The effects of known type 2 diabetes susceptibility loci on complications of the disease

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Thesis for the Degree of Master of Science
School of Health Sciences
Faculty of Medicine

HÁSKÓLI ÍSLANDS

Áhrif þekktra erfðamarka sykursýki 2 á fylgikvilla sjúkdómsins

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Ritgerð til meistaragráðu í líf- og læknavísindum

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Ágrip

Algengi sykursýki 2 (SS2) fer ört vaxandi um allan heim, með tilheyrandi aukningu á fylgikvillum sjúkdómsins, sem eru meðal annars æðaskemmdir í hjarta, heila, nýrum og augum. Fundist hafa milli 40 og 50 erfðamörk sem auka áhættu á SS2. Þessi erfðamörk geta hugsanlega stuðlað að þróun fylgikvillanna á sjálfstæðan máta en fáar rannsóknir, ef einhverjar, hafa kannað á skipulegan hátt áhrif þekktra erfðamarka SS2 á fylgikvilla sjúkdómsins, sem var markmið þessarar rannsóknar.

Valin voru 47 erfðamörk sem uppgötvuð hafa verið í yfirgripsmiklum erfðarannsóknum síðari ára og tengsl þeirra við SS2 og tengdar breytur könnuð í þýði Öldrunarrannsóknar Hjartaverndar (AGES-Reykjavík). Annað þýði, REFINE-Reykjavík, var notað til að staðfesta niðurstöður þegar hægt var. Rannsóknin náði til 30 breyta sem lýstu efnaskiptum, líkamsformi og blóðfitum þáttakenda auk ástandi hjarta, nýra, augna og heila. Sérstök áhersla var lögð á erfðamark í geninu *TCF7L2,* þar sem það sýnir sterkasta sambandið við SS2 af þeim erfðamörkum sem fundist hafa, og kannað var hvort utanaðkomandi þættir gætu stjórnað áhrifum þess að einhverju leyti. Búin var til áhættuskali úr öllum erfðamörkunum 47 (e. *genetic risk score)* en síðan voru undirhópar búnir til, eftir tengslum erfðamarkanna við beta frumu virkni annars vegar (áætluð með HOMA-B) og insúlinþols hins vegar (áætlað með HOMA-IR). Skipting erfðamarkanna í undirhópana var bæði gerð eftir áður birtum niðurstöðum og tengslum sem komu fram í þessari rannsókn. Könnuð voru tengsl milli skorunar á áhættuskalanum við sömu breytur og áður, og einnig hvort að líkamsform hefði áhrif á tengsl skorunarinnar við SS2 og blóðsykur.

Meirihluti erfðamarkanna (38 af 47) sýndi samband við SS2 í þá átt sem búist var við og 12 þeirra sýndu tölfræðilega marktækt samband við sjúkdóminn. Sex erfðamörk með óþekkt hlutverk í sjúkdómsmyndun SS2 sýndu vísbendingu um að hafa áhrif á beta frumu virkni og þrjú virtust hafa áhrif á insúlinþol. Áður uppgötvuð sambönd erfðamarka við líkamsþyngdarstuðul, þríglýseríð, HDL kólesteról og æðakölkun voru staðfest í þessari rannsókn og eitt áður óþekkt samband við minnkað mittisummál var fundið. Fleiri áður óþekkt sambönd fundust þar sem $P < 0.05$ en ekkert þeirra var tölfræðilega marktækt eftir að leiðrétt hafði verið fyrir fjölda tölfræðiprófa. Aukaleg greining á áhrifum *TCF7L2* erfðamarksins sýndi að líkamsþyngdarstuðull og blóðfitur virtust ekki breyta miklu um áhrif þess á SS2 eða kransæðasjúkdóm. Erfðamarkið sýndi samband við aukna áhættu á kransæðasjúkdóm í REFINE-Reykjavík þýðinu en ekki í AGES-Reykjavík.

Skorun á áhættuskalanum úr erfðamörkunum 47 sýndi sterkt samband við blóðsykur, SS2 og HOMA-B. Skorunin virtist hafa meiri áhrif á blóðsykur í of feitum einstaklingum í samanburði við einstaklinga í ofþyngd eða kjörþyngd. Sama samband sást ekki á áhrif skorunarinnar á SS2, hugsanlega út af minnkuðum tölfræðilegum styrk. Þegar erfðamörkunum var skipt í hópa eftir hlutverki þeirra í sjúkdómsmyndun SS2 sást sterkt samband við minnkað HOMA-B fyrir annan þeirra og við hækkað HOMA-IR fyrir hinn. Skorun á skalanum sem var tengdur insúlinþoli, en ekki hinum áhættuskölunum, sýndi marktækt samband við aukna áhættu á nýrnasjúkdómi. Mendelísk slembigreining gaf til kynna að orsakasamband væri til staðar milli aukins insúlinþols og minnkaðs gaukulsíunarhraða. Sambandið var enn tölfræðilega marktækt eftir að sykursjúkum einstaklingum var

sleppt úr greiningunni og leiðrétt fyrir líkamsþyngdarstuðli og tríglýseríðum, sem mætti túlka sem svo að sambandið væri óháð SS2. Þessar niðurstöður gætu þýtt að insúlinþol sé mikilvægur þáttur í sjúkdómsmyndun nýrnasjúkdóms, jafnvel áður en SS2 kemur til sögunnar, og að einstaklingar með erfðafræðilega áhættu fyrir insúlinþoli gætu því einnig verið í áhættuhópi fyrir nýrnaskaða. Kanna þarf samband insúlínþols erfðamarkanna við nýrnasjúkdóm í öðru þýði sem væri sambærilegt AGES-Reykjavík með tilliti til aldurs og algengi sjúkdómsins, til að staðfesta niðurstöðurnar.

Abstract

The prevalence of type 2 diabetes (T2D) continues to grow throughout the world with the subsequent increase in burden of diabetic complications, including microvascular and macrovascular diseases of the heart, brain, kidneys and eyes. To date, between 40 and 50 genetic variants have been discovered which increase the risk of T2D. These variants may contribute to the development of T2D complications in independent ways but few studies, if any, have systematically investigated the effect of known T2D susceptibility variants on complications of the disease, which was the aim of this study.

The effects of 47 T2D susceptibility variants selected from genome-wide association studies (GWAS) on T2D and its associated phenotypes were examined in the population based AGES-Reykjavík cohort, and the REFINE-Reykjavík cohort was used for replication of observed results when possible. Association analysis was conducted for 30 metabolic, anthropometric, blood lipid, cardiovascular, renal, retinal and brain volume variables. The *TCF7L2* locus was given special attention in the analysis, as it harbors the strongest risk variants for T2D identified to date, and tested for modulating factors of its effect. A genetic risk score was constructed from the total set of single nucleotide polymorphisms (SNPs), and different subscores created based on their individual associations with beta cell function (BCF), as estimated by HOMA-B, or insulin resistance (IR), as estimated by HOMA-IR, both from literature references and those observed in this analysis.

The majority of the variants (38 of 47) showed the expected direction of effect for T2D in the multiple regression analysis, and 12 showed a statistically significant association with the disease. Six variants with unknown role in T2D pathogenesis were found to possibly influence BCF and three were identified as possible IR risk variants. Previously reported single SNP associations with BMI, triglycerides, HDL and coronary calcium were corroborated in this analysis and one novel association was observed with decreased waist circumference. A number of other novel associations were observed where $P < 0.05$ but none survived correction for multiple testing. Additional analysis on the effects of the *TCF7L2* locus showed that there was no interaction between the genotype and BMI or blood lipids on T2D or coronary heart disease (CHD) risk. The variant was associated with CHD risk in REFINE-Reykjavík, but not in the AGES-Reykjavík cohort.

