	All	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	DM Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-DM Only	NS	NS	NS	**0.039**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CRVE	All	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-Hyt Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	DM Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Non-DM Only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.009**	NS	NS

Abbreviations: B, blacks; Hyt, hypertension; DM, diabetes mellitus; FN, focal narrowing; NS, non significant; W, whites.

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
TCF 7L2	rs7903 146	W	CRAE	ref genotype	163.25 (162.42, 164.08)	162.38 (161.36, 163.41)	164.38 (160.97, 167.79)	164.67 (162.7, 166.64)	162.89 (161.96, 163.83)
				het genotype	162.97 (162.16, 163.79)	162 (160.99, 163.02)	164.15 (160.76, 167.53)	164.89 (163.04, 166.74)	162.46 (161.53, 163.39)
				hom genotype	162.69 (161.65, 163.73)	161.62 (160.18, 163.07)	163.91 (160.45, 167.38)	165.11 (162.71, 167.5)	162.02 (160.85, 163.2)
				p value	0.2363	0.3069	0.4483	0.7172	0.0926
TCF 7L2	rs7903 146	W	CRVE	ref genotype	197.52 (196.69, 198.35)	198.44 (197.4, 199.49)	196.89 (193.48, 200.29)	198.18 (196.13, 200.23)	197.66 (196.72, 198.59)
				het genotype	197.54 (196.72, 198.36)	198.63 (197.59, 199.67)	196.83 (193.45, 200.21)	197.93 (196, 199.86)	197.69 (196.76, 198.62)
				hom genotype	197.56 (196.51, 198.6)	198.82 (197.35, 200.28)	196.77 (193.31, 200.24)	197.68 (195.18, 200.18)	197.72 (196.55, 198.89)
				p value	0.9360	0.6223	0.8523	0.6896	0.9026
TCF 7L2	rs7903 146	W	retinopathy	het vs. ref	1.09 (0.94, 1.26)	1.04 (0.85, 1.26)	1.12 (0.9, 1.39)	1.05 (0.82, 1.34)	1.01 (0.84, 1.22)
				hom vs. ref	1.18 (0.88, 1.58)	1.08 (0.73, 1.6)	1.26 (0.81, 1.94)	1.1 (0.67, 1.8)	1.02 (0.7, 1.48)
				p value	0.2679	0.7133	0.3075	0.7008	0.9142
TCF 7L2	rs7903 146	W	A/V nicking	het vs. ref	1.03 (0.93, 1.14)	1.18 (1.02, 1.36)	0.91 (0.78, 1.05)	0.99 (0.78, 1.26)	1.04 (0.92, 1.16)
				hom vs. ref	1.06 (0.86, 1.3)	1.39 (1.04, 1.86)	0.83 (0.61, 1.11)	0.98 (0.61, 1.58)	1.07 (0.85, 1.35)
				p value	0.5775	0.0270	0.2092	0.9402	0.5577
TCF 7L2	rs7903 146	W	Focal narrowing	het vs. ref	1.11 (1, 1.23)	1.25 (1.09, 1.44)	0.96 (0.82, 1.12)	0.85 (0.65, 1.11)	1.18 (1.06, 1.32)
				hom vs. ref	1.23 (1, 1.51)	1.56 (1.18, 2.06)	0.92 (0.68, 1.25)	0.73 (0.42, 1.24)	1.4 (1.12, 1.75)
				p value	0.0501	0.0017	0.5916	0.2409	0.0030
TCF 7L2	rs7903 146	B	CRAE	ref genotype	161.61 (160.07, 163.15)	160.17 (158.37, 161.98)	158.38 (150.5, 166.27)	163.78 (161.1, 166.46)	160.91 (158.95, 162.88)
				het genotype	161.94 (160.4, 163.48)	161 (159.22, 162.78)	157.52 (149.61, 165.44)	163.74 (161.08, 166.41)	161.34 (159.37, 163.32)
				hom genotype	162.27 (160.24, 164.29)	161.82 (159.43, 164.21)	156.66 (148.41, 164.92)	163.71 (160.18, 167.24)	161.77 (159.23, 164.31)
				p value	0.4882	0.1607	0.2811	0.9666	0.4548
TCF 7L2	rs7903 146	B	CRVE	ref genotype	200.36 (198.79, 201.94)	201.72 (199.9, 203.55)	204.75 (196.45, 213.06)	201.01 (198.08, 203.93)	199.43 (197.47, 201.4)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				het genotype	199.86 (198.28, 201.45)	200.92 (199.11, 202.73)	205.05 (196.71, 213.39)	201.57 (198.68, 204.46)	198.43 (196.45, 200.42)
				hom genotype	199.36 (197.25, 201.47)	200.11 (197.62, 202.6)	205.35 (196.64, 214.05)	202.14 (198.25, 206.03)	197.43 (194.85, 200.01)
				p value	0.3175	0.1949	0.7263	0.5509	0.0872
TCF 7L2	rs7903 146	B	retinopathy	het vs. ref	1.1 (0.9, 1.35)	1.07 (0.84, 1.35)	1.31 (0.86, 2)	1.22 (0.91, 1.64)	0.9 (0.66, 1.22)
				hom vs. ref	1.21 (0.81, 1.81)	1.14 (0.71, 1.82)	1.71 (0.73, 4)	1.5 (0.84, 2.68)	0.81 (0.44, 1.5)
				p value	0.3598	0.5848	0.2154	0.1734	0.5018
TCF 7L2	rs7903 146	B	A/V nicking	het vs. ref	1.12 (0.93, 1.35)	1.1 (0.88, 1.38)	1.13 (0.78, 1.64)	1.31 (0.94, 1.82)	1.03 (0.82, 1.3)
				hom vs. ref	1.26 (0.86, 1.83)	1.22 (0.78, 1.9)	1.28 (0.61, 2.69)	1.71 (0.88, 3.3)	1.07 (0.67, 1.7)
				p value	0.2355	0.3866	0.5054	0.1105	0.7831
TCF 7L2	rs7903 146	B	Focal narrowing	het vs. ref	1.1 (0.88, 1.36)	1.12 (0.88, 1.43)	1 (0.63, 1.6)	1.18 (0.79, 1.76)	1.08 (0.84, 1.4)
				hom vs. ref	1.2 (0.78, 1.85)	1.26 (0.77, 2.06)	1 (0.39, 2.57)	1.38 (0.62, 3.09)	1.18 (0.7, 1.97)
				p value	0.4028	0.3626	0.9917	0.4272	0.5413
CDK N2A	rs1081 166	W	CRAE	ref genotype	163.13 (162.33, 163.93)	161.99 (161, 162.97)	164.25 (160.87, 167.63)	164.81 (162.97, 166.65)	162.71 (161.79, 163.62)
				het genotype	163.16 (162.31, 164.02)	162.41 (161.31, 163.5)	164.04 (160.64, 167.44)	165.31 (163.24, 167.37)	162.67 (161.7, 163.63)
				hom genotype	163.19 (162, 164.39)	162.83 (161.12, 164.53)	163.82 (160.26, 167.38)	165.8 (162.65, 168.94)	162.63 (161.32, 163.94)
				p value	0.9111	0.3364	0.5568	0.5311	0.8945
CDK N2A	rs1081 166	W	CRVE	ref genotype	197.52 (196.72, 198.33)	198.65 (197.64, 199.65)	196.97 (193.59, 200.34)	197.92 (196, 199.85)	197.65 (196.75, 198.56)
				het genotype	197.29 (196.43, 198.15)	197.96 (196.85, 199.07)	197.04 (193.65, 200.43)	196.76 (194.6, 198.91)	197.61 (196.65, 198.57)
				hom genotype	197.06 (195.87, 198.26)	197.27 (195.55, 199)	197.11 (193.56, 200.66)	195.59 (192.3, 198.88)	197.56 (196.26, 198.86)
				p value	0.4103	0.1203	0.8441	0.1565	0.8805
CDK N2A	rs1081 166	W	retinopathy	het vs. ref	1.08 (0.91, 1.29)	1.02 (0.81, 1.29)	1.16 (0.9, 1.5)	0.87 (0.63, 1.21)	1.25 (1.01, 1.53)
				hom vs. ref	1.17 (0.83, 1.66)	1.04 (0.65, 1.65)	1.35 (0.81, 2.26)	0.76 (0.39, 1.47)	1.55 (1.03, 2.34)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				p value	0.3604	0.8724	0.2469	0.4157	0.0362
CDKN2A	rs1081166	W	A/V nicking	het vs. ref	0.93 (0.82, 1.06)	0.93 (0.78, 1.11)	0.95 (0.79, 1.13)	0.9 (0.65, 1.24)	0.94 (0.82, 1.08)
				hom vs. ref	0.87 (0.68, 1.12)	0.86 (0.6, 1.23)	0.9 (0.63, 1.28)	0.81 (0.43, 1.54)	0.89 (0.68, 1.17)
				p value	0.2869	0.4047	0.5542	0.5239	0.3943
CDKN2A	rs1081166	W	Focal narrowing	het vs. ref	1.08 (0.96, 1.22)	1.12 (0.95, 1.32)	1.06 (0.89, 1.27)	1.21 (0.87, 1.69)	1.06 (0.93, 1.21)
				hom vs. ref	1.18 (0.92, 1.5)	1.25 (0.9, 1.73)	1.13 (0.79, 1.62)	1.47 (0.76, 2.85)	1.13 (0.87, 1.47)
				p value	0.1877	0.1811	0.5128	0.2478	0.3642
CDKN2A	rs1081166	B	CRAE	ref genotype	162.07 (160.6, 163.54)	160.91 (159.21, 162.6)	158.38 (150.48, 166.28)	164.52 (161.96, 167.07)	161.12 (159.23, 163)
				het genotype	163.13 (161.03, 165.22)	162.28 (159.73, 164.83)	158.59 (150.16, 167.03)	162.94 (159.14, 166.73)	163.23 (160.63, 165.83)
				hom genotype	164.19 (160.67, 167.7)	163.65 (159.3, 168.01)	158.81 (149.01, 168.6)	161.36 (154.88, 167.83)	165.35 (161.1, 169.59)
				p value	0.2187	0.2013	0.8813	0.3231	0.0391
CDKN2A	rs1081166	B	CRVE	ref genotype	199.98 (198.48, 201.48)	201.14 (199.43, 202.85)	204.75 (196.46, 213.03)	200.67 (197.89, 203.45)	199.02 (197.13, 200.9)
				het genotype	199 (196.81, 201.18)	199.73 (197.07, 202.38)	204.84 (195.99, 213.7)	202.3 (198.13, 206.48)	197.12 (194.47, 199.76)
				hom genotype	198.01 (194.32, 201.71)	198.31 (193.72, 202.9)	204.94 (194.64, 215.24)	203.93 (196.72, 211.15)	195.21 (190.87, 199.56)
				p value	0.2792	0.2127	0.9485	0.3647	0.0695
CDKN2A	rs1081166	B	retinopathy	het vs. ref	1.11 (0.77, 1.6)	1.06 (0.7, 1.62)	1.13 (0.53, 2.43)	1.39 (0.82, 2.36)	0.89 (0.51, 1.56)
				hom vs. ref	1.23 (0.59, 2.56)	1.13 (0.48, 2.62)	1.28 (0.28, 5.9)	1.92 (0.67, 5.56)	0.8 (0.26, 2.44)
				p value	0.5767	0.7825	0.7541	0.2267	0.6890
CDKN2A	rs1081166	B	A/V nicking	het vs. ref	0.93 (0.65, 1.33)	0.8 (0.52, 1.24)	1.44 (0.76, 2.72)	0.95 (0.5, 1.83)	0.94 (0.61, 1.44)
				hom vs. ref	0.87 (0.43, 1.77)	0.64 (0.27, 1.53)	2.08 (0.58, 7.42)	0.91 (0.25, 3.34)	0.88 (0.38, 2.08)
				p value	0.6992	0.3174	0.2609	0.8836	0.7785
CDKN2A	rs1081166	B	Focal narrowing	het vs. ref	1.17 (0.8, 1.71)	1.22 (0.8, 1.87)	0.92 (0.37, 2.26)	1.39 (0.69, 2.77)	1.1 (0.7, 1.73)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				hom vs. ref	1.37 (0.64, 2.92)	1.5 (0.65, 3.49)	0.84 (0.14, 5.11)	1.92 (0.48, 7.67)	1.21 (0.49, 2.99)
				p value	0.4197	0.3461	0.8513	0.3540	0.6867
IGF2 BP	rs4402 960	W	CRAE	ref genotype	163.3 (162.3, 164.3)	162.51 (161.13, 163.89)	164.14 (160.67, 167.61)	163.57 (161.23, 165.91)	163.15 (162.03, 164.27)
				het genotype	163.15 (162.34, 163.95)	162.25 (161.25, 163.24)	164.1 (160.72, 167.47)	164.61 (162.79, 166.44)	162.8 (161.88, 163.71)
				hom genotype	162.99 (162.16, 163.82)	161.98 (160.94, 163.02)	164.05 (160.67, 167.44)	165.65 (163.69, 167.62)	162.44 (161.5, 163.38)
				p value	0.4986	0.4616	0.8855	0.0784	0.1560
IGF2 BP	rs4402 960	W	CRVE	ref genotype	197.12 (196.11, 198.12)	197.57 (196.18, 198.97)	197.09 (193.63, 200.55)	197.76 (195.3, 200.21)	197.16 (196.04, 198.27)
				het genotype	197.35 (196.55, 198.16)	198.09 (197.07, 199.1)	197.08 (193.71, 200.45)	197.6 (195.69, 199.5)	197.48 (196.56, 198.39)
				hom genotype	197.59 (196.75, 198.42)	198.6 (197.54, 199.66)	197.07 (193.69, 200.45)	197.44 (195.38, 199.49)	197.8 (196.86, 198.73)
				p value	0.3088	0.1575	0.9753	0.7964	0.1980
IGF2 BP	rs4402 960	W	retinopathy	het vs. ref	1.08 (0.94, 1.25)	1.09 (0.9, 1.32)	1.07 (0.86, 1.34)	1.08 (0.85, 1.37)	1.04 (0.86, 1.24)
				hom vs. ref	1.17 (0.88, 1.56)	1.19 (0.82, 1.75)	1.15 (0.74, 1.79)	1.16 (0.71, 1.88)	1.08 (0.75, 1.55)
				p value	0.2755	0.3622	0.5379	0.5531	0.6943
IGF2 BP	rs4402 960	W	A/V nicking	het vs. ref	0.98 (0.88, 1.08)	0.93 (0.8, 1.07)	1.05 (0.91, 1.21)	0.86 (0.68, 1.09)	1.01 (0.9, 1.13)
				hom vs. ref	0.96 (0.78, 1.17)	0.86 (0.64, 1.14)	1.1 (0.83, 1.47)	0.74 (0.46, 1.2)	1.01 (0.81, 1.27)
				p value	0.6791	0.2947	0.5100	0.2195	0.9245
IGF2 BP	rs4402 960	W	Focal narrowing	het vs. ref	1 (0.9, 1.11)	0.97 (0.84, 1.11)	1.05 (0.9, 1.22)	0.93 (0.72, 1.2)	1.02 (0.91, 1.14)
				hom vs. ref	1 (0.82, 1.23)	0.94 (0.71, 1.23)	1.1 (0.81, 1.49)	0.86 (0.52, 1.43)	1.03 (0.83, 1.29)
				p value	0.9881	0.6294	0.5437	0.5579	0.7658
IGF2 BP	rs4402 960	B	CRAE	ref genotype	161.84 (160.13, 163.55)	160.43 (158.42, 162.44)	158.45 (150.44, 166.45)	163.27 (160.27, 166.26)	161.45 (159.28, 163.61)
				het genotype	161.87 (160.39, 163.34)	160.67 (158.95, 162.38)	157.81 (149.96, 165.65)	163.75 (161.17, 166.32)	161.19 (159.3, 163.08)
				hom genotype	161.89 (160.2, 163.59)	160.9 (158.88, 162.93)	157.17 (149.23, 165.1)	164.22 (161.24, 167.21)	160.94 (158.79, 163.08)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				p value	0.9536	0.6611	0.3768	0.5374	0.6283
IGF2 BP	rs4402960	B	CRVE	ref genotype	200.03 (198.26, 201.81)	201.52 (199.46, 203.58)	204.77 (196.27, 213.26)	201.89 (198.6, 205.19)	198.55 (196.36, 200.75)
				het genotype	200.07 (198.56, 201.59)	201.38 (199.64, 203.11)	205.18 (196.87, 213.5)	201.31 (198.49, 204.12)	198.96 (197.05, 200.86)
				hom genotype	200.11 (198.36, 201.87)	201.24 (199.16, 203.32)	205.6 (197.18, 214.01)	200.72 (197.4, 204.03)	199.36 (197.2, 201.53)
				p value	0.9302	0.8090	0.5909	0.5048	0.4563
IGF2 BP	rs4402960	B	retinopathy	het vs. ref	0.82 (0.68, 0.99)	0.76 (0.61, 0.94)	1.05 (0.7, 1.57)	0.64 (0.48, 0.85)	1.06 (0.81, 1.4)
				hom vs. ref	0.67 (0.46, 0.98)	0.57 (0.37, 0.89)	1.1 (0.49, 2.45)	0.41 (0.23, 0.71)	1.13 (0.65, 1.95)
				p value	0.0396	0.0126	0.8216	0.0017	0.6734
IGF2 BP	rs4402960	B	A/V nicking	het vs. ref	0.92 (0.77, 1.09)	0.9 (0.73, 1.11)	0.97 (0.7, 1.35)	0.82 (0.6, 1.12)	0.97 (0.79, 1.2)
				hom vs. ref	0.84 (0.59, 1.2)	0.81 (0.54, 1.23)	0.94 (0.49, 1.82)	0.67 (0.36, 1.26)	0.94 (0.62, 1.44)
				p value	0.3419	0.3286	0.8572	0.2111	0.7887
IGF2 BP	rs4402960	B	Focal narrowing	het vs. ref	1.09 (0.89, 1.33)	1.13 (0.9, 1.42)	0.95 (0.62, 1.45)	1.15 (0.79, 1.69)	1.07 (0.84, 1.36)
				hom vs. ref	1.19 (0.79, 1.78)	1.28 (0.81, 2.03)	0.9 (0.38, 2.12)	1.33 (0.62, 2.85)	1.15 (0.71, 1.85)
				p value	0.4028	0.2928	0.8084	0.4588	0.5759
CDKAL1	rs7754840	W	CRAE	ref genotype	162.86 (162.03, 163.69)	162.17 (161.13, 163.21)	163.74 (160.35, 167.13)	165.13 (163.22, 167.04)	162.35 (161.41, 163.29)
				het genotype	163.19 (162.39, 163.99)	162.21 (161.23, 163.19)	164.26 (160.89, 167.64)	164.68 (162.86, 166.5)	162.84 (161.93, 163.75)
				hom genotype	163.52 (162.52, 164.52)	162.25 (160.91, 163.6)	164.78 (161.32, 168.24)	164.24 (161.89, 166.59)	163.33 (162.2, 164.46)
				p value	0.1502	0.9052	0.0832	0.4361	0.0509
CDKAL1	rs7754840	W	CRVE	ref genotype	197.6 (196.77, 198.43)	198.54 (197.48, 199.61)	197.08 (193.69, 200.46)	197.57 (195.55, 199.58)	197.81 (196.88, 198.74)
				het genotype	197.41 (196.6, 198.21)	198.27 (197.27, 199.27)	196.93 (193.56, 200.3)	197.69 (195.77, 199.62)	197.54 (196.63, 198.45)
				hom genotype	197.21 (196.21, 198.22)	197.99 (196.62, 199.36)	196.79 (193.33, 200.25)	197.82 (195.34, 200.3)	197.27 (196.15, 198.4)
				p value	0.3995	0.4406	0.6316	0.8355	0.2819