Finally, a risk score consisting of 47 T2D susceptibility gene variants showed a strong association with fasting glucose, T2D and HOMA-B. Interaction analysis suggested that its effect on fasting glucose levels might be modulated by BMI. The score was associated with greater increase in fasting glucose in obese individuals compared to overweight or normal individuals. The same effect was not observed for T2D risk, possibly because of reduced statistical power. When the variants were partitioned by their proposed physiologic mechanism in the disease, a strong association was found with decreased HOMA-B for one set of SNPs and with increased HOMA-IR for another. The genetic risk score associated with IR, but not the other genetic risk scores, showed a strong association with chronic kidney disease (CKD). Mendelian randomization analysis suggested a causal effect of IR on impaired glomerular filtration rate. The association was statistically significant after the exclusion of diabetic individuals when adjusted for BMI and triglyceride levels, indicating that the effect might be

independent of T2D. These results could suggest that IR is an important factor in the pathogenesis of CKD, even before the onset of T2D, and that individuals with genetic susceptibility for IR might be predisposed to renal damage. The association between the IR associated variants and CKD should be investigated in a cohort comparable to AGES-Reykjavík with regard to age and CKD prevalence, to confirm these findings.

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Abbreviations

ACR	Albumin/creatinine ratio
ATP	Adenosine triphosphate
BCF	Beta cell function
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular disease
ER	Endoplasmic reticulum
FFA	Free fatty acid
GFR	Glomerular filtration rate
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
GRS	Genetic risk score
GWAS	Genome-wide association study
HbA1c	Hemoglobin A1c
HDL	High density lipoprotein
HOMA-B	Homeostasis model assessment of beta cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IHA	Icelandic Heart Association
IL-6	Interleukin-6
IR	Insulin resistance
IRS1-6	Insulin receptor substrate protein 1-6
LDL	Low density lipoprotein
MADGE	Microplate-array diagonal-gel electrophoresis
MI	Myocardial infarction
MODY	Maturity-onset diabetes of the young
NHGRI	National Human Genome Research Institute
OLS	Ordinary least squares
OR	Odds ratio
PI(3)K	Phosphatidylinositol 3-kinase
ROS	Reactive oxygen species
SD	Standard deviation
SE	Standard error

SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes
TNFα	Tumor necrosis factor-α
TRAF2	Tumor-necrosis-factor eceptor-associated-factor-2
WHO	World Health Organization
WML	White matter lesion

Human genes

ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9
ADCY5	Adenylate cyclase 5
ADIPOQ	Adiponectin, C1Q and collagen domain containing
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
C2CD4B	C2 calcium-dependent domain containing 4B
CDC123,CAMK1D	Cell division cycle 123 homolog (S. cerevisiae), calcium/calmodulin-dependent protein kinase ID
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1
CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
CENTD2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1
CETN3	Centrin, EF-hand protein, 3
CHCHD9	Coiled-coil-helix-coiled-coil-helix domain containing 2 pseudogene 9
DCD	Dermcidin
DGKB,TMEM195	Diacylglycerol kinase, beta 90kDa, transmembrane protein 195
DUSP9	Dual specificity phosphatase 9
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1
FTO	Fat mass and obesity associated
GCK	Glucokinase (hexokinase 4)
GCKR	Glucokinase (hexokinase 4) regulator
HHEX	Hematopoietically expressed homeobox
HMGA2	High mobility group AT-hook 2
HNF1A	HNF1 homeobox A
HNF1B,TCF2	HNF1 homeobox B, transcription factor 2
IGF1	Insulin-like growth factor 1 (somatomedin C)
IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2
JAZF1	Juxtaposed with another zinc finger protein 1
KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
KLF14	Kruppel-like factor 14
MTNR1B	Melatonin receptor 1B

NOTCH2	Notch gene homolog 2 (Drosophila)
PPARG	Peroxisome proliferator-activated receptor gamma
PRC1	Protein regulator of cytokinesis 1
PROX1	Prospero homeobox 1
PTPRD	Protein tyrosine phosphatase, receptor type, D
RBMS1, ITGB6	RNA binding motif, single stranded interacting protein 1, integrin, beta 6
SLC30A8	Solute carrier family 30 (zinc transporter), member 8
SPRY2	Sprouty homolog 2 (Drosophila)
SRR	Serine racemase
SYN2, PPARG	Synapsin II, peroxisome proliferator-activated receptor gamma
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)
THADA	Thyroid adenoma associated
TP53INP1	Tumor protein p53 inducible nuclear protein 1
TSPAN8, LGR5	Tetraspanin 8, leucine-rich repeat containing G protein-coupled receptor 5
VEGFA	Vascular endothelial growth factor A
WFS1	Wolfram syndrome 1 (wolframin)
ZBED3	Zinc finger, BED-type containing 3
ZFAND6	Zinc finger, AN1-type domain 6

1 Introduction

1.1 The burden and cost of type 2 diabetes

Type 2 diabetes (T2D) is one of the greatest health problems the western world faces today as the continuous increase in the disease prevalence has reached pandemic proportions, following the steady increase of obesity as a result of the abundance of nutrition available and sedentary lifestyle associated with the modern style of living. T2D is a chronic disease caused by defects in glucose regulation, either as a consequence of impaired insulin secretion from pancreatic beta cells, reduced insulin sensitivity in its target tissues, or both. The result is increased blood glucose, which over time is associated with damage of various body systems.

Diabetes affected 366 million individuals around the world in 2011 [1], 90% out of which suffered of T2D [2]. The prevalence of the disease continues to grow and the disease is predicted to affect 552 million individuals by the year 2030 [1]. A similar pattern has been observed in Iceland during the last few decades. The T2D prevalence in Icelandic individuals aged 25 – 84 years was estimated to be 4% during the period 2006 – 2007 and had then doubled in men and increased by two thirds in women since 30 years earlier (1967 – 1972) [3]. It is estimated that for every two diabetic individual in the Icelandic population there is one individual undiagnosed [3]. Although the T2D prevalence in Iceland is well below the European average, which was 8.1% in 2011 for age 20 – 79 [4], a steady increase has been observed (Figure 1). The observed rise in T2D prevalence is in accordance with the increasing BMI of the population, as data from the Icelandic Heart Association (IHA) has shown (Figure 2). The proportion of obese individuals (BMI ≥ 30) within the Icelandic population has similarly increased, as a recent study showed clearly [5]. In 1990 the proportion of obese individuals was 7.2% for males and 9.5% for females. In 2007, the obese proportion had risen to 18.9% and 21.3%, for males and females respectively (aged 18 – 79 years).

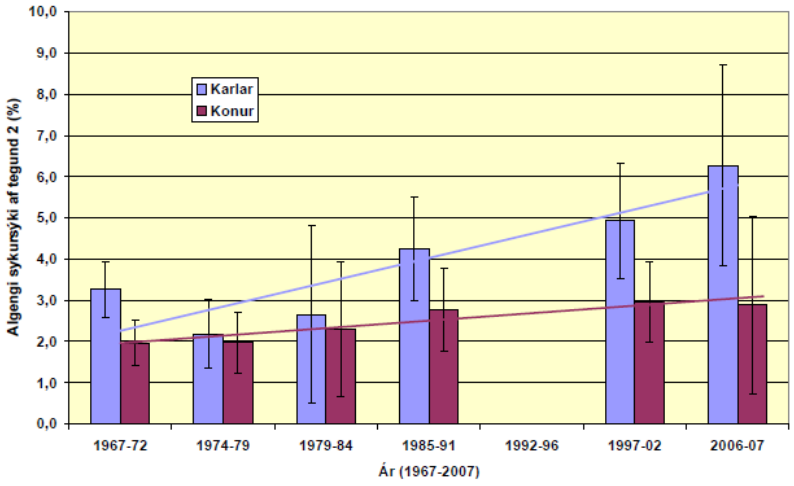

Figure 1 The increased T2D prevalence in Iceland over the 40 year period from 1967 to 2007. The figure shows the T2D prevalence for males (blue) and females (purple) with 95% CI [3].

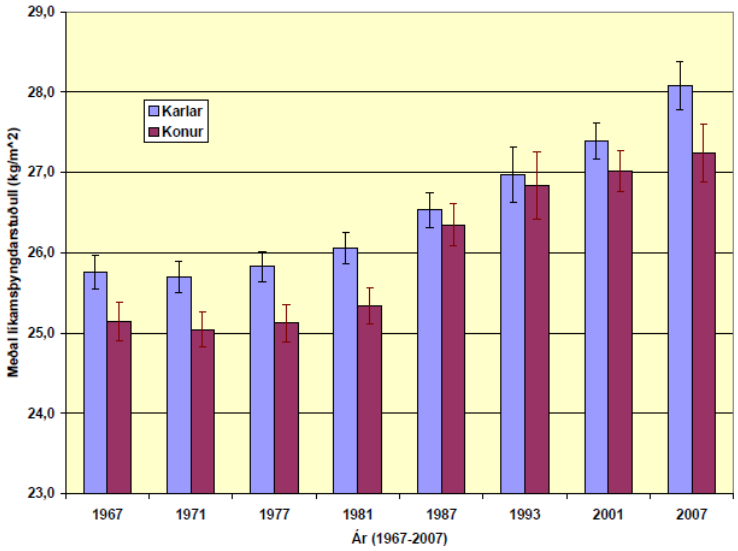

Figure 2 The increase in mean BMI over the 40 year period from 1967 to 2007. The figure shows the mean BMI (error bars represent 95% CI) for Icelandic males (blue) and females (purple) [3].

The current diagnostic criteria for T2D is based on recommendations from the World Health Organization (WHO) [6] and the American Diabetes Association [7], where fasting glucose ≥ 7.0 mmol/L or 2-hour post-glucose-load (75 g glucose orally) ≥ 11.1 mmol/L is classified as T2D. Recently, having HbA1c ≥ 6.5% was also added as a definition of T2D [7]. Before the onset of T2D, individuals may have impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) for a long period, often termed as the prediabetic state. IGT is defined as fasting glucose < 7.0 mmol/L (if measured) and 2-hour post-glucose load ≥ 7.8 mmol/L and < 11.1 mmol/L. IFG is defined as fasting glucose ≥ 6.1 mmol/L and < 7.0 mmol/L, and 2-hour post-glucose-load < 7.8 mmol/L (if measured) [6]. It is estimated that approximately 7% of prediabetic individuals progress to T2D every year, although a lifestyle intervention has been shown to be effective in lowering the risk for these individuals [8, 9].

The estimated direct medical cost of T2D and its complications in the U.S. in 2007 was as much as $116 billion, out of which 50% were cost for treating complications of the disease [10] and it is estimated that 11% of health care expenditure around the world in adults (20 – 79 years) in 2011 was used for diabetes and its associated complications [4].

1.2 Diabetic complications

The majority of the socioeconomic and medical burden of T2D arises from complications of the disease. T2D increases risk of both microvascular complications, which affect brain, nerves, eyes, kidneys and cause ischemia in the heart, as well as macrovascular complications, such as cardiovascular disease (CVD), stroke and peripheral vascular disease (Figure 3). Microvascular changes in the brain increase risk of dementia, and are possibly linked to white matter lesions and hyperintensities which are more common in diabetic individuals [11]. Diabetic neuropathy affects 50% of diabetic individuals [2] and > 60% of T2D patients develop some sort of retinopathy during the first two decades of disease [12]. Approximately one third of diabetic individuals develop kidney disease

[13] and 10 – 20% die of kidney failure [2]. Macrovascular complications are responsible for the majority of deaths associated with T2D, but according to WHO's 2011 diabetes fact sheet, approximately 50% of diabetic individuals die of CVD [2]. CVD can either affect the heart and coronary circulation, increasing risk of atherosclerosis and myocardial infarction (MI), or the brain and the cerebral circulation, often leading to a stroke. Peripheral vascular disease is also common, where decreased blood supply to the legs can eventually lead to gangrene and limb amputation.

The prevalence of macrovascular diabetic complications in Iceland is similar as reported elsewhere, in particular the prevalence of CVD [14], but microvascular complications are generally less common or are less likely to progress to an end stage, as has been seen for chronic kidney disease (CKD) [15]. Similarly, the progression of diabetic retinopathy to full blindness is uncommon in Iceland. The 10-year incident of retinopathy in Icelandic T2D patients was estimated to be 41.9% [16] but the prevalence of full blindness because of diabetic retinopathy was estimated to be only 1.6% [17]. Finally, peripheral neuropathy has a much lower prevalence in Iceland than elsewhere [18].

Although the prevalence of some of the diabetic complications is lower in Iceland than elsewhere in the world, the number of affected individuals can be expected to rise in the near future as the prevalence of T2D increases and the proportion of the elderly within the population continues to grow. It is clear that the complications that arise as a consequence of T2D are both common and serious, and contribute significantly to the overall mortality of the disease. It is therefore extremely important to understand the mechanisms by which these complications develop. Being able to identify individuals at high risk of developing diabetic complications could also be of aid in the clinical setting, as those individuals would benefit from more intense therapy while less susceptible individuals could be spared the inconveniences associated with strict glycemic control.