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
CDKAL1	rs7754840	W	retinopathy	het vs. ref	1.01 (0.87, 1.16)	1.2 (1, 1.44)	0.75 (0.59, 0.95)	0.99 (0.78, 1.25)	0.99 (0.82, 1.19)
				hom vs. ref	1.01 (0.76, 1.35)	1.44 (1, 2.09)	0.56 (0.35, 0.91)	0.97 (0.6, 1.57)	0.97 (0.68, 1.41)
				p value	0.9444	0.0507	0.0181	0.9099	0.8920
CDKAL1	rs7754840	W	A/V nicking	het vs. ref	1.01 (0.92, 1.12)	0.93 (0.81, 1.08)	1.11 (0.96, 1.28)	1.08 (0.86, 1.35)	1 (0.9, 1.12)
				hom vs. ref	1.03 (0.84, 1.26)	0.87 (0.66, 1.16)	1.22 (0.92, 1.63)	1.16 (0.73, 1.83)	1 (0.8, 1.26)
				p value	0.7739	0.3465	0.1701	0.5304	0.9762
CDKAL1	rs7754840	W	Focal narrowing	het vs. ref	0.91 (0.82, 1)	0.9 (0.79, 1.03)	0.92 (0.79, 1.07)	0.71 (0.55, 0.93)	0.95 (0.85, 1.06)
				hom vs. ref	0.82 (0.67, 1.01)	0.81 (0.62, 1.06)	0.85 (0.63, 1.16)	0.51 (0.3, 0.86)	0.91 (0.73, 1.13)
				p value	0.0620	0.1284	0.3016	0.0114	0.3949
CDKAL1	rs7754840	B	CRAE	ref genotype	161.67 (159.92, 163.42)	160.49 (158.4, 162.59)	157.46 (149.39, 165.54)	165.6 (162.46, 168.73)	160.27 (158.06, 162.47)
				het genotype	161.99 (160.53, 163.45)	160.79 (159.09, 162.49)	157.95 (150.08, 165.83)	164.36 (161.81, 166.91)	161.11 (159.23, 162.99)
				hom genotype	162.31 (160.68, 163.94)	161.08 (159.17, 162.98)	158.45 (150.52, 166.38)	163.12 (160.2, 166.03)	161.95 (159.9, 164)
				p value	0.4629	0.5884	0.4959	0.1347	0.0997
CDKAL1	rs7754840	B	CRVE	ref genotype	199.81 (198, 201.62)	200.68 (198.51, 202.84)	205.5 (196.99, 214)	200.26 (196.8, 203.73)	198.96 (196.73, 201.2)
				het genotype	199.92 (198.42, 201.41)	201.09 (199.37, 202.81)	204.84 (196.54, 213.13)	200.93 (198.16, 203.7)	198.93 (197.04, 200.82)
				hom genotype	200.03 (198.35, 201.71)	201.51 (199.57, 203.44)	204.18 (195.83, 212.54)	201.6 (198.43, 204.77)	198.89 (196.82, 200.96)
				p value	0.8106	0.4664	0.3929	0.4738	0.9443
CDKAL1	rs7754840	B	retinopathy	het vs. ref	1.06 (0.88, 1.28)	1 (0.81, 1.24)	1.24 (0.84, 1.84)	1 (0.75, 1.33)	1.18 (0.9, 1.54)
				hom vs. ref	1.13 (0.78, 1.65)	1 (0.65, 1.54)	1.54 (0.7, 3.39)	1 (0.56, 1.78)	1.39 (0.82, 2.36)
				p value	0.5189	0.9872	0.2792	0.9977	0.2223
CDKAL1	rs7754840	B	A/V nicking	het vs. ref	0.95 (0.8, 1.13)	0.91 (0.74, 1.12)	1.06 (0.76, 1.48)	0.88 (0.63, 1.23)	0.98 (0.8, 1.21)
				hom vs. ref	0.9 (0.63, 1.27)	0.84 (0.55, 1.26)	1.13 (0.58, 2.2)	0.77 (0.39, 1.51)	0.96 (0.64, 1.45)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				p value	0.5461	0.3966	0.7245	0.4492	0.8552
CDKAL1	rs7754840	B	Focal narrowing	het vs. ref	0.94 (0.77, 1.14)	0.97 (0.78, 1.22)	0.85 (0.55, 1.31)	0.82 (0.55, 1.22)	0.98 (0.78, 1.23)
				hom vs. ref	0.88 (0.59, 1.31)	0.95 (0.6, 1.49)	0.72 (0.3, 1.72)	0.67 (0.3, 1.49)	0.95 (0.6, 1.51)
				p value	0.5233	0.8140	0.4591	0.3222	0.8407
HHEX	rs1111875	W	CRAE	ref genotype	163.25 (162.39, 164.1)	162.38 (161.29, 163.47)	164.25 (160.75, 167.75)	164.52 (162.53, 166.5)	162.94 (161.97, 163.91)
				het genotype	163.03 (162.24, 163.81)	162.09 (161.14, 163.04)	164.09 (160.61, 167.57)	164.77 (162.97, 166.58)	162.65 (161.76, 163.54)
				hom genotype	162.8 (161.88, 163.73)	161.8 (160.57, 163.02)	163.92 (160.38, 167.47)	165.03 (162.76, 167.31)	162.36 (161.32, 163.39)
				p value	0.3046	0.3919	0.5590	0.6587	0.2111
HHEX	rs1111875	W	CRVE	ref genotype	197.13 (196.28, 197.99)	198.15 (197.04, 199.26)	196.25 (192.75, 199.75)	197.4 (195.31, 199.49)	197.25 (196.29, 198.22)
				het genotype	197.51 (196.72, 198.3)	198.5 (197.52, 199.47)	196.64 (193.16, 200.12)	197.71 (195.81, 199.61)	197.66 (196.77, 198.55)
				hom genotype	197.89 (196.96, 198.82)	198.85 (197.59, 200.1)	197.02 (193.48, 200.57)	198.02 (195.62, 200.42)	198.07 (197.04, 199.1)
				p value	0.0835	0.3090	0.1668	0.6129	0.0788
HHEX	rs1111875	W	retinopathy	het vs. ref	1.04 (0.91, 1.2)	1.03 (0.86, 1.24)	1.08 (0.88, 1.33)	1.06 (0.83, 1.34)	1.07 (0.91, 1.27)
				hom vs. ref	1.09 (0.83, 1.43)	1.06 (0.74, 1.53)	1.17 (0.77, 1.78)	1.12 (0.69, 1.81)	1.15 (0.82, 1.62)
				p value	0.5380	0.7461	0.4598	0.6527	0.4152
HHEX	rs1111875	W	A/V nicking	het vs. ref	0.89 (0.81, 0.98)	0.81 (0.71, 0.93)	0.99 (0.86, 1.13)	0.87 (0.68, 1.1)	0.9 (0.81, 1)
				hom vs. ref	0.8 (0.66, 0.97)	0.66 (0.5, 0.87)	0.97 (0.74, 1.28)	0.75 (0.47, 1.22)	0.81 (0.65, 1)
				p value	0.0212	0.0030	0.8452	0.2471	0.0454
HHEX	rs1111875	W	Focal narrowing	het vs. ref	0.94 (0.86, 1.04)	0.92 (0.8, 1.04)	0.98 (0.86, 1.13)	0.85 (0.66, 1.11)	0.96 (0.87, 1.07)
				hom vs. ref	0.89 (0.74, 1.08)	0.84 (0.65, 1.08)	0.97 (0.73, 1.28)	0.73 (0.43, 1.22)	0.92 (0.75, 1.13)
				p value	0.2356	0.1799	0.8233	0.2295	0.4536
HHEX	rs1111875	B	CRAE	ref genotype	162.07 (160.55, 163.59)	160.83 (159.06, 162.59)	157.93 (149.91, 165.95)	164.52 (161.9, 167.14)	161.02 (159.06, 162.98)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				het genotype	162.11 (160.53, 163.7)	161.02 (159.18, 162.85)	157.74 (149.73, 165.76)	163.9 (161.09, 166.71)	161.43 (159.42, 163.43)
				hom genotype	162.15 (159.97, 164.33)	161.21 (158.64, 163.77)	157.56 (149.15, 165.97)	163.29 (159.37, 167.21)	161.84 (159.16, 164.51)
				p value	0.9379	0.7592	0.8434	0.5018	0.5111
HHEX	rs1111875	B	CRVE	ref genotype	199.86 (198.3, 201.42)	200.94 (199.15, 202.74)	204.84 (196.45, 213.23)	200.58 (197.73, 203.43)	199.07 (197.1, 201.03)
				het genotype	200.1 (198.48, 201.73)	201.17 (199.3, 203.04)	204.9 (196.51, 213.29)	201.62 (198.58, 204.66)	198.9 (196.88, 200.92)
				hom genotype	200.35 (198.08, 202.61)	201.4 (198.74, 204.07)	204.97 (196.16, 213.77)	202.65 (198.35, 206.95)	198.74 (196.02, 201.46)
				p value	0.6529	0.7262	0.9486	0.3144	0.7962
HHEX	rs1111875	B	retinopathy	het vs. ref	0.91 (0.73, 1.14)	0.94 (0.73, 1.21)	0.85 (0.5, 1.45)	1 (0.73, 1.37)	0.86 (0.62, 1.2)
				hom vs. ref	0.83 (0.53, 1.3)	0.88 (0.53, 1.46)	0.72 (0.25, 2.1)	1 (0.53, 1.89)	0.75 (0.39, 1.45)
				p value	0.4078	0.6174	0.5515	0.9933	0.3889
HHEX	rs1111875	B	A/V nicking	het vs. ref	0.93 (0.75, 1.14)	0.96 (0.76, 1.22)	0.79 (0.51, 1.22)	1.27 (0.89, 1.79)	0.77 (0.6, 1)
				hom vs. ref	0.86 (0.57, 1.3)	0.93 (0.58, 1.49)	0.62 (0.26, 1.5)	1.6 (0.8, 3.22)	0.6 (0.36, 1)
				p value	0.4680	0.7630	0.2928	0.1842	0.0511
HHEX	rs1111875	B	Focal narrowing	het vs. ref	1.1 (0.88, 1.38)	1.15 (0.89, 1.47)	0.88 (0.51, 1.5)	1.2 (0.79, 1.82)	1.07 (0.81, 1.4)
				hom vs. ref	1.2 (0.77, 1.89)	1.32 (0.8, 2.17)	0.77 (0.26, 2.25)	1.44 (0.62, 3.31)	1.14 (0.66, 1.96)
				p value	0.4201	0.2850	0.6302	0.3970	0.6405
SLC30A	rs1326663	W	CRAE	ref genotype	163.23 (162.39, 164.06)	162.07 (161.03, 163.12)	164.56 (161.16, 167.96)	164.8 (162.89, 166.71)	162.82 (161.87, 163.77)
				het genotype	162.95 (162.15, 163.75)	162.11 (161.12, 163.1)	164.01 (160.63, 167.39)	165.09 (163.24, 166.94)	162.48 (161.56, 163.39)
				hom genotype	162.67 (161.67, 163.67)	162.15 (160.79, 163.52)	163.46 (160, 166.92)	165.38 (162.92, 167.84)	162.13 (161.02, 163.24)
				p value	0.2294	0.9139	0.0658	0.6307	0.1650
SLC30A	rs1326663	W	CRVE	ref genotype	197.45 (196.61, 198.29)	198.54 (197.48, 199.6)	196.74 (193.35, 200.13)	198 (195.99, 200.01)	197.55 (196.61, 198.5)
				het genotype	197.62 (196.81, 198.42)	198.51 (197.5, 199.51)	197.09 (193.72, 200.47)	197.46 (195.51, 199.4)	197.85 (196.94, 198.75)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				hom genotype	197.78 (196.78, 198.78)	198.47 (197.09, 199.86)	197.44 (193.99, 200.9)	196.92 (194.33, 199.5)	198.14 (197.03, 199.24)
				p value	0.4719	0.9273	0.2367	0.3984	0.2380
SLC 30A	rs1326663	W	retinopathy	het vs. ref	0.97 (0.84, 1.12)	1 (0.83, 1.21)	0.92 (0.73, 1.14)	1 (0.78, 1.28)	1 (0.83, 1.19)
				hom vs. ref	0.94 (0.71, 1.26)	1 (0.68, 1.46)	0.84 (0.54, 1.31)	0.99 (0.6, 1.63)	0.99 (0.69, 1.42)
				p value	0.6979	0.9972	0.4385	0.9775	0.9725
SLC 30A	rs1326663	W	A/V nicking	het vs. ref	0.99 (0.89, 1.1)	0.95 (0.82, 1.09)	1.03 (0.9, 1.19)	0.93 (0.73, 1.19)	1 (0.9, 1.12)
				hom vs. ref	0.98 (0.8, 1.2)	0.9 (0.67, 1.2)	1.07 (0.8, 1.42)	0.87 (0.53, 1.42)	1 (0.8, 1.25)
				p value	0.8489	0.4675	0.6506	0.5695	0.9862
SLC 30A	rs1326663	W	Focal narrowing	het vs. ref	1.15 (1.05, 1.28)	1.1 (0.96, 1.26)	1.23 (1.06, 1.42)	1.07 (0.83, 1.39)	1.17 (1.05, 1.3)
				hom vs. ref	1.33 (1.09, 1.63)	1.21 (0.92, 1.58)	1.51 (1.12, 2.03)	1.15 (0.69, 1.94)	1.36 (1.1, 1.69)
				p value	0.0044	0.1642	0.0060	0.5883	0.0049
SLC 30A	rs1326663	B	CRAE	ref genotype	162.3 (160.8, 163.81)	161.03 (159.3, 162.75)	158.3 (150.34, 166.26)	164.66 (162.07, 167.25)	161.39 (159.44, 163.33)
				het genotype	161.41 (159.47, 163.35)	159.78 (157.47, 162.1)	158.31 (149.94, 166.68)	163.42 (160, 166.83)	160.57 (158.13, 163)
				hom genotype	160.51 (157.31, 163.7)	158.54 (154.64, 162.44)	158.33 (148.76, 167.9)	162.17 (156.37, 167.98)	159.75 (155.87, 163.63)
				p value	0.2631	0.2072	0.9914	0.4049	0.3901
SLC 30A	rs1326663	B	CRVE	ref genotype	199.46 (197.92, 201)	200.63 (198.87, 202.38)	204.61 (196.35, 212.86)	200.18 (197.32, 203.04)	198.74 (196.8, 200.69)
				het genotype	200.44 (198.44, 202.45)	202.25 (199.86, 204.63)	204.33 (195.64, 213.02)	201.73 (197.95, 205.51)	199.5 (197.05, 201.94)
				hom genotype	201.42 (198.08, 204.76)	203.87 (199.78, 207.95)	204.05 (194.1, 214)	203.28 (196.76, 209.8)	200.25 (196.32, 204.19)
				p value	0.2470	0.1217	0.8482	0.3607	0.4383
SLC 30A	rs1326663	B	retinopathy	het vs. ref	1.16 (0.83, 1.62)	1.34 (0.93, 1.93)	0.68 (0.29, 1.58)	1.51 (0.92, 2.5)	1 (0.61, 1.64)
				hom vs. ref	1.35 (0.69, 2.63)	1.79 (0.86, 3.73)	0.46 (0.09, 2.49)	2.29 (0.84, 6.26)	1 (0.37, 2.69)
				p value	0.3756	0.1191	0.3709	0.1059	0.9947

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
SLC 30A	rs1326663	B	A/V nicking	het vs. ref	0.77 (0.54, 1.09)	0.84 (0.57, 1.26)	0.59 (0.29, 1.21)	0.56 (0.28, 1.13)	0.83 (0.56, 1.25)
				hom vs. ref	0.59 (0.3, 1.19)	0.71 (0.32, 1.58)	0.35 (0.09, 1.46)	0.31 (0.08, 1.29)	0.7 (0.31, 1.56)
				p value	0.1414	0.4042	0.1517	0.1074	0.3777
SLC 30A	rs1326663	B	Focal narrowing	het vs. ref	1.02 (0.7, 1.47)	1.09 (0.72, 1.65)	0.84 (0.36, 1.94)	0.64 (0.27, 1.56)	1.14 (0.76, 1.71)
				hom vs. ref	1.04 (0.5, 2.16)	1.19 (0.52, 2.72)	0.7 (0.13, 3.77)	0.42 (0.07, 2.43)	1.3 (0.57, 2.93)
				p value	0.9232	0.6730	0.6784	0.3291	0.5346
FTO	rs1225537	W	CRAE	ref genotype	163.05 (162.22, 163.88)	162.06 (161.03, 163.09)	164.28 (160.88, 167.68)	164.44 (162.48, 166.41)	162.75 (161.81, 163.69)
				het genotype	162.9 (162.09, 163.72)	162.02 (161.01, 163.04)	164.06 (160.68, 167.44)	164.69 (162.85, 166.52)	162.47 (161.54, 163.41)
				hom genotype	162.76 (161.72, 163.8)	161.99 (160.55, 163.43)	163.84 (160.37, 167.3)	164.93 (162.56, 167.3)	162.2 (161.03, 163.37)
				p value	0.5436	0.9261	0.4711	0.6850	0.2917
FTO	rs1225537	W	CRVE	ref genotype	197.57 (196.74, 198.4)	198.54 (197.49, 199.59)	196.94 (193.55, 200.34)	198.11 (196.05, 200.18)	197.7 (196.76, 198.63)
				het genotype	197.51 (196.69, 198.33)	198.51 (197.47, 199.54)	196.89 (193.52, 200.26)	197.69 (195.77, 199.61)	197.67 (196.74, 198.6)
				hom genotype	197.45 (196.4, 198.49)	198.47 (197, 199.94)	196.83 (193.38, 200.29)	197.27 (194.79, 199.75)	197.64 (196.47, 198.81)
				p value	0.7969	0.9228	0.8587	0.5017	0.9092
FTO	rs1225537	W	retinopathy	het vs. ref	1.06 (0.91, 1.23)	0.94 (0.77, 1.15)	1.21 (0.97, 1.51)	1 (0.78, 1.28)	1 (0.83, 1.21)
				hom vs. ref	1.12 (0.84, 1.5)	0.88 (0.59, 1.32)	1.47 (0.95, 2.28)	1 (0.61, 1.64)	1 (0.69, 1.46)
				p value	0.4473	0.5450	0.0873	0.9882	0.9950
FTO	rs1225537	W	A/V nicking	het vs. ref	1.05 (0.95, 1.17)	1.14 (0.99, 1.32)	0.99 (0.85, 1.14)	0.98 (0.77, 1.25)	1.07 (0.95, 1.2)
				hom vs. ref	1.11 (0.9, 1.37)	1.3 (0.97, 1.74)	0.97 (0.72, 1.31)	0.96 (0.59, 1.55)	1.15 (0.91, 1.45)
				p value	0.3226	0.0783	0.8493	0.8630	0.2452
FTO	rs1225537	W	Focal narrowing	het vs. ref	1.08 (0.97, 1.2)	1.23 (1.07, 1.41)	0.92 (0.78, 1.07)	0.83 (0.63, 1.08)	1.15 (1.03, 1.29)
				hom vs. ref	1.16 (0.95, 1.43)	1.51 (1.14, 1.99)	0.84 (0.61, 1.15)	0.68 (0.4, 1.17)	1.33 (1.06, 1.66)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				p value	0.1517	0.0038	0.2709	0.1638	0.0138
FTO	rs1225537	B	CRAE	ref genotype	161.49 (159.93, 163.04)	160.23 (158.41, 162.05)	157.4 (149.52, 165.28)	163.62 (160.94, 166.3)	160.69 (158.69, 162.69)
				het genotype	162.1 (160.59, 163.62)	160.88 (159.12, 162.65)	158.12 (150.29, 165.94)	163.7 (161.04, 166.36)	161.61 (159.67, 163.55)
				hom genotype	162.72 (160.76, 164.68)	161.54 (159.2, 163.88)	158.83 (150.77, 166.89)	163.78 (160.22, 167.34)	162.53 (160.1, 164.96)
				p value	0.1846	0.2609	0.3571	0.9246	0.0986
FTO	rs1225537	B	CRVE	ref genotype	200.36 (198.76, 201.96)	201.58 (199.74, 203.43)	204.94 (196.6, 213.27)	201.43 (198.49, 204.36)	199.31 (197.31, 201.31)
				het genotype	199.83 (198.27, 201.39)	200.92 (199.13, 202.71)	204.53 (196.26, 212.81)	200.96 (198.03, 203.89)	198.7 (196.76, 200.65)
				hom genotype	199.3 (197.26, 201.35)	200.26 (197.83, 202.69)	204.13 (195.6, 212.66)	200.5 (196.51, 204.48)	198.09 (195.63, 200.55)
				p value	0.2857	0.2817	0.6276	0.6353	0.2852
FTO	rs1225537	B	retinopathy	het vs. ref	1.11 (0.91, 1.35)	1.12 (0.89, 1.4)	1.07 (0.7, 1.65)	1.09 (0.81, 1.47)	1.11 (0.83, 1.47)
				hom vs. ref	1.23 (0.82, 1.83)	1.25 (0.79, 1.97)	1.15 (0.49, 2.71)	1.19 (0.65, 2.15)	1.22 (0.69, 2.17)
				p value	0.3105	0.3364	0.7527	0.5746	0.4925
FTO	rs1225537	B	A/V nicking	het vs. ref	1 (0.83, 1.21)	1.09 (0.87, 1.35)	0.78 (0.54, 1.14)	1.08 (0.76, 1.52)	0.97 (0.77, 1.21)
				hom vs. ref	1 (0.69, 1.46)	1.18 (0.76, 1.83)	0.61 (0.29, 1.29)	1.16 (0.58, 2.32)	0.94 (0.6, 1.47)
				p value	0.9825	0.4540	0.1978	0.6721	0.7727
FTO	rs1225537	B	Focal narrowing	het vs. ref	0.99 (0.8, 1.22)	1.08 (0.85, 1.38)	0.67 (0.41, 1.09)	1.12 (0.75, 1.67)	0.94 (0.73, 1.22)
				hom vs. ref	0.98 (0.64, 1.5)	1.18 (0.73, 1.9)	0.45 (0.17, 1.2)	1.25 (0.56, 2.8)	0.89 (0.54, 1.48)
				p value	0.9176	0.5099	0.1101	0.5827	0.6538
KCNJ11	rs5219	W	CRAE	ref genotype	163.27 (162.43, 164.11)	162.13 (161.05, 163.21)	164.29 (160.89, 167.68)	165.53 (163.5, 167.56)	162.75 (161.8, 163.7)
				het genotype	163.25 (162.46, 164.04)	162.21 (161.25, 163.16)	164.18 (160.81, 167.56)	165.11 (163.32, 166.91)	162.79 (161.89, 163.69)
				hom genotype	163.23 (162.28, 164.19)	162.29 (161.03, 163.54)	164.08 (160.63, 167.52)	164.7 (162.46, 166.94)	162.83 (161.75, 163.9)
				p value	0.9336	0.8182	0.7112	0.4806	0.8664