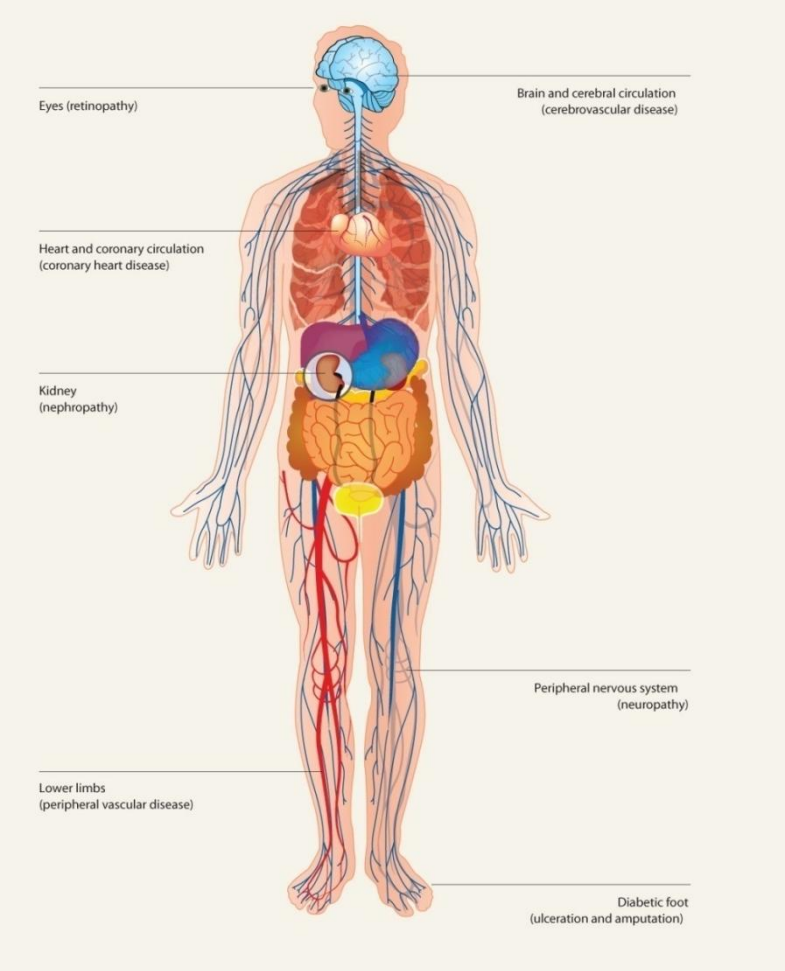

Figure 3 The complications of T2D affect many different body systems [4].

1.3 Type 2 diabetes susceptibility

T2D is caused by a complex interplay of genetic and environmental factors [19]. The T2D pandemic is most likely explained by changes in lifestyle throughout the world which is best demonstrated by the significant increase in T2D prevalence that has been observed in populations that move from traditional rural environments, where the prevalence is low, to urban areas [20, 21]. High energy foods and physical inactivity associated with the westernized lifestyle give rise to obesity, which is the greatest risk factor for developing T2D. However, not all obese individuals develop T2D and 30 – 40% of T2D patients are non-obese (although this percentage varies by populations), so it is clear that some individuals are more susceptible to the disease than others. The heritability of T2D is estimated to be more than 50% [22] and epigenetics and neonatal programming are also gaining increasing attention when estimating T2D risk [19]. Hence, excess consumption of food and obesity may trigger the disease in genetically and epigenetically susceptible individuals and some even develop the disease without fitting into these lifestyle patterns.

Other environmental factors may also contribute to the T2D risk. Vitamin D deficiency and other micronutrient imbalances as well as exposure to some synthetic pesticides and plasticizers have been associated with T2D [23-26]. Low socioeconomic status and depression are known to increase risk of T2D [27, 28]. Finally, the gut microbiota may affect body composition and T2D susceptibility, and this relationship is currently being investigated intensively [29, 30].

1.4 The type 2 diabetes process

1.4.1 Normal glucose homeostasis

Under normal circumstances the hormone insulin is produced and secreted from the pancreatic beta cells as a response to increased blood glucose. The glucose is taken up by the beta cells through the GLUT2 hexose transporter. Glycolysis increases, but the rate limiting step in insulin secretion is the phosphorylation of glucose by the glucokinase enzyme. ATP levels rise, leading to the inactivation of ATP-sensitive potassium channels in the cell membrane. The following depolarization of the membrane causes the opening of calcium channels, and the calcium influx triggers exocytotic release of insulin from the cell (Figure 4) [31].

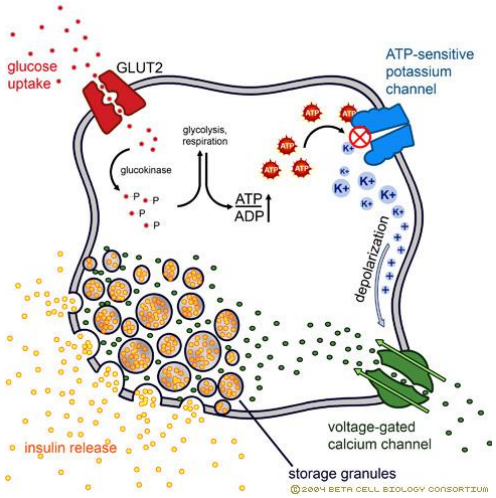

Figure 4 Glucose dependent insulin release from the pancreatic beta cell [32].

Insulin plays a part in a large and complex signaling network (Figure 5) [33]. It binds to insulin receptors in the membrane of cells in target tissues, which are mainly liver, skeletal muscle and adipose tissue, although insulin receptors are found in various other tissues [34]. The binding to the receptor initiates phosphorylation of insulin receptor substrate proteins (IRS proteins). The most studied IRS protein is IRS1 but five additional IRS

proteins have been identified, IRS2-6, which exhibit different tissue distribution [35]. The IRS proteins continue the signaling cascade that, through different mediators, regulates various metabolic functions of the cell. Glucose uptake is stimulated in muscle and adipose tissue through the GLUT4 transporter, glycolysis and glycogen synthesis is stimulated and hepatic glycogenolysis and gluconeogenesis is reduced. Similarly for lipid metabolism, insulin signaling induces fatty acid synthesis in liver and adipose tissue, stimulates re-esterification of free fatty acids (FFAs) into triglycerides and reduces lipolysis in adipose cells, thus reducing FFA flux to the liver. Furthermore, insulin stimulates uptake of certain amino acids into liver, muscle and adipose tissue, increases protein synthesis and reduces proteolysis. In summary, these mechanisms promote energy utilization from glucose breakdown rather than from other fuel sources and the overall effect is a reduction in plasma glucose [31].

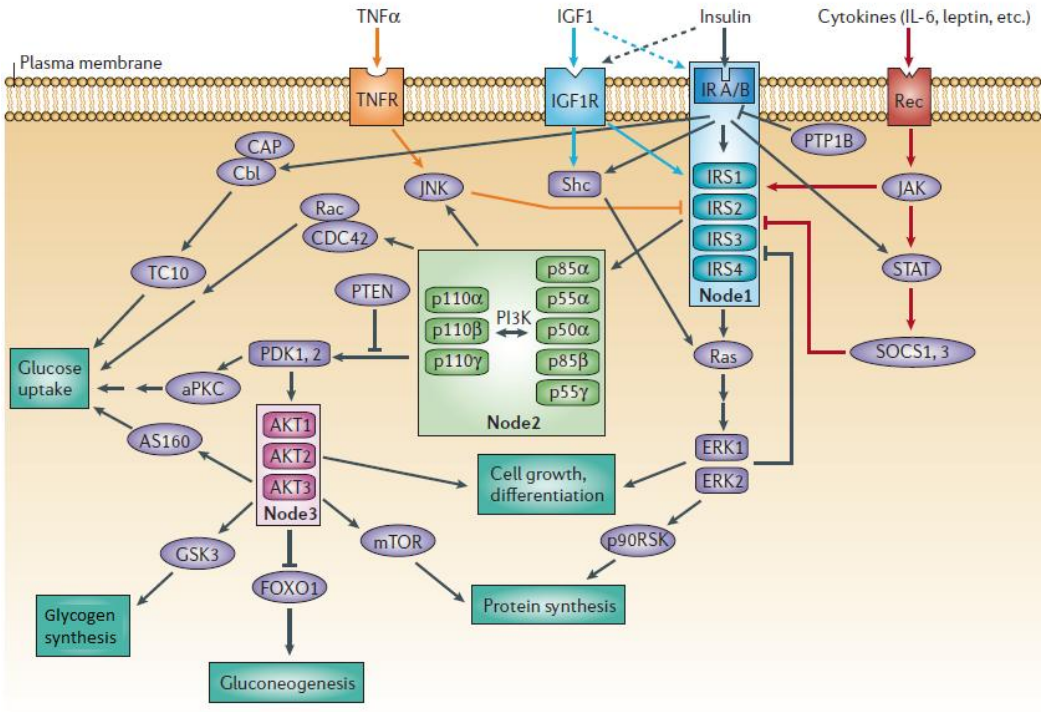

Figure 5 The insulin signaling network [33]. Insulin binds to its receptor, which starts a complex signaling cascade. The final result is increased glucose uptake, glycogen synthesis, protein synthesis and cell growth, and reduced gluconeogenesis.

1.4.2 Pathophysiology of type 2 diabetes

T2D is characterized by two concurrent physiologic conditions, namely increased insulin resistance (IR) and impaired pancreatic beta cell function (BCF). IR is the state where target tissues become less sensitive to the effect of insulin because of impaired insulin signaling, resulting in reduced glucose disposal by skeletal muscle and impaired suppression of glucose production in the liver [36]. Normally, the pancreatic beta cells can adapt well to changes in insulin sensitivity and increased IR is met with an increase in insulin secretion. This state of IR and hyperinsulinemia can persist for decades. However, the triggering event that usually causes progression from the IR state to T2D is when the beta cells fail to secrete enough insulin to compensate for the reduced insulin sensitivity, so blood sugar levels start to rise, leading to impaired glucose tolerance and eventually T2D (Figure 6) [37]. The metabolic abnormalities that are mainly observed as a consequence of IR and reduced insulin secretion are hyperglycemia and dyslipidemia. The abnormal concentrations of glucose and lipids further disrupt the normal energy homeostasis in the body, hence adding to the problem [37]. It is currently thought that IR weighs more heavily in the pathogenesis of T2D in obese individuals, whereas non-obese T2D patients are more likely to have greater defects in BCF, often of genetic origin.

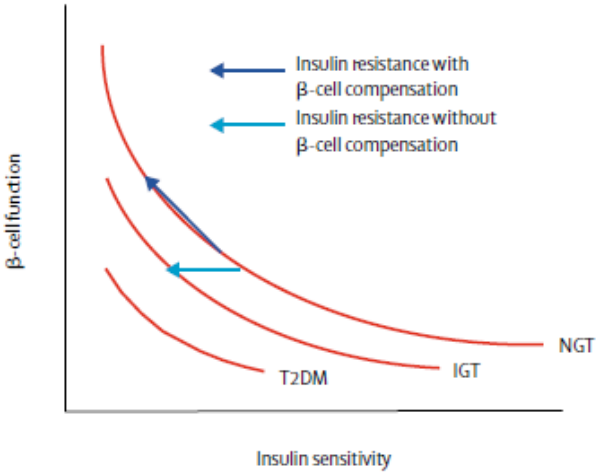

Figure 6 The hyperbolic relationship between beta cell function and insulin sensitivity [36]. NGT: normal glucose tolerance, IGT: impaired glucose tolerance, T2DM: type 2 diabetes mellitus.

1.4.2.1 Insulin resistance

The complex mechanisms of IR progression are not completely understood, although research in the field is continuously adding to our knowledge. Obesity is strongly associated with IR and various factors have been suggested that link the two states [38-40]. Metabolic overload and inflammation, both of which are present in obesity, seem to contribute to IR through interconnected pathways. Adipose tissue has increasingly gained central focus in lipid and glucose metabolism research over the last years [40] but adipocytes play an important role in the regulation of overall energy homeostasis, as they produce a number of different regulatory hormones and cytokines, usually termed as adipokines. Two of the most studied ones are the hormones leptin and adiponectin. Leptin mainly affects the hypothalamus and regulates appetite and energy intake [41, 42], although it also stimulates lipid oxidation in liver and lipolysis in both skeletal muscle and adipose tissue [43]. Adiponectin has an insulin sensitizing effect through various mechanisms, such as suppression of hepatic glucose production, stimulation of fatty acid oxidation and by increasing energy expenditure

[44]. Both hormones are anti-diabetogenic, however, it has been shown that leptin levels are increased in obese individuals and adiponectin levels are decreased [45, 46], indicating that leptin resistance and adiponectin deficiency are associated with the IR progress, although the precise mechanisms are not known.

Obesity is strongly associated with a chronic, low inflammatory state [47]. Adipose tissue in obese individuals contains more of macrophages than adipose tissue in lean individuals and the secretion of proinflammatory cytokines from both adipocytes and macrophages increases with obesity [40]. Tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) are well known proinflammatory cytokines, secreted by adipocytes and macrophages, and elevated levels are associated with IR and T2D [48, 49]. The cytokines can activate serine/threonine kinases which phosphorylate IRS1 or IRS2 and interfere with the insulin signaling pathway (Figure 5) [50]. Furthermore, TNFα can reduce insulin sensitivity by inhibiting gene expression that is necessary for adipocyte differentiation and insulin signaling, such as *PPARG* and *IRS1* [51].

Although not completely understood, it is thought that endoplasmic reticulum (ER) stress plays an important role in the development of inflammation and metabolic disease [52]. The rough ER is the site for protein synthesis and folding, whereas lipids and steroids are synthesized in the smooth ER and carbohydrates and steroids metabolized. An accumulation of misfolded proteins and lipids along with nutrient fluctuations and hypoxia, all of which are present in obesity, can trigger the ER stress response. This response is a signaling mechanism linking the ER with the cytoplasm and nucleus and is called the unfolded protein response. In obese animal models, markers for ER stress have been detected in liver and adipose tissue, but not in muscle, indicating a tissue specific mechanism [53]. Furthermore, ER stress has been shown to cause decreased insulin action in liver cells [53].

Enlarged adipocytes, which are common in obese individuals, release greater amounts of FFAs into the bloodstream. Elevated levels of FFAs have been strongly associated with IR [54, 55]. FFAs can activate macrophage secretion of TNFα, which in turn activates adipocytes and induces further lipolysis, leading to a vicious cycle. The increased circulating levels of FFAs result in increased FFA flux into both liver and skeletal muscle cells. In the liver, β-oxidation of fatty acids becomes impaired and as a consequence, fatty acid metabolites (such diacylglycerols, fatty acyl-coenzyme A and ceramides) accumulate in the cytosol and the ER, inducing ER stress. The stress response activates a serine/threonine kinase cascade that inhibits the interaction between IRS1 or IRS2 with PI(3)K and thus affects downstream events in the insulin signaling network [37, 55] and contributes to hepatic IR, which has a central role in T2D pathophysiology [36].

A slightly different mechanism has been proposed for skeletal muscle. Unlike in liver, FFA influx into muscle cells promotes β-oxidation of fatty acids. However, without sufficient citric acid cycle flux, metabolic by-products and reactive oxygen species (ROS) accumulate in the mitochondria. This accumulation possibly induces a mitochondrial stress response that can activate serine kinases that again interfere with insulin signaling and impede glucose uptake through the GLUT4 transporter. Exercise increases citric acid cycle flux and thus can reverse the peripheral IR progress [37].