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
KCNJ11	rs5219	W	CRVE	ref genotype	197.8 (196.95, 198.64)	198.91 (197.81, 200)	197.27 (193.88, 200.67)	197.73 (195.59, 199.86)	198.05 (197.11, 199)
				het genotype	197.42 (196.63, 198.22)	198.4 (197.42, 199.37)	197.01 (193.63, 200.38)	197.59 (195.7, 199.47)	197.61 (196.71, 198.51)
				hom genotype	197.05 (196.09, 198.01)	197.89 (196.61, 199.16)	196.74 (193.29, 200.19)	197.45 (195.1, 199.8)	197.17 (196.1, 198.24)
				p value	0.0917	0.1443	0.3490	0.8226	0.0616
KCNJ11	rs5219	W	retinopathy	het vs. ref	1.06 (0.92, 1.21)	0.95 (0.79, 1.15)	1.19 (0.97, 1.47)	1.04 (0.81, 1.32)	1.05 (0.89, 1.25)
				hom vs. ref	1.12 (0.85, 1.47)	0.91 (0.63, 1.32)	1.42 (0.94, 2.15)	1.07 (0.66, 1.74)	1.11 (0.79, 1.56)
				p value	0.4242	0.6081	0.0990	0.7797	0.5563
KCNJ11	rs5219	W	A/V nicking	het vs. ref	1 (0.91, 1.11)	1 (0.88, 1.15)	1 (0.87, 1.15)	1.15 (0.91, 1.45)	0.98 (0.88, 1.09)
				hom vs. ref	1.01 (0.83, 1.22)	1.01 (0.77, 1.32)	1 (0.76, 1.31)	1.32 (0.83, 2.1)	0.95 (0.77, 1.18)
				p value	0.9323	0.9542	0.9908	0.2441	0.6619
KCNJ11	rs5219	W	Focal narrowing	het vs. ref	1.02 (0.92, 1.12)	0.95 (0.84, 1.09)	1.1 (0.96, 1.27)	0.96 (0.74, 1.24)	1.03 (0.93, 1.14)
				hom vs. ref	1.03 (0.85, 1.25)	0.91 (0.7, 1.18)	1.22 (0.92, 1.62)	0.92 (0.55, 1.54)	1.06 (0.86, 1.3)
				p value	0.7411	0.4735	0.1725	0.7529	0.5912
KCNJ11	rs5219	B	CRAE	ref genotype	161.95 (160.43, 163.46)	160.78 (159.02, 162.54)	157.89 (149.97, 165.82)	164.08 (161.5, 166.65)	161.15 (159.19, 163.11)
				het genotype	163.14 (161.09, 165.18)	161.73 (159.19, 164.27)	159.33 (151.22, 167.44)	165.31 (161.58, 169.05)	162.45 (159.9, 164.99)
				hom genotype	164.33 (160.93, 167.73)	162.68 (158.34, 167.02)	160.76 (151.69, 169.83)	166.55 (160.25, 172.85)	163.75 (159.62, 167.88)
				p value	0.1601	0.3810	0.2816	0.4240	0.2014
KCNJ11	rs5219	B	CRVE	ref genotype	200.26 (198.72, 201.81)	201.36 (199.59, 203.13)	205.28 (196.9, 213.67)	200.9 (198.12, 203.68)	199.39 (197.43, 201.36)
				het genotype	198.77 (196.64, 200.9)	200 (197.37, 202.64)	203.5 (194.91, 212.09)	202.17 (198.07, 206.27)	196.68 (194.09, 199.27)
				hom genotype	197.27 (193.69, 200.85)	198.65 (194.09, 203.21)	201.72 (192.08, 211.36)	203.44 (196.43, 210.46)	193.97 (189.74, 198.2)
				p value	0.0939	0.2378	0.2090	0.4641	0.0091
KCNJ11	rs5219	B	retinopathy	het vs. ref	0.99 (0.69, 1.43)	0.96 (0.63, 1.48)	1.05 (0.52, 2.12)	0.98 (0.58, 1.67)	1.02 (0.6, 1.73)

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
				hom vs. ref	0.99 (0.47, 2.06)	0.93 (0.39, 2.2)	1.09 (0.27, 4.48)	0.97 (0.33, 2.81)	1.04 (0.36, 3)
				p value	0.9723	0.8656	0.9023	0.9542	0.9370
KCNJ11	rs5219	B	A/V nicking	het vs. ref	1.12 (0.81, 1.56)	1.25 (0.85, 1.83)	0.85 (0.43, 1.68)	1.78 (1.03, 3.08)	0.9 (0.59, 1.39)
				hom vs. ref	1.27 (0.65, 2.45)	1.56 (0.72, 3.35)	0.73 (0.19, 2.84)	3.18 (1.07, 9.49)	0.82 (0.35, 1.92)
				p value	0.4853	0.2594	0.6468	0.0378	0.6396
KCNJ11	rs5219	B	Focal narrowing	het vs. ref	0.97 (0.66, 1.44)	0.97 (0.62, 1.53)	1 (0.44, 2.25)	1.02 (0.49, 2.1)	0.95 (0.59, 1.52)
				hom vs. ref	0.94 (0.43, 2.08)	0.95 (0.39, 2.33)	1 (0.2, 5.08)	1.04 (0.24, 4.41)	0.9 (0.35, 2.31)
				p value	0.8870	0.9078	0.9967	0.9624	0.8241
Intergenic	rs9300039	W	CRAE	ref genotype	163.11 (162.32, 163.9)	162.26 (161.3, 163.22)	164.05 (160.67, 167.43)	165.08 (163.28, 166.87)	162.61 (161.71, 163.51)
				het genotype	163.08 (162.09, 164.07)	161.67 (160.32, 163.02)	164.41 (160.96, 167.85)	164.59 (162.16, 167.03)	162.7 (161.59, 163.8)
				hom genotype	163.06 (161.5, 164.61)	161.08 (158.77, 163.39)	164.76 (161.02, 168.51)	164.11 (160.09, 168.13)	162.78 (161.09, 164.48)
				p value	0.9426	0.3077	0.4575	0.6253	0.8292
Intergenic	rs9300039	W	CRVE	ref genotype	197.52 (196.73, 198.31)	198.47 (197.49, 199.45)	197.12 (193.74, 200.49)	197.66 (195.78, 199.54)	197.72 (196.83, 198.62)
				het genotype	197.06 (196.07, 198.06)	198.11 (196.73, 199.48)	196.58 (193.14, 200.02)	196.86 (194.31, 199.42)	197.3 (196.2, 198.4)
				hom genotype	196.6 (195.05, 198.16)	197.74 (195.4, 200.09)	196.05 (192.31, 199.79)	196.07 (191.85, 200.29)	196.87 (195.18, 198.55)
				p value	0.2174	0.5357	0.2664	0.4448	0.2806
Intergenic	rs9300039	W	retinopathy	het vs. ref	1.1 (0.88, 1.37)	0.81 (0.58, 1.14)	1.42 (1.04, 1.93)	0.95 (0.63, 1.45)	1.2 (0.91, 1.57)
				hom vs. ref	1.2 (0.77, 1.88)	0.66 (0.34, 1.3)	2 (1.08, 3.71)	0.91 (0.39, 2.11)	1.43 (0.83, 2.45)
				p value	0.4263	0.2301	0.0272	0.8246	0.1957
Intergenic	rs9300039	W	A/V nicking	het vs. ref	0.92 (0.78, 1.08)	0.91 (0.72, 1.15)	0.93 (0.73, 1.18)	1.06 (0.71, 1.59)	0.89 (0.74, 1.07)
				hom vs. ref	0.84 (0.6, 1.17)	0.82 (0.51, 1.33)	0.86 (0.54, 1.38)	1.13 (0.51, 2.52)	0.79 (0.55, 1.15)
				p value	0.3073	0.4239	0.5333	0.7607	0.2158

Table 23. Retinal lesions, caliber and diabetes susceptibility polymorphisms, by hypertension and diabetes status in the ARIC Study.

					OR (95%CI) or Mean Caliber (95%CI) with P value				
Gene	**SNP**	**R**	**Phenotype**	**Geno-type**	**All**	**Hypertension Only**	**Non-Hypertension Only**	**Diabetes Only**	**Non-Diabetes only**
Intergenic	rs9300039	W	Focal narrowing	het vs. ref	0.98 (0.83, 1.15)	1.01 (0.81, 1.26)	0.94 (0.73, 1.2)	1.01 (0.65, 1.59)	0.97 (0.82, 1.16)
				hom vs. ref	0.95 (0.69, 1.32)	1.03 (0.66, 1.59)	0.88 (0.54, 1.43)	1.03 (0.42, 2.53)	0.94 (0.67, 1.34)
				p value	0.7649	0.9082	0.6023	0.9539	0.7487
Intergenic	rs9300039	B	CRAE	ref genotype	161.96 (160.46, 163.45)	160.79 (159.06, 162.51)	158.07 (150.16, 165.97)	163.94 (161.33, 166.55)	161.22 (159.3, 163.13)
				het genotype	161.98 (160.19, 163.77)	160.98 (158.87, 163.08)	157.66 (149.48, 165.84)	164.47 (161.25, 167.7)	161.09 (158.85, 163.32)
				hom genotype	162 (159.24, 164.76)	161.17 (157.86, 164.48)	157.25 (148.2, 166.31)	165 (159.92, 170.09)	160.96 (157.61, 164.31)
				p value	0.9744	0.8146	0.7322	0.6674	0.8709
Intergenic	rs9300039	B	CRVE	ref genotype	200.07 (198.54, 201.6)	201.05 (199.29, 202.81)	204.92 (196.67, 213.18)	200.87 (198.03, 203.72)	199.27 (197.35, 201.18)
				het genotype	199.43 (197.58, 201.29)	200.87 (198.7, 203.04)	203.35 (194.79, 211.91)	201.57 (198.02, 205.12)	198.01 (195.75, 200.27)
				hom genotype	198.79 (195.89, 201.69)	200.69 (197.21, 204.16)	201.78 (192.27, 211.28)	202.27 (196.59, 207.95)	196.76 (193.34, 200.18)
				p value	0.3662	0.8339	0.2073	0.6189	0.1260
Intergenic	rs9300039	B	retinopathy	het vs. ref	0.8 (0.59, 1.09)	0.83 (0.59, 1.16)	0.67 (0.31, 1.42)	0.78 (0.5, 1.22)	0.81 (0.52, 1.27)
				hom vs. ref	0.64 (0.34, 1.18)	0.68 (0.34, 1.35)	0.44 (0.1, 2)	0.6 (0.25, 1.49)	0.66 (0.27, 1.61)
				p value	0.1549	0.2746	0.2900	0.2718	0.3640
Intergenic	rs9300039	B	A/V nicking	het vs. ref	0.79 (0.59, 1.05)	0.8 (0.57, 1.11)	0.78 (0.43, 1.42)	0.82 (0.49, 1.39)	0.76 (0.54, 1.08)
				hom vs. ref	0.62 (0.35, 1.1)	0.63 (0.33, 1.22)	0.62 (0.19, 2.02)	0.68 (0.24, 1.93)	0.58 (0.29, 1.16)
				p value	0.1030	0.1755	0.4239	0.4671	0.1251
Intergenic	rs9300039	B	Focal narrowing	het vs. ref	1.13 (0.83, 1.52)	1.07 (0.76, 1.5)	1.36 (0.73, 2.55)	0.82 (0.43, 1.57)	1.23 (0.88, 1.74)
				hom vs. ref	1.27 (0.7, 2.3)	1.14 (0.58, 2.25)	1.85 (0.53, 6.5)	0.68 (0.19, 2.47)	1.52 (0.77, 3.02)
				p value	0.4398	0.7082	0.3344	0.5556	0.2269

Abbreviations: het, heterozygote; hom, homozygote; ref, reference.

APPENDICES

A. IRB approval

To: Yu Yan
Epidemiology
CB:8050

From: Public Health-Nursing IRB

Date: 4/14/2008

RE: Determination that Research or Research-Like Activity does not require IRB Approval
Study #: 08-0649

Study Title: Diabetes Susceptibility Polymorphisms and Risk of Prediabetes and Diabetes Complications in the Atherosclerosis Risk in Communities (ARIC) Study

This submission was reviewed by the above-referenced IRB. The IRB has determined that this submission does not constitute human subjects research as defined under federal regulations [45 CFR 46.102 (d or f)] and does not require IRB approval.

Study Description:

Purpose: Our goal is to measure the association between diabetes-related single nucleotide polymorphisms (SNPs) . Participants: A total of 15,792 men and women randomly selected from the residents of four U.S. communities: Washington County, Maryland, Forsyth County, North Carolina; suburbs of Minneapolis, Minnesota; and Jackson, Mississippi in 1987-1989 and followed for 9 years. Procedures: Using behavioral, biochemical and clinical data as well as stored bio specimens from these population samples we propose to conduct a series of data analyses to estimate the relative risks of prediabetes/retinal abnormalities associated with these variants, and use the population attributable fraction to estimate the population impact of diabetes-related SNPs on retinal abnormalities.

If your study protocol changes in such a way that this determination will no longer apply, you should contact the above IRB before making the changes.

CC:
Kari North, Epidemiology

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.

2. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. NatGenet 2006;38:320-3.

3. Yan Y, North KE, Ballantyne CM, et al. Transcription Factor 7-Like 2 (TCF7L2) Polymorphism and Context-Specific Risk of Type 2 Diabetes in African American and Caucasian Adults: The Atherosclerosis Risk in Communities (ARIC) Study. Diabetes 2008.

4. Cauchi S, El Achhab Y, Choquet H, et al. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. Journal of molecular medicine (Berlin, Germany) 2007.

5. Cauchi S, Froguel P. TCF7L2 genetic defect and type 2 diabetes. Current diabetes reports 2008;8:149-55.

6. Helgason A, Palsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet 2007;39:218-25.

7. ADA. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2004;27:5S-10.

8. Douaihy K. Prediabetes & atherosclerosis: what's the connection? Nurse Pract 2005;30:24-35.

9. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.

10. Sjoholm A, Nystrom T. Inflammation and the etiology of type 2 diabetes. Diabetes Metab Res Rev 2006;22:4-10.

11. Buchanan TA. Pancreatic beta-cell loss and preservation in type 2 diabetes. ClinTher 2003;25 Suppl B:B32-B46.

12. Kissebah AH, Tulloch BR, Hope-Gill H, Clarke PV, Vydelingum N, Fraser TR. Mode of insulin action. Lancet 1975;1:144-7.

13. Zimmet P. The burden of type 2 diabetes: are we doing enough? Diabetes Metab 2003;29:6S9-18.

14. Boyle JP, Honeycutt AA, Narayan KM, et al. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the U.S. Diabetes Care 2001;24:1936-40.

15. Winer N, Sowers JR. Epidemiology of diabetes. J ClinPharmacol 2004;44:397-405.

16. ADA. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.

17. Genuth S, Alberti KG, Bennett P, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160-7.

18. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-31.

19. Anderson RN, Smith BL. Deaths: leading causes for 2002. NatlVital StatRep 2005;53:1-89.

20. ADA. Economic Costs of Diabetes in the U.S. in 2002. Diabetes Care 2003;26:917-32.

21. Cdc. National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2005.Centers for Disease Control and Prevention. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2005; 2005.

22. Lipton RB, Liao Y, Cao G, Cooper RS, McGee D. Determinants of incident non-insulin-dependent diabetes mellitus among blacks and whites in a national sample. The NHANES I Epidemiologic Follow-up Study. Am J Epidemiol 1993;138:826-39.

23. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. JAMA 2003;290:1884-90.

24. Resnick HE, Valsania P, Halter JB, Lin X. Differential effects of BMI on diabetes risk among black and white Americans. Diabetes Care 1998;21:1828-35.

25. Brancati FL, Kao WH, Folsom AR, Watson RL, Szklo M. Incident type 2 diabetes mellitus in African American and white adults: the Atherosclerosis Risk in Communities Study. JAMA 2000;283:2253-9.

26. Tull ES, Roseman JM. Diabetes in African Americans. In: National Diabetes Data Group, ed. Diabetes in America Bethesda, Md: US Dept of Health and Human Services, Public Health Service, National Institutes of Health; 1995.

27. ADA. Standards of medical care in diabetes--2006. Diabetes Care 2006;29 Suppl 1:S4-42.

28. Engelgau MM, Narayan KM, Herman WH. Screening for type 2 diabetes. Diabetes Care 2000;23:1563-80.

29. ADA. Screening for type 2 diabetes. Diabetes Care 2000;23 Suppl 1:S20-3.

30. Abuissa H, Bel DS, O'Keefe JH, Jr. Strategies to prevent type 2 diabetes. CurrMed ResOpin 2005;21:1107-14.

31. Fletcher B, Gulanick M, Lamendola C. Risk factors for type 2 diabetes mellitus. J CardiovascNurs 2002;16:17-23.

32. National Diabetes Surveillance System: Prevalence of Diabetes. Centers for Disease Control and Prevention. (Accessed at http://www.cdc.gov/diabetes/statistics/prev/national/index.htm)

33. Vivian EM. Type 2 diabetes in children and adolescents--the next epidemic? CurrMed ResOpin 2006;22:297-306.

34. Schmidt MI, Duncan BB, Bang H, et al. Identifying individuals at high risk for diabetes: The Atherosclerosis Risk in Communities study. Diabetes Care 2005;28:2013-8.

35. Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2006;113:e85-151.

36. Slynkova K, Mannino DM, Martin GS, Morehead RS, Doherty DE. The role of body mass index and diabetes in the development of acute organ failure and subsequent mortality in an observational cohort. Critical care (London, England) 2006;10:R137.

37. Carnethon MR, Palaniappan LP, Burchfiel CM, Brancati FL, Fortmann SP. Serum Insulin, Obesity, and the Incidence of Type 2 Diabetes in Black and White Adults: The Atherosclerosis Risk in Communities Study: 1987-1998. Diabetes Care 2002;25:1358-64.

38. Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes 2000;49:2201-7.

39. Karter AJ, Rowell SE, Ackerson LM, et al. Excess maternal transmission of type 2 diabetes. The Northern California Kaiser Permanente Diabetes Registry. Diabetes Care 1999;22:938-43.

40. Kim DJ, Cho NH, Noh JH, Lee MS, Lee MK, Kim KW. Lack of excess maternal transmission of type 2 diabetes in a Korean population. Diabetes Research and Clinical Practice 2004;65:117-24.

41. Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF. Consequences of Fetal Exposure to Maternal Diabetes in Offspring. Journal of Clinical Endocrinology Metabolism 2006;91:3718-24.

42. Jeon CY, Lokken RP, Hu FB, van Dam RM. Physical activity of moderate intensity and risk of type 2 diabetes: a systematic review. Diabetes Care 2007;30:744-52.

43. Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical Activity/Exercise and Type 2 Diabetes: A consensus statement from the American Diabetes Association. Diabetes Care 2006;29:1433-8.

44. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA 2001;286:1218-27.

45. Boule NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. Diabetologia 2003;46:1071-81.

46. LaMonte MJ, Blair SN, Church TS. Physical activity and diabetes prevention. J Appl Physiol 2005;99:1205-13.

47. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.

48. Marshall MC, Jr. Diabetes in African Americans. PostgradMedJ 2005;81:734-40.

49. Carter JS, Pugh JA, Monterrosa A. Non-insulin-dependent diabetes mellitus in minorities in the United States. AnnInternMed 1996;125:221-32.

50. Harris MI. Racial and ethnic differences in health care access and health outcomes for adults with type 2 diabetes. Diabetes Care 2001;24:454-9.

51. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up Report on the Diagnosis of Diabetes Mellitus. Diabetes Care 2003;26:3160-7.

52. Phillips LS, Weintraub WS, Ziemer DC, et al. All Pre-Diabetes Is Not the Same: Metabolic and Vascular Risks of Impaired Fasting Glucose at 100 Versus 110 mg/dl: The Screening for Impaired Glucose Tolerance Study 1 (SIGT 1). Diabetes Care 2006;29:1405-7.

53. Tuomilehto J, Gao W, Qiao Q. Assessing the preprandial glucose target: 100 mg/dL versus 110 mg/dL. Endocr Pract 2006;12 Suppl 1:67-70.

54. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of Diabetes and Impaired Fasting Glucose in Adults in the U.S. Population: National Health and Nutrition Examination Survey 1999-2002. Diabetes Care 2006;29:1263-8.

55. Hutchinson RG, Watson RL, Davis CE, et al. Racial differences in risk factors for atherosclerosis. The ARIC Study. Atherosclerosis Risk in Communities. Angiology 1997;48:279-90.

56. Nathan DM, Davidson MB, DeFronzo RA, et al. Impaired Fasting Glucose and Impaired Glucose Tolerance: Implications for care. Diabetes Care 2007;30:753-9.

57. Decode. Glucose tolerance and mortality: comparison of WHO and American Diabetic Association diagnostic criteria.The DECODE study group on behalf of the Europe an Diabetes Epidemiology Group. The Lancet 1999;354:617-21.

58. Dunstan DW, Zimmet PZ, Welborn TA, et al. The rising prevalence of diabetes and impaired glucose tolerance: the Australian Diabetes, Obesity and Lifestyle Study. Diabetes Care 2002;25:829-34.

59. Gabir MM, Hanson RL, Dabelea D, et al. The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 2000;23:1108-12.

60. Harris MI, Eastman RC, Cowie CC, Flegal KM, Eberhardt MS. Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria. Diabetes Care 1997;20:1859-62.

61. Ko GT, Chan JC, Woo J, Cockram CS. Use of the 1997 American Diabetes Association diagnostic criteria for diabetes in a Hong Kong Chinese population. Diabetes Care 1998;21:2094-7.

62. Ko GT, Chan JC, Woo J, et al. The reproducibility and usefulness of the oral glucose tolerance test in screening for diabetes and other cardiovascular risk factors. AnnClinBiochem 1998;35 (Pt 1):62-7.