1.4.2.2 Impaired beta cell function

Various different mechanisms have been suggested that can lead to pancreatic beta cell failure, which is usually the turning point that leads to T2D and is thought to be the major defect in non-obese T2D individuals. Chronic exposure to high glucose and lipid levels, sometimes termed glucolipotoxicity [56], is known to reduce BCF [37]. Increased glucose and FFA flux can increase mitochondrial ROS production, which is toxic for the cell, and the effort to reduce ROS production comes at a price of less ATP synthesis and hence reduced insulin secretion [19]. ER stress is also thought to be a contributing factor to reduced beta cell mass by inducing apoptosis [52]. Amyloid fibrils are possibly involved in beta cell failure, but these deposits, comprised from the protein amylin, are present in islet samples from T2D patients and are thought to reduce beta cell mass [37]. Genetic variants may also influence beta cell mass and their general function [37]. Unlike in type 1 diabetes, the beta cell failure in T2D is usually not of autoimmune etiology [19, 37].

1.4.3 Pathophysiology of diabetic complications

The central component of diabetic complication pathophysiology is hyperglycemia. Increased glucose concentration surrounds all of the body's cells, but only the cell types that cannot efficiently control glucose transport into the cell are vulnerable to glycemic toxicity. These cells are for example capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, neurons and Schwann cells in peripheral nerves [57].

Intracellular hyperglycemia causes overload in the citric acid cycle flux, resulting in such an increase in the voltage gradient across the mitochondrial membrane that it rises above a critical threshold, which is met with increased production of superoxide (O_2^-). Superoxide is a ROS which, when produced in great amounts, cannot be converted efficiently to H_2O and O_2 and instead exerts its toxic effect on the cell. Superoxide interferes with the glycolytic pathway so glucose and glycolytic intermediates accumulate within the cell. This accumulation activates pathways that are known to have damaging effects on the cell through oxidative stress, cellular signaling dysfunction, inflammation and unfavorable changes in gene expression [57].

Although hyperglycemia is largely responsible for the development of diabetic microvascular complications, it is not the major determinant of macrovascular disease [58] where additional pathogenic mechanisms are evidently present. In fact, IR is an independent risk factor for cardiovascular disease in individuals without diabetes and IGT, even after adjusting for multiple known risk factors [59]. The increased FFA flux from adipocytes associated with IR accelerates FFA uptake in arterial endothelial cells. In macrovascular endothelial cells, but not in microvascular, this increased FFA uptake stimulates β-oxidation, causing mitochondrial overload and ROS production and thus adding to the progression of macrovascular disease [57]. It is therefore possible that pathogenic mechanisms associated with IR already take place in the prediabetic state, and are then enhanced when the individuals finally develop impaired glucose tolerance and diabetes with the accompanying hyperglycemia. Even though this is a simplified explanation of the complex mechanisms that drive diabetic complication pathobiology, it gives an idea of how hyperglycemia and IR contribute to the progress.

Hyperglycemia may be the main culprit in microvascular diabetic complications but IR seems also to play a part of the pathogenesis in certain tissues. IR is associated with increased risk of both vascular dementia and Alzheimer's disease and is gaining increasing attention as a risk factor for neurodegenerative disease [60]. IR has also been consistently associated with increased risk of CKD, independently of T2D [61-65]. CKD is a heterogeneous disease characterized by reduced kidney function, measured as a decrease in glomerular filtration rate (GFR), and/or the presence of kidney damage, measured as an increase of albumin in urine (i.e. microalbuminuria) [66]. It has been shown in animal models that uremia can induce IR [67] so there has been some debate on whether IR is a cause or a consequence of CKD. Prospective studies however suggest that IR is predictive for CKD [62] and thus most likely adds to the pathogenesis of the disease, although CKD in its late stages could further reduce insulin sensitivity. The causality within the relationship is though yet to be proven and the mechanisms behind the association are poorly understood.

1.5 Genetics

1.5.1 Genetics of type 2 diabetes

A number of rare monogenetic disorders causing diabetes have been identified, such as the maturity-onset diabetes of the young (MODY) and mutations of the insulin receptor gene, but the focus here will be on common genetic risk variants for T2D. Early genetic studies on T2D mainly focused on candidate genes, selected from biological pathways known to be involved in glucose metabolism, both via BCF and insulin action. These studies yielded a number of genetic loci that were extensively studied but few of the detected associations with the disease were replicated, as has been the case for many candidate gene studies on various traits. The most successful findings from these studies were the discoveries of coding variants in the *KCNJ11* and *PPARG* loci [68, 69], both of which have consistently been associated with T2D, although the effect size is very modest.

KCNJ11 encodes for a subunit of the ATP-sensitive potassium channel complex, which has an important role in the glucose dependent secretion of insulin in pancreatic beta cells (Figure 4), and variation in the missense single nucleotide polymorphism (SNP) rs5219 increases risk of T2D through impaired insulin secretion [70]. The *PPARG* gene product, the transcription factor PPARγ, is highly expressed in adipose tissue and activates genes that are involved in adipocyte differentiation and the trapping of fatty acids. PPARγ can be activated with a class of drugs called thiazolidinediones, which are PPARγ ligands. The activation results in increased insulin sensitivity, through adipocyte differentiation and increased adiponectin levels [40]. The minor G allele of the missense SNP rs1801282 is associated with decreased risk of T2D and improved insulin sensitivity [71].

In the surge of genome-wide association studies (GWAS) since the year 2006, the number of T2D susceptibility variants dramatically increased and to date there are more than 40 SNPs known that have been convincingly associated with T2D [22, 72]. The genetic variant showing the strongest effect on T2D risk discovered to date through GWAS is the rs7903146 SNP within intron 3 of the *TCF7L2* gene, with a per allele odds ratio (OR) of approximately 1.4 in a recent meta-analysis of 48,117 individuals [73]. The majority of the other T2D SNPs that have been identified in GWAS only have an OR of ~1.05 – 1.20 for increased T2D risk.

A large number of studies have been conducted to explain the role of these newly identified variants in the pathogenesis of T2D. The majority of the SNPs are situated outside of coding sequences and thus do not have a clear molecular role in cellular mechanisms. This is true for many variants identified in GWAS, although disease associated SNPs often point to a region which might then overlap a transcript and can thus indicate the involvement of a particular gene in the respective association. However, often it has not been fully demonstrated through which gene the variant exerts its effect, and the locus is then referred to by the nearest gene, as will be done in this text. Variants located outside of coding sequences that have been associated with disease are thought to possibly affect gene regulation and expression, or even epigenetic changes. Although the exact mechanisms are not fully understood for the majority of the T2D variants, association studies have been able to give clues to how some of them mediate their risk on T2D. An overview of the T2D variants discovered to date is shown in Table 1, along with their proposed function in T2D pathophysiology. Most of them seem to act through impaired BCF and only a small number has been associated with insulin sensitivity [74]. The SNPs affecting insulin secretion can do so through reduced beta cell mass, beta cell dysfunction or impaired insulin processing and/or release. For example, the loci *TCF7L2, SLC30A8, C2CD4B* and *CENTD2* have recently been associated with proinsulin levels [75], indicating a role in insulin processing and secretion. Only six of the T2D associated loci have been convincingly associated with IR. The variant rs35767 in the *IGF1* locus is also included in Table 1, although not a T2D susceptibility SNP per se but was associated with a measurement of IR in a recent GWAS [76]. It is possible that environmental factors play a bigger role than genetics in the development of IR, however its heritability has been estimated to be as much as 44% in the Framingham Offspring Study [77]. Some of the variants have in addition been associated with anthropometric indices. The *FTO* locus is strongly associated with increased BMI [78, 79], and also increases the probability of developing IR [80]. Variants in the *IRS1* and *SPRY2* loci have recently been associated with decreased body fat percentage [81] and variants in the *ADAMTS9* and *VEGFA* regions have been associated with increased waist to hip ratio [82]. However, a large portion of the variants still has no established role in the T2D pathogenesis.

Pathway analysis was applied in the large scale meta-analysis by Voight et al. [73] in the hope to identify biological processes connecting the GWAS signals to the etiology of the disease. Throughout a number of different analyses, cell cycle regulation pathways were the only consistent signal that appeared to be enriched in the gene set. This observation fits with the fact that the majority of the genes are associated with BCF. Faults in cell cycle regulation may lead to reduced beta cell mass, consequently increasing the individual's susceptibility to T2D.

As already mentioned, the heritability of T2D has been estimated to be > 50%, but the genetic variants that have been discovered to date only explain approximately 10% of the estimated heritability [73]. The low proportion of the T2D heritability explained by the discovered genetic risk variants is a classic example of the missing heritability problem that genetic researchers face today. More low effect variants are expected to be discovered with increased sample sizes in GWAS, and rare variants exhibiting greater effects will likely be discovered following the increase in whole-genome sequencing studies, through efforts such as the 1000 Genomes Project [83]. Other biological

mechanisms, such as epigenetics, are increasingly gaining notion as a part of the solution to this problem.

Even though the discovered genetic variants only explain a small proportion of the predicted heritability, they have been shown to have an additive effect on T2D risk. It has been demonstrated that T2D genetic risk scores (GRSs) predict diabetes in the general population, although they have generally not been significantly more effective than clinical measurement predictions [84-86]. Gene variants that are independently associated with impaired BCF also show an additive effect on insulin secretion [87, 88] but a similar effect has not been demonstrated for variants associated with insulin sensitivity. It has recently been reported that the predictability of T2D-GRSs does improve with additional genetic information and that the disease risk prediction using GRSs might be more effective for younger individuals [89], before they develop clinical symptoms.

It is possible that environmental factors modulate the effect of some of the genetic variants. Studies have reported modulating factors on the effect of *PPARG* and *TCF7L2* variants, such as lipid profile [90], nutrition [91, 92] and obesity status [73, 93-95], but interestingly, the *TCF7L2* rs7903146 T2D risk variant has been associated with decreased BMI and is thought to exert a greater effect on T2D risk in lean individuals compared to obese.

The role of obesity as a modulating factor is unclear for most of the other T2D loci. It has been suggested by Cauchi et al. [93] that variants affecting BCF show a greater effect size in lean individuals, whereas loci affecting insulin sensitivity may have a more robust effect in obese people. This study was conducted before many of the IR associated SNPs were discovered and four out of the five insulin action variants in the study were selected from candidate gene studies rather than GWAS. Furthermore, only 10 loci were included in the study. In the large meta-analysis by the DIAGRAM+ consortium only variants in the *TCF7L2* and *BCL11A* loci were shown to have statistically significant different effect size in lean and obese individuals [73], although 23 of the 30 loci that were analyzed showed numerically greater risk estimates in non-obese individuals. There was not a definite different pattern observed for variants associated with BCF or IR. It is challenging to study gene-environment interaction effects on human physiology and it is still unclear if obesity is a modulating factor for T2D genetic variants.

1.5.2 Genetics of diabetic complications

Diabetic complications are the main cause of T2D mortality and reduced quality of life for patients. However, people differ in susceptibility to the various complications, and genetics play a role in this variability. Efforts to identify risk variants in candidate genes have not been very successful, although variations in a few genes have been identified which increase risk of diabetic retinopathy, nephropathy and cardiovascular events [96]. A couple of GWAS have been conducted for diabetic complications, using a type 1 diabetic study sample where individuals who develop the relevant complication are considered as cases but individuals who have suffered from T2D for a long time without developing the complication are used as controls. A recent GWAS meta-analysis on diabetic retinopathy did not observe any association at the genome-wide significance level, although a few variants were suggested for follow-up [97]. Four distinct loci were associated with diabetic nephropathy in a GWAS

by the GoKinD initiative in 2009 and two of those, *FRMD3* and *CARS*, were replicated in an independent cohort [98]. A third one, on chromosome 13q, was replicated in a separate Japanese study [99]. No GWAS have been conducted in a diabetic population on coronary artery disease (CAD), although a couple of variants identified in studies of the general population may be of special interest in T2D. The rs10811661 and rs564398 variants in the 9p21 locus, the closest genes being *CDKN2B* and *CDKN2A*, are associated with T2D [79] but other variants in a similar region are strongly associated with CAD [100, 101]. The T2D rs564398 variant is in linkage disequilibrium (LD) with some of the CAD associated variants, whereas the rs10811661 SNP is not, as calculated with the Ensembl genome browser [102]. Furthermore, the 9p21 locus may have a stronger effect on CAD risk in diabetic individuals than others [103], possibly indicating an interaction with some component of T2D.

Interestingly, there are more T2D associated loci than just the 9p21 locus that are associated with more than one disease trait. Voight et al. have taken together T2D associated loci which show a significant associations with other phenotypes [73] (supplementary data) and Figure 7 gives an overview of genes harboring variants both associated with T2D and other phenotypes at the genome-wide significance level. Given the fact that such a great number of the known T2D variants exert a pleiotropic effect, it is possible that some of them might increase risk of a certain diabetic complication independently of their effect on T2D risk. Few studies, if any, have systematically investigated the effect of known T2D susceptibility variants on individual complications of the disease.

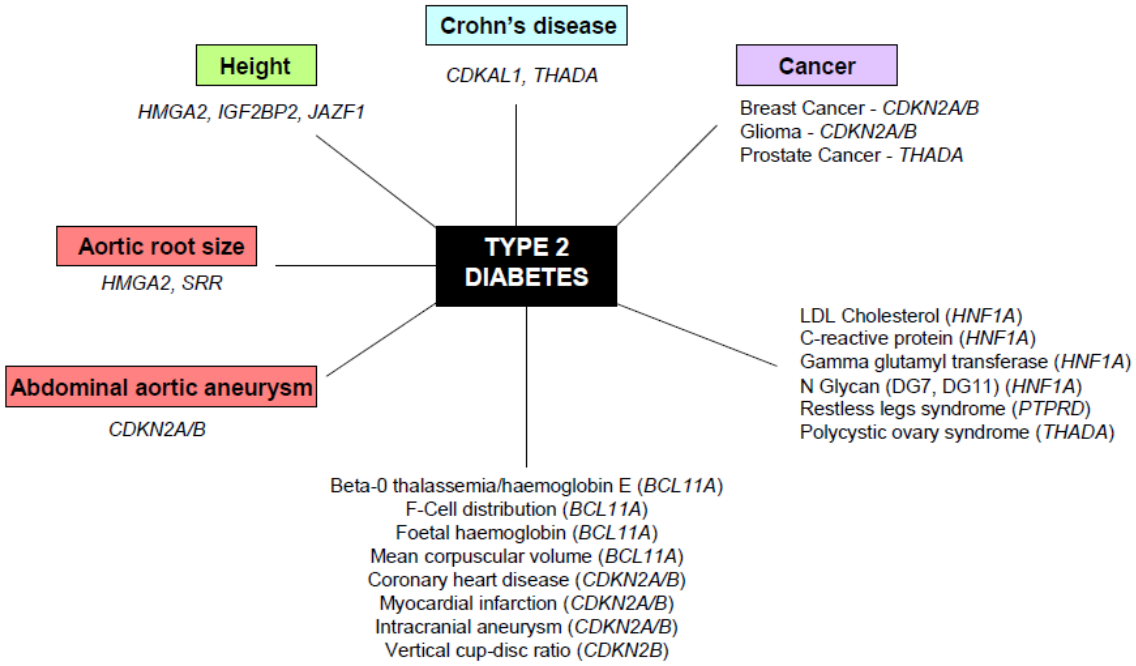

Figure 7 Genes with variants associated with both T2D and other phenotypes at the genome-wide significance level [104].