63. Larsson H, Berglund G, Lindgarde F, Ahren B. Comparison of ADA and WHO criteria for diagnosis of diabetes and glucose intolerance. Diabetologia 1998;41:1124-5.

64. Shaw JE, Zimmet PZ, de C, et al. Impaired fasting glucose or impaired glucose tolerance. What best predicts future diabetes in Mauritius? Diabetes Care 1999;22:399-402.

65. de Vegt F, Dekker JM, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. The 1997 American Diabetes Association criteria versus the 1985 World Health Organization criteria for the diagnosis of abnormal glucose tolerance: poor agreement in the Hoorn Study. Diabetes Care 1998;21:1686-90.

66. Eschwege E, Charles MA, Simon D, Thibult N, Balkau B. Reproducibility of the diagnosis of diabetes over a 30-month follow-up: the Paris Prospective Study. Diabetes Care 2001;24:1941-4.

67. Gimeno SG, Ferreira SR, Franco LJ, Iunes M. Comparison of glucose tolerance categories according to World Health Organization and American Diabetes Association

diagnostic criteria in a population-based study in Brazil. The Japanese-Brazilian Diabetes Study Group. Diabetes Care 1998;21:1889-92.

68. Vaccaro O, Ruffa G, Imperatore G, Iovino V, Rivellese AA, Riccardi G. Risk of diabetes in the new diagnostic category of impaired fasting glucose: a prospective analysis. Diabetes Care 1999;22:1490-3.

69. Shaw JE, Zimmet PZ, Hodge AM, et al. Impaired fasting glucose: how low should it go? Diabetes Care 2000;23:34-9.

70. Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care 2006;29:1130-9.

71. Schmidt MI, Duncan BB, Vigo A, et al. Detection of Undiagnosed Diabetes and Other Hyperglycemia States: The Atherosclerosis Risk in Communities Study. Diabetes Care 2003;26:1338-43.

72. ADA. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2005;28:S37-42.

73. Engelgau MM, Herman WH, Smith PJ, German RR, Aubert RE. The epidemiology of diabetes and pregnancy in the U.S., 1988. Diabetes Care 1995;18:1029-33.

74. Solomon CG, Willett WC, Carey VJ, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. JAMA 1997;278:1078-83.

75. Dooley SL, Metzger BE, Cho NH. Gestational diabetes mellitus. Influence of race on disease prevalence and perinatal outcome in a U.S. population. Diabetes 1991;40 Suppl 2:25-9.

76. Bloomgarden ZT. American Diabetes Association 60th Scientific Sessions, 2000: diabetes and pregnancy. Diabetes Care 2000;23:1699-702.

77. Kahn HS, Williamson DF, Brancati FL, et al. Race, Parity, and Gestational Diabetes as Risk Factors for Type 2 Diabetes Mellitus. JAMA 2000;284:2318-9.

78. Jandeleit-Dahm K, Cooper ME. Hypertension and diabetes. CurrOpinNephrolHypertens 2002;11:221-8.

79. Jandeleit-Dahm K, Cooper ME. Hypertension and diabetes: role of the renin-angiotensin system. EndocrinolMetab ClinNorth Am 2006;35:469-90, vii.

80. Mancia G. The association of hypertension and diabetes: prevalence, cardiovascular risk and protection by blood pressure reduction. Acta Diabetol 2005;42 Suppl 1:S17-S25.

81. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. NEnglJ Med 2000;342:905-12.

82. Padwal R, Majumdar SR, Johnson JA, Varney J, McAlister FA. A Systematic Review of Drug Therapy to Delay or Prevent Type 2 Diabetes. Diabetes Care 2005;28:736-44.

83. The Dream Trial Investigators. Effect of Ramipril on the Incidence of Diabetes. N Engl J Med 2006;355:1551-62.

84. Niemeijer-Kanters SDJM, Banga JD, Erkelens DW. Lipid-lowering therapy in diabetes mellitus. The Netherlands Journal of Medicine 2001;58:214-22.

85. Syvanne M, Taskinen MR. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. Lancet 1997;350 Suppl 1:SI20-SI3.

86. Boden G. Fatty acids and insulin resistance. Diabetes Care 1996;19:394-5.

87. Kolovou GD, Anagnostopoulou KK, Cokkinos DV. Pathophysiology of dyslipidaemia in the metabolic syndrome. PostgradMedJ 2005;81:358-66.

88. Goldberg RB, Mellies MJ, Sacks FM, et al. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. The Care Investigators. Circulation 1998;98:2513-9.

89. Koskinen P, Manttari M, Manninen V, Huttunen JK, Heinonen OP, Frick MH. Coronary heart disease incidence in NIDDM patients in the Helsinki Heart Study. Diabetes Care 1992;15:820-5.

90. Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). Diabetes Care 1997;20:614-20.

91. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 2003;361:2005-16.

92. Pelusi B, Gambineri A, Pasquali R. Type 2 diabetes and the polycystic ovary syndrome. Minerva Ginecol 2004;56:41-51.

93. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141-6.

94. Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN, Pcos/Troglitazone Study G. Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome. The Journal Of Clinical Endocrinology And Metabolism 2005;90:66-71.

95. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. The Journal Of Clinical Endocrinology And Metabolism 1999;84:165-9.

96. Legro RS. Type 2 diabetes and polycystic ovary syndrome. Fertility and Sterility 2006;86:S16-S7.

97. Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN, for the PTSG. Effects of Race and Family History of Type 2 Diabetes on Metabolic Status of Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2005;90:66-71.

98. Sir-Petermann T. Polycystic ovary syndrome, a pathway to type 2 diabetes. Nutrition 2005;21:1160-3.

99. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. Diabetes 1995;44:369-74.

100. Jarrett RJ, Shipley MJ. Type 2 (non-insulin-dependent) diabetes mellitus and cardiovascular disease--putative association via common antecedents; further evidence from the Whitehall Study. Diabetologia 1988;31:737-40.

101. Yudkin JS. Is insulin vasculotoxic? Diabetologia 1997;40 Suppl 2:S145-6.

102. Duncan BB, Schmidt MI, Offenbacher S, Wu KK, Savage PJ, Heiss G. Factor VIII and other hemostasis variables are related to incident diabetes in adults. The Atherosclerosis Risk in Communities (ARIC) Study. Diabetes Care 1999;22:767-72.

103. Ross R. Atherosclerosis -- An Inflammatory Disease. The New England Journal of Medicine 1999;340:115-26.

104. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286-92.

105. Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. The New England Journal of Medicine 2005;352:1685-95.

106. Freeman H, Shimomura K, Horner E, Cox RD, Ashcroft FM. Nicotinamide nucleotide transhydrogenase: a key role in insulin secretion. Cell metabolism 2006;3:35-45.

107. Gunton JE, Kulkarni RN, Yim S, et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 2005;122:337-49.

108. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998;391:900-4.

109. Groop LC, Tuomi T. Non-insulin-dependent diabetes mellitus--a collision between thrifty genes and an affluent society. Annals of medicine 1997;29:37-53.

110. Knowler WC, Pettitt DJ, Lillioja S. Genetic and environmental factors in the development of diabetes mellitus in Pima Indians. In: Smith U, Eriksson S, Lindgarde F, eds. Genetic susceptibility to environmental factors-a challenge for public intervention. Stockhol,: Almqvist and Wiksell International; 1998:67-76.

111. Viswanathan M, Mohan V, Snehalatha C, Ramachandran A. High prevalence of type 2 (non-insulin-dependent) diabetes among the offspring of conjugal type 2 diabetic parents in India. Diabetologia 1985;28:907-10.

112. Barnett AH, Eff C, Leslie RD, Pyke DA. Diabetes in identical twins. A study of 200 pairs. Diabetologia 1981;20:87-93.

113. Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia 1987;30:763-8.

114. Committee on Diabetic Twins. Diabetes mellitus in twins: a cooperative study in Japan. Committee on Diabetic Twins, Japan Diabetes Society. Diabetes Res Clin Pract 1988;5:271-80.

115. Kaprio J, Tuomilehto J, Koskenvuo M, et al. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 1992;35:1060-7.

116. Matsuda A, Kuzuya T. Diabetic twins in Japan. Diabetes Res Clin Pract 1994;24 Suppl:S63-7.

117. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. Diabetologia 1999;42:139-45.

118. Medici F, Hawa M, Ianari A, Pyke DA, Leslie RD. Concordance rate for type II diabetes mellitus in monozygotic twins: actuarial analysis. Diabetologia 1999;42:146-50.

119. Ghosh S, Schork NJ. Genetic analysis of NIDDM. The study of quantitative traits. Diabetes 1996;45:1-14.

120. Elbein SC, Chiu KC, Hoffman MD, Mayorga RA, Bragg KL, Leppert MF. Linkage analysis of 19 candidate regions for insulin resistance in familial NIDDM. Diabetes 1995;44:1259-65.

121. Lesage S, Hani EH, Philippi A, et al. Linkage analyses of the MODY3 locus on chromosome 12q with late-onset NIDDM. Diabetes 1995;44:1243-7.

122. Jackson R, Chambless LE, Yang K, et al. Differences between respondents and nonrespondents in a multicenter community-based study vary by gender ethnicity. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. J ClinEpidemiol 1996;49:1441-6.

123. Florez JC, Hirschhorn J, Altshuler D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Annual review of genomics and human genetics 2003;4:257-91.

124. Hanis CL, Boerwinkle E, Chakraborty R, et al. A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nat Genet 1996;13:161-6.

125. Horikawa Y, Oda N, Cox NJ, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 2000;26:163-75.

126. Cox NJ, Hayes MG, Roe CA, Tsuchiya T, Bell GI. Linkage of Calpain 10 to Type 2 Diabetes: The Biological Rationale. Diabetes 2004;53:S19-25.

127. Gloyn AL, Weedon MN, Owen KR, et al. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 2003;52:568-72.

128. Baier LJ, Permana PA, Yang X, et al. A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. J ClinInvest 2000;106:R69-R73.

129. Cassell PG, Jackson AE, North BV, et al. Haplotype combinations of calpain 10 gene polymorphisms associate with increased risk of impaired glucose tolerance and type 2 diabetes in South Indians. Diabetes 2002;51:1622-8.

130. Elbein SC, Chu W, Ren Q, et al. Role of calpain-10 gene variants in familial type 2 diabetes in Caucasians. J ClinEndocrinolMetab 2002;87:650-4.

131. Evans JC, Frayling TM, Cassell PG, et al. Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. Am J HumGenet 2001;69:544-52.

132. Fingerlin TE, Erdos MR, Watanabe RM, et al. Variation in three single nucleotide polymorphisms in the calpain-10 gene not associated with type 2 diabetes in a large Finnish cohort. Diabetes 2002;51:1644-8.

133. Garant MJ, Kao WH, Brancati F, et al. SNP43 of CAPN10 and the risk of type 2 Diabetes in African-Americans: the Atherosclerosis Risk in Communities Study. Diabetes 2002;51:231-7.

134. Hegele RA, Harris SB, Zinman B, Hanley AJ, Cao H. Absence of association of type 2 diabetes with CAPN10 and PC-1 polymorphisms in Oji-Cree. Diabetes Care 2001;24:1498-9.

135. Horikawa Y, Oda N, Cox NJ, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. NatGenet 2000;26:163-75.

136. Xiang K, Fang Q, Zheng T, et al. [The impact of calpain-10 gene combined-SNP variation on type 2 diabetes mellitus and its related metabolic traits]. Zhonghua YiXueYiChuan XueZa Zhi 2001;18:426-30.

137. Weedon MN, Schwarz PE, Horikawa Y, et al. Meta-analysis and a large association study confirm a role for calpain-10 variation in type 2 diabetes susceptibility. Am J Hum Genet 2003;73:1208-12.

138. Song Y, Niu T, Manson JE, Kwiatkowski DJ, Liu S. Are variants in the CAPN10 gene related to risk of type 2 diabetes? A quantitative assessment of population and family-based association studies. Am J Hum Genet 2004;74:208-22.

139. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225-9.

140. Hanson RL, Ehm MG, Pettitt DJ, et al. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet 1998;63:1130-8.

141. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. Diabetes 1999;48:1175-82.

142. Vionnet N, Hani EH, Dupont S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet 2000;67:1470-80.

143. Wiltshire S, Hattersley AT, Hitman GA, et al. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. Am J Hum Genet 2001;69:553-69.

144. Meigs JB, Panhuysen CI, Myers RH, Wilson PW, Cupples LA. A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees: The Framingham Offspring Study. Diabetes 2002;51:833-40.

145. Xiang K, Wang Y, Zheng T, et al. Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21-q23 and chromosome 1q21-q24. Diabetes 2004;53:228-34.

146. Bowden DW, Sale M, Howard TD, et al. Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes 1997;46:882-6.

147. Mahtani MM, Widen E, Lehto M, et al. Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat Genet 1996;14:90-4.

148. Rotimi CN, Chen G, Adeyemo AA, et al. A genome-wide search for type 2 diabetes susceptibility genes in West Africans: the Africa America Diabetes Mellitus (AADM) Study. Diabetes 2004;53:838-41.

149. Shaw JT, Lovelock PK, Kesting JB, et al. Novel susceptibility gene for late-onset NIDDM is localized to human chromosome 12q. Diabetes 1998;47:1793-6.

150. Wiltshire S, Frayling TM, Groves CJ, et al. Evidence from a large U.K. family collection that genes influencing age of onset of type 2 diabetes map to chromosome 12p and to the MODY3/NIDDM2 locus on 12q24. Diabetes 2004;53:855-60.

151. Ji L, Malecki M, Warram JH, Yang Y, Rich SS, Krolewski AS. New susceptibility locus for NIDDM is localized to human chromosome 20q. Diabetes 1997;46:876-81.

152. Klupa T, Malecki MT, Pezzolesi M, et al. Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. Diabetes 2000;49:2212-6.

153. Mori Y, Otabe S, Dina C, et al. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes 2002;51:1247-55.

154. Zouali H, Hani EH, Philippi A, et al. A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. Human molecular genetics 1997;6:1401-8.

155. Altshuler D, Hirschhorn JN, Klannemark M, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. NatGenet 2000;26:76-80.

156. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. NatGenet 1998;20:284-7.

157. Douglas JA, Erdos MR, Watanabe RM, et al. The peroxisome proliferator-activated receptor-gamma2 Pro12A1a variant: association with type 2 diabetes and trait differences. Diabetes 2001;50:886-90.

158. Hani EH, Boutin P, Durand E, et al. Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia 1998;41:1511-5.

159. Nielsen EM, Hansen L, Carstensen B, et al. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes 2003;52:573-7.

160. Love-Gregory L, Wasson J, Lin J, Skolnick G, Suarez B, Permutt MA. E23K single nucleotide polymorphism in the islet ATP-sensitive potassium channel gene (Kir6.2) contributes as much to the risk of Type II diabetes in Caucasians as the PPARgamma Pro12Ala variant. Diabetologia 2003;46:136-7.

161. van Dam RM, Hoebee B, Seidell JC, Schaap MM, de Bruin TWA, Feskens EJM. Common variants in the ATP-sensitive K+ channel genes KCNJ11 (Kir6.2) and ABCC8 (SUR1) in relation to glucose intolerance: population-based studies and meta-analyses1. Diabetic Medicine 2005;22:590-8.

162. Sale MM, Smith SG, Mychaleckyj JC, et al. Variants of the Transcription Factor 7-Like 2 (TCF7L2) Gene Are Associated With Type 2 Diabetes in an African-American Population Enriched for Nephropathy. Diabetes 2007;56:2638-42.

163. Barroso I, Luan J, Middelberg RP, et al. Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. PLoSBiol 2003;1:E20.

164. Zhu Q, Yamagata K, Miura A, et al. T130I mutation in HNF-4alpha gene is a loss-of-function mutation in hepatocytes and is associated with late-onset Type 2 diabetes mellitus in Japanese subjects. Diabetologia 2003;46:567-73.

165. Silander K, Mohlke KL, Scott LJ, et al. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. Diabetes 2004;53:1141-9.

166. Love-Gregory LD, Wasson J, Ma J, et al. A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 alpha gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an ashkenazi jewish population. Diabetes 2004;53:1134-40.

167. Johansen A, Jensen DP, Bergholdt R, et al. IRS1, KCNJ11, PPARγ2 and HNF-1α: do amino acid polymorphisms in these candidate genes support a shared aetiology between type 1 and type 2 diabetes? Diabetes, Obesity and Metabolism 2006;8:75-82.

168. Hansen T, Ambye L, Grarup N, et al. Genetic variability of the SUR1 promoter in relation to beta-cell function and Type II diabetes mellitus. Diabetologia 2001;44:1330-4.

169. Huxtable SJ, Saker PJ, Haddad L, et al. Analysis of parent-offspring trios provides evidence for linkage and association between the insulin gene and type 2 diabetes mediated exclusively through paternally transmitted class III variable number tandem repeat alleles. Diabetes 2000;49:126-30.

170. Kao WHL, Coresh J, Shuldiner AR, Boerwinkle E, Bray MS, Brancati FL. Pro12Ala of the Peroxisome Proliferator-Activated Receptor-{gamma}2 Gene Is Associated With Lower Serum Insulin Levels in Nonobese African Americans: The Atherosclerosis Risk in Communities Study. Diabetes 2003;52:1568-72.

171. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. Nat Rev Genet 2007;8:657-62.

172. Laukkanen O, Pihlajamaki J, Lindstrom J, et al. Polymorphisms of the SUR1 (ABCC8) and Kir6.2 (KCNJ11) Genes Predict the Conversion from Impaired Glucose Tolerance to Type 2 Diabetes. The Finnish Diabetes Prevention Study. J Clin Endocrinol Metab 2004;89:6286-90.

173. Urhammer SA, Fridberg M, Hansen T, et al. A prevalent amino acid polymorphism at codon 98 in the hepatocyte nuclear factor-1alpha gene is associated with reduced serum C-peptide and insulin responses to an oral glucose challenge. Diabetes 1997;46:912-6.

174. Schmitz-Peiffer C, Whitehead JP. IRS-1 regulation in health and disease. IUBMB life 2003;55:367-74.

175. Amos CI. Successful design and conduct of genome-wide association studies. Hum Mol Genet 2007;16:R220-5.

176. A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/26525384. Accessed 02/05/2009. (Accessed at

177. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341-5.

178. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638-45.

179. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 2008;40:1098-102.

180. Yasuda K, Miyake K, Horikawa Y, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 2008;40:1092-7.

181. Krishnamurthy J, Ramsey MR, Ligon KL, et al. p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 2006;443:453-7.

182. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of Genome-Wide Association Signals in UK Samples Reveals Risk Loci for Type 2 Diabetes. Science 2007;316:1336-41.

183. Meigs JB, Manning AK, Fox CS, et al. Genome-wide association with diabetes-related traits in the Framingham Heart Study. BMC medical genetics 2007;8 Suppl 1:S16.

184. Salonen JT, Uimari P, Aalto JM, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. Am J Hum Genet 2007;81:338-45.

185. Saxena R, Voight BF, Lyssenko V, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331-6.

186. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881-5.

187. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007;39:770-5.

188. Timpson NJ, Lindgren CM, Weedon MN, et al. Adiposity-related heterogeneity in patterns of type 2 diabetes susceptibility observed in genome-wide association data. Diabetes 2009;58:505-10.

189. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.

190. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336-41.

191. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889-94.

192. Hanson RL, Bogardus C, Duggan D, et al. A search for variants associated with young-onset type 2 diabetes in American Indians in a 100K genotyping array. Diabetes 2007;56:3045-52.

193. Hayes MG, Pluzhnikov A, Miyake K, et al. Identification of type 2 diabetes genes in Mexican Americans through genome-wide association studies. Diabetes 2007;56:3033(12).

194. Rampersaud E, Damcott CM, Fu M, et al. Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: evidence for replication from diabetes-related quantitative traits and from independent populations. Diabetes 2007;56:3053-62.

195. Reynisdottir I, Thorleifsson G, Benediktsson R, et al. Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34-q35.2. Am J Hum Genet 2003;73:323-35.

196. Chandak GR, Janipalli CS, Bhaskar S, et al. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia 2007;50:63-7.

197. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S. Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. Diabetologia 2007;50:980-4.

198. Lehman DM, Hunt KJ, Leach RJ, et al. Haplotypes of transcription factor 7-like 2 (TCF7L2) gene and its upstream region are associated with type 2 diabetes and age of onset in Mexican Americans. Diabetes 2007;56:389-93.

199. Helgason A, Palsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet 2007;39:218-25.

200. Damcott CM, Pollin TI, Reinhart LJ, et al. Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. Diabetes 2006;55:2654-9.

201. Scott LJ, Bonnycastle LL, Willer CJ, et al. Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. Diabetes 2006;55:2649-53.

202. Guo T, Hanson RL, Traurig M, et al. TCF7L2 Is Not a Major Susceptibility Gene for Type 2 Diabetes in Pima Indians: Analysis of 3,501 Individuals. Diabetes 2007;56:3082-8.

203. Florez JC, Jablonski KA, Bayley N, et al. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. NEnglJMed 2006;355:241-50.

204. Humphries SE, Gable D, Cooper JA, et al. Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. J MolMed 2006;84:1-10.

205. Elbein SC, Chu WS, Das SK, et al. Transcription factor 7-like 2 polymorphisms and type 2 diabetes, glucose homeostasis traits and gene expression in US participants of European and African descent. Diabetologia 2007.

206. Elbein SC. Evaluation of polymorphisms known to contribute to risk for diabetes in African and African-American populations. Current opinion in clinical nutrition and metabolic care 2007;10:415-9.

207. Marzi C, Huth C, Kolz M, et al. Variants of the transcription factor 7-like 2 gene (TCF7L2) are strongly associated with type 2 diabetes but not with the metabolic syndrome in the MONICA/KORA surveys. Hormone and metabolic research Hormon- und Stoffwechselforschung 2007;39:46-52.

208. Melzer D, Murray A, Hurst A, et al. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. BMC Medicine 2006;4:34.

209. Barber TM, Bennett AJ, Groves CJ, et al. Disparate genetic influences on polycystic ovary syndrome (PCOS) and type 2 diabetes revealed by a lack of association between common variants within the TCF7L2 gene and PCOS. Diabetologia 2007;50:2318-22.

210. Bielinski SJ, Pankow JS, Folsom AR, North KE, Boerwinkle E. TCF7L2 single nucleotide polymorphisms, cardiovascular disease and all-cause mortality: the Atherosclerosis Risk in Communities (ARIC) study. Diabetologia 2008;51:968-70.

211. Bodhini D, Radha V, Dhar M, Narayani N, Mohan V. The rs12255372(G/T) and rs7903146(C/T) polymorphisms of the TCF7L2 gene are associated with type 2 diabetes mellitus in Asian Indians. Metabolism 2007;56:1174-8.

212. Cauchi S, Meyre D, Dina C, et al. Transcription Factor TCF7L2 Genetic Study in the French Population: Expression in Human {beta}-Cells and Adipose Tissue and Strong Association With Type 2 Diabetes. Diabetes 2006;55:2903-8.

213. Cauchi S, Meyre D, Choquet H, et al. TCF7L2 variation predicts hyperglycemia incidence in a french general population: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes 2006;55:3189-92.

214. Cauchi S, Choquet H, Gutierrez-Aguilar R, et al. Effects of TCF7L2 Polymorphisms on Obesity in European Populations. Obesity 2008;16:476-82.

215. Chandak GR, Janipalli CS, Bhaskar S, et al. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia 2006.

216. Dahlgren A, Zethelius B, Jensevik K, Syvänen AC, Berne C. Variants of the TCF7L2 gene are associated with beta cell dysfunction and confer an increased risk of type 2 diabetes mellitus in the ULSAM cohort of Swedish elderly men. Diabetologia 2007;50:1852-7.

217. De Silva NM, Steele A, Shields B, et al. The transcription factor 7-like 2 (TCF7L2) gene is associated with Type 2 diabetes in UK community-based cases, but the risk allele

frequency is reduced compared with UK cases selected for genetic studies. Diabet Med 2007;24:1067-72.

218. Duan QL, Dube MP, Frasure-Smith N, et al. Additive effects of obesity and TCF7L2 variants on risk for type 2 diabetes among cardiac patients. Diabetes Care 2007.

219. Field SF, Howson JM, Smyth DJ, Walker NM, Dunger DB, Todd JA. Analysis of the type 2 diabetes gene, TCF7L2, in 13,795 type 1 diabetes cases and control subjects. Diabetologia 2006.

220. Fisher E, Boeing H, Fritsche A, Doering F, Joost HG, Schulze MB. Whole-grain consumption and transcription factor-7-like 2 (TCF7L2) rs7903146: gene-diet interaction in modulating type 2 diabetes risk. Br J Nutr 2009;101:478-81.

221. Folsom AR, Pankow JS, Peacock JM, Bielinski SJ, Heiss G, Boerwinkle E. Variation in TCF7L2 and increased risk of colon cancer: the Atherosclerosis Risk in Communities (ARIC) Study. Diabetes Care 2008;31:905-9.

222. Groves CJ, Zeggini E, Minton J, et al. Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes 2006;55:2640-4.

223. Horikoshi M, Hara K, Ito C, Nagai R, Froguel P, Kadowaki T. A genetic variation of the transcription factor 7-like 2 gene is associated with risk of type 2 diabetes in the Japanese population. Diabetologia 2007.

224. Kimber CH, Doney AS, Pearson ER, et al. TCF7L2 in the Go-DARTS study: evidence for a gene dose effect on both diabetes susceptibility and control of glucose levels. Diabetologia 2007;50:1186-91.

225. Kirchhoff K, Machicao F, Haupt A, et al. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 2008.

226. Korner A, Berndt J, Stumvoll M, Kiess W, Kovacs P. TCF7L2 gene polymorphisms confer an increased risk for early impairment of glucose metabolism and increased height in obese children. J Clin Endocrinol Metab 2007;92:1956-60.

227. Kottgen A, Hwang SJ, Rampersaud E, et al. TCF7L2 variants associate with CKD progression and renal function in population-based cohorts. J Am Soc Nephrol 2008;19:1989-99.

228. Kunika K, Tanahashi T, Numata S, et al. Common coding variant in the TCF7L2 gene and study of the association with type 2 diabetes in Japanese subjects. J Hum Genet 2008;53:972-82.

229. Loos RJ, Franks PW, Francis RW, et al. TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British Europid population. Diabetes 2007;56:1943-7.

230. Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. The Journal of clinical investigation 2007;117:2155-63.

231. Mayans S, Lackovic K, Lindgren P, et al. TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden. Eur J Hum Genet 2007;15:342-6.

232. Marquezine GF, Pereira AC, Sousa AG, Mill JG, Hueb WA, Krieger JE. TCF7L2 variant genotypes and type 2 diabetes risk in Brazil: significant association, but not a significant tool for risk stratification in the general population. BMC medical genetics 2008;9:106.

233. Munoz J, Lok KH, Gower BA, et al. Polymorphism in the Transcription Factor 7-Like 2 (TCF7L2) Gene Is Associated With Reduced Insulin Secretion in Nondiabetic Women. Diabetes 2006;55:3630-4.

234. Ng MC, Tam CH, Lam VK, So WY, Ma RC, Chan JC. Replication and identification of novel variants at TCF7L2 associated with type 2 diabetes in Hong Kong Chinese. J Clin Endocrinol Metab 2007;92:3733-7.

235. Palmer ND, Lehtinen AB, Langefeld CD, et al. Association of TCF7L2 Gene Polymorphisms with Reduced Acute Insulin Response in Hispanic Americans. J Clin Endocrinol Metab 2008;93:304-9.

236. Qu HQ, Polychronakos C. The TCF7L2 locus and type 1 diabetes. BMC medical genetics 2007;8:51.

237. Raitakari OT, Ronnemaa T, Huupponen R, et al. Variation of the transcription factor 7-like 2 (TCF7L2) gene predicts impaired fasting glucose in healthy young adults: the Cardiovascular Risk in Young Finns Study. Diabetes Care 2007;30:2299-301.

238. Rees SD, Bellary S, Britten AC, et al. Common variants of the TCF7L2 gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population. BMC medical genetics 2008;9:8.

239. Saadi H, Nagelkerke N, Carruthers SG, et al. Association of TCF7L2 polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects. Diabetes Res Clin Pract 2008.

240. Saxena R, Gianniny L, Burtt NP, et al. Common Single Nucleotide Polymorphisms in TCF7L2 Are Reproducibly Associated With Type 2 Diabetes and Reduce the Insulin Response to Glucose in Nondiabetic Individuals. Diabetes 2006;55:2890-5.

241. Schafer SA, Tschritter O, Machicao F, et al. Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. Diabetologia 2007.

242. Shaat N, Lernmark A, Karlsson E, et al. A variant in the transcription factor 7-like 2 (TCF7L2) gene is associated with an increased risk of gestational diabetes mellitus. Diabetologia 2007;50:972-9.

243. Thorsby PM, Midthjell K, Gjerlaugsen N, et al. Comparison of genetic risk in three candidate genes (TCF7L2, PPARG, KCNJ11) with traditional risk factors for type 2 diabetes in a population-based study - the HUNT study. Scand J Clin Lab Invest 2008:1-6.

244. van Vliet-Ostaptchouk JV, Shiri-Sverdlov R, Zhernakova A, et al. Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. Diabetologia 2007;50:59-62.

245. Watanabe RM, Allayee H, Xiang AH, et al. Transcription Factor 7-Like 2 (TCF7L2) Is Associated With Gestational Diabetes Mellitus and Interacts With Adiposity to Alter Insulin Secretion in Mexican Americans. Diabetes 2007;56:1481-5.

246. Wang J, Kuusisto J, Vanttinen M, et al. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007;50:1192-200.

247. Weedon MN, McCarthy MI, Hitman G, et al. Combining Information from Common Type 2 Diabetes Risk Polymorphisms Improves Disease Prediction. PLoSMed 2006;3.

248. Zhang C, Qi L, Hunter DJ, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. Diabetes 2006;55:2645-8.

249. Weedon MN. The importance of TCF7L2. Diabetic Medicine 2007;24:1062-6.

250. Munoz J, Lok KH, Gower BA, et al. Polymorphism in the transcription factor 7-like 2 (TCF7L2) gene is associated with reduced insulin secretion in nondiabetic women. Diabetes 2006;55:3630-4.

251. Cauchi S, Meyre D, Dina C, et al. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes 2006;55:2903-8.

252. Smith U. TCF7L2 and type 2 diabetes-we WNT to know. Diabetologia 2006.

253. Polakis P. Wnt signaling and cancer. Genes and Development 2000;14:1837-51.

254. Wong NA, Pignatelli M. Beta-catenin--a linchpin in colorectal carcinogenesis? Am J Pathol 2002;160:389-401.

255. Korinek V, Barker N, Moerer P, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998;19:379-83.

256. Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J BiolChem 2005;280:1457-64.

257. Freathy RM, Weedon MN, Bennett A, et al. Type 2 Diabetes TCF7L2 Risk Genotypes Alter Birth Weight: A Study of 24,053 Individuals. The American Journal of Human Genetics 2007;80:1150-61.

258. Bailes BK. Diabetes mellitus and its chronic complications. AORN J 2002;76:266-82.

259. Ferris FL, III, Davis MD, Aiello LM. Treatment of diabetic retinopathy. The New England Journal of Medicine 1999;341:667-78.

260. Fong DS, Aiello LP, Ferris FL, III, Klein R. Diabetic retinopathy. Diabetes Care 2004;27:2540-53.

261. Frank RN. Diabetic Retinopathy. N Engl J Med 2004;350:48-58.

262. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol 1984;102:527-32.

263. Klein BE. Overview of epidemiologic studies of diabetic retinopathy. Ophthalmic epidemiology 2007;14:179-83.

264. Klein R, Klein BE, Davis MD. Is cigarette smoking associated with diabetic retinopathy? American journal of epidemiology 1983;118:228-38.

265. The Eye Diseases Prevalence Research Group. The Prevalence of Diabetic Retinopathy Among Adults in the United States. Arch Ophthalmol 2004;122:552-63.

266. Wong TY, Klein R, Amirul Islam FM, et al. Three-Year Incidence and Cumulative Prevalence of Retinopathy: The Atherosclerosis Risk in Communities Study. American Journal of Ophthalmology 2007;143:970-6.

267. Fong DS, Aiello L, Gardner TW, et al. Diabetic Retinopathy. Diabetes Care 2003;26:226-9.

268. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol 1984;102:520-6.

269. Tooke JE. Microvascular function in human diabetes. A physiological perspective. Diabetes 1995;44:721-6.

270. Hsueh WA, Law RE. Diabetes is a vascular disease. J Investig Med 1998;46:387-90.

271. Balletshofer BM, Rittig K, Enderle MD, et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation 2000;101:1780-4.

272. Caballero AE, Arora S, Saouaf R, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999;48:1856-62.

273. Jaap AJ, Shore AC, Tooke JE. Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycaemia. Diabetologia 1997;40:238-43.

274. Wong TY, Klein R, Sharrett AR, et al. Retinal Arteriolar Narrowing and Risk of Diabetes Mellitus in Middle-aged Persons. JAMA 2002;287:2528-33.

275. Wong TY, Mohamed Q, Klein R, Couper DJ. Do retinopathy signs in non-diabetic individuals predict the subsequent risk of diabetes? Br J Ophthalmol 2006;90:301-3.

276. Sharrett AR, Hubbard LD, Cooper LS, et al. Retinal Arteriolar Diameters and Elevated Blood Pressure: The Atherosclerosis Risk in Communities Study. Am J Epidemiol 1999;150:263-70.

277. Wong TY, Shankar A, Klein R, Klein BEK, Hubbard LD. Retinal Arteriolar Narrowing, Hypertension, and Subsequent Risk of Diabetes Mellitus. Arch Intern Med 2005;165:1060-5.

278. Wong TY, Klein R, Sharrett AR, et al. Retinal Arteriolar Diameter and Risk for Hypertension. Ann Intern Med 2004;140:248-55.

279. Klein R, Klein BEK, Moss SE, et al. The Relation of Retinal Vessel Caliber to the Incidence and Progression of Diabetic Retinopathy: XIX: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. Arch Ophthalmol 2004;122:76-83.

280. Klein R, Klein BEK, Moss SE, Cruickshanks KJ. The Wisconsin epidemiologic study of diabetic retinopathy: XVII: The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. Ophthalmology 1998;105:1801-15.

281. Lee KE, Klein BEK, Klein R, Knudtson MD. Familial Aggregation of Retinal Vessel Caliber in the Beaver Dam Eye Study. Invest Ophthalmol Vis Sci 2004;45:3929-33.

282. Rema M, Saravanan G, Deepa R, Mohan V. Familial clustering of diabetic retinopathy in South Indian Type 2 diabetic patients. Diabet Med 2002;19:910-6.

283. The Diabetes Control and Complications Trial Research Group. Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. . Diabetes 1997;46:1829-39.

284. Hallman DM, Huber JC, Jr., Gonzalez VH, Klein BE, Klein R, Hanis CL. Familial aggregation of severity of diabetic retinopathy in Mexican Americans from Starr County, Texas. Diabetes Care 2005;28:1163-8.

285. Warpeha KM, Chakravarthy U. Molecular genetics of microvascular disease in diabetic retinopathy. Eye;17:305-11.

286. Patel S, Chen H, Tinkham NH, Zhang K. Genetic susceptibility of diabetic retinopathy. Current diabetes reports 2008;8:257-62.

287. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480-7.

288. Melzer D, Murray A, Hurst AJ, et al. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. BMC Med 2006;4:34.

289. Liew G, Shankar A, Wang JJ, et al. Apolipoprotein E Gene Polymorphisms Are Not Associated With Diabetic Retinopathy: The Atherosclerosis Risk in Communities Study. American Journal of Ophthalmology 2006;142:105-11.

290. Liew G, Shankar A, Wang JJ, et al. Apolipoprotein E Gene Polymorphisms and Retinal Vascular Signs: The Atherosclerosis Risk in Communities (ARIC) Study. Arch Ophthalmol 2007;125:813-8.

291. Rothman KJ, Greenland S. Modern Epidemiology. 2nd ed. Philadelphia, PA: Lippincott-Raven; 1998.

292. Grarup N, Andersen G. Gene-environment interactions in the pathogenesis of type 2 diabetes and metabolism. Current opinion in clinical nutrition and metabolic care 2007;10:420-6.

293. Yan Y, North KE, Ballantyne CM, et al. Transcription factor 7-like 2 (TCF7L2) polymorphism and context-specific risk of type 2 diabetes in African American and Caucasian adults: the Atherosclerosis Risk in Communities study. Diabetes 2009;58:285-9.

294. ADA. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2007;30 Suppl 1:S42-7.

295. World Health Organization. Physical status: the use and interpretation of anthropometry WHO: Geneva; 1995.

296. Chobanian AV, Bakris GL, Black HR, et al. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.

297. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2005;28:S37-42.

298. Li R, Chambless L. Test for additive interaction in proportional hazards models. Annals of epidemiology 2007;17:227-36.

299. DiCiccio TJ, Efron B. Bootstrap confidence intervals.: Technical Report No. 175. Division of Biostatistics, Stanford University; 1995.

300. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology 1999;106:2269-80.

301. US Department of Health and Human Service. The practical guide—identification, evaluation, and treatment of overweight and obesity in adults. NIH publication no. 004084. Bethesda, MD: National Institutes of Health; 2000.

302. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement: Executive Summary. Circulation 2005;112:e285-90.

303. Eckfeldt JH, Chambless LE, Shen YL. Short-term, within-person variability in clinical chemistry test results. Experience from the Atherosclerosis Risk in Communities Study. ArchPatholLab Med 1994;118:496-500.

304. Expert Panel on Detection EaToHBCiA. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

305. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J ClinNutr 1982;36:936-42.

306. Hosking L, Lumsden S, Lewis K, et al. Detection of genotyping errors by Hardy-Weinberg equilibrium testing. Eur J Hum Genet 2004;12:395-9.

307. Deng HW, Chen WM, Recker RR. Population admixture: detection by Hardy-Weinberg test and its quantitative effects on linkage-disequilibrium methods for localizing genes underlying complex traits. Genetics 2001;157:885-97.

308. Pencina MJ, Larson MG, D'Agostino RB. Choice of time scale and its effect on significance of predictors in longitudinal studies. Statistics in medicine 2007;26:1343-59.

309. Klein R, Sharrett AR, Klein BEK, et al. The association of atherosclerosis, vascular risk factors, and retinopathy in adults with diabetes : the atherosclerosis risk in communities study. Ophthalmology 2002;109:1225-34.

310. Greenland S. Tests for interaction in epidemiologic studies: a review and a study of power. Statistics in medicine 1983;2:243-51.