Table 1 An overview of the 47 T2D susceptibility SNPs and the HOMA-IR associated SNP *IGF1* rs35767 that were chosen for the analysis from the NHGRI catalog.

SNP	Gene	Risk allele	T2D reference	Function	Function reference
rs11708067	*ADCY5*	A	[76]	B	[76]
rs1436955	*C2CD4B*	C	[105]	B	[76]
rs12779790	*CDC123,CAMK1D*	G	[106]	B	[73, 107]
rs7756992	*CDKAL1*	G	[79]	B	[108-111]
rs10811661	*CDKN2B*	T	[79]	B	[110-112]
rs564398	*CDKN2B*	T	[79]	B	[111]
rs1552224	*CENTD2*	A	[73]	B	[73]
rs2191349	*DGKB, TMEM195*	T	[76]	B	[76]
rs5945326	*DUSP9*	A	[73]	B	[113]
rs4607517	*GCK*	A	[76]	B	[76]
rs1111875	*HHEX*	C	[114]	B	[109-111]
rs5015480	*HHEX*	C	[79]	B	
rs4430796	*HNF1B,TCF2*	G	[73]	B	[73]
rs4402960	*IGF2BP2*	T	[79]	B	[73, 115]
rs864745	*JAZF1*	T	[106]	B	[107]
rs5219	*KCNJ11*	T	[116]	B	[70, 111]
rs231362	*KCNQ1*	G	[73]	B	[73]
rs1387153	*MTNR1B*	T	[73]	B	[76, 117]
rs340874	*PROX1*	C	[76]	B	[76]
rs13266634	*SLC30A8*	C	[114]	B	[73, 110, 115, 118]
rs7903146	*TCF7L2*	T	[114]	B	[73, 110, 111, 119, 120]
rs7578597	*THADA*	T	[106]	B	[73]
rs7961581	*TSPAN8,LGR5*	C	[106]	B	[107]
rs1801214	*WFS1*	T	[73]	B	[121]
rs4607103	*ADAMTS9*	C	[106]	IR	[122, 123]
rs8050136	*FTO*	A	[79]	IR	[73]
rs780094	*GCKR*	C	[76]	IR	[76, 124]
rs35767	*IGF1*	G	-	IR	[76, 124]
rs972283	*KLF14*	G	[73]	IR	[73]
rs2943641	*LOC64673, IRS1*	C	[73]	IR	[73, 125]
rs1801282	*PPARG*	C	[79]	IR	[71, 73]
rs17036101	*SYN2, PPARG*	G	[106]	IR	
rs1531343	*HMGA2*	C	[73]	IR*	[73, 126]
rs10490072	*BCL11A*	T	[106]	Unknown	
rs13292136	*CHCHD9*	C	[73]	Unknown	
rs1153188	*DCD*	A	[106]	Unknown	
rs7957197	*HNF1A*	T	[73]	Unknown	
rs12518099	*LOC729011, CETN3*	G	[125]	Unknown	
rs10923931	*NOTCH2*	T	[106]	Unknown	
rs8042680	*PRC1*	A	[73]	Unknown	
rs17584499	*PTPRD*	T	[127]	Unknown	
rs7593730	*RBMS1, ITGB6*	C	[128]	Unknown	
rs1359790	*SPRY2*	G	[129]	Unknown	
rs391300	*SRR*	C	[127]	Unknown	
rs896854	*TP53INP1*	T	[73]	Unknown	
rs9472138	*VEGFA*	T	[106]	Unknown	
rs4457053	*ZBED3*	G	[73]	Unknown	
rs11634397	*ZFAND6*	G	[73]	Unknown	

Gene refers to the gene closest to the respective variant and does not necessarily mean that the gene itself is involved in the reported association. Function denotes the proposed mechanism of the SNP in T2D pathogenesis: B, role in beta cell function impairment; IR, role in IR development; IR*, Possible role in IR development; Unknown, function unknown.

2 Aim of this study

The genetic architecture of T2D is complex and consists of many genes, which may exert their effect through different pathways. Some of the genes are known to contribute independently to the development of certain complications but a systematic analysis on all the identified T2D associated loci in this respect has not been conducted. A clearer picture of the biological mechanisms behind T2D complications is crucial for a better understanding of the differential individual susceptibility to the diseases. The aim of this study was to systematically investigate the effect of known T2D susceptibility variants on T2D and its associated phenotypes in the population based AGES-Reykjavík study cohort.

Specific aims of the study:

1. To examine the contribution of known T2D risk variants identified from GWAS to T2D in an Icelandic population based sample of individuals aged 66 to 98 years old.

2. To examine the association of known T2D risk variants with quantitative metabolic variables, anthropometric indices and blood lipids.

3. To examine the association of known T2D risk variants with microvascular and macrovascular complications of T2D in the heart, brain, kidneys and eyes.

3 Methods

3.1 Cohorts and study design

The Age, Gene/Environment Susceptibility (AGES)-Reykjavík study has been described in detail elsewhere [130]. AGES-Reykjavík is an epidemiological population-based cohort study conducted by the IHA and the National Institute of Aging, USA. The aim of the study is to focus on the effect of genetic and environmental factors on the vascular, neurocognitive, musculoskeletal and body composition/metabolism biological systems in old age.

The Reykjavík study is a population based cohort, which consists of 30,795 men and women born in 1907-1935 who lived in Reykjavík when the study was initiated in 1967 by the IHA. In 2002, the surviving individuals were invited to participate in the AGES-Reykjavík study, which was then concluded in 2006 with a final sample size of 5,764 individuals, resulting in 71% participation rate. An overview of the phenotypic data collected in the study examination has been published [130]. The surviving individuals were then invited for a second examination (AGESII) five years later, in the years 2007-2011. AGES-Reykjavík was approved by the Icelandic National Bioethics Committee (VSN 00-063), the Icelandic Data Protection Authority and the institutional review board of the U.S. National Institute on Aging, National Institute of Health. Informed consent was obtained from all participants.

The Risk Evaluation For INfarct Estimates (REFINE)-Reykjavik study is a prospective randomized controlled cohort trial on the risk factors and etiology of atherosclerotic disease, which consists of 6,490 men and women (68% participation rate) born in 1935-1985 and living in Reykjavík in November 2005.

Data from the AGES-Reykjavík examination was used in the current study. The study was cross-sectional in nature, except for one part in the analysis where data from the AGESII 5 year follow up examination was added to look at a specific longitudinal effect. Data was collected as previously described [130]. Individuals who did not give informed consent for use of hospital records were excluded from the analysis as well as individuals with no laboratory measurements, so the final study cohort included 5,703 individuals. In parts of the analysis a replication of results was attempted in a separate study sample, using data from the interim analysis of the REFINE-Reykjavík cohort (n = 4,753).

3.2 Phenotypes

The following variables were chosen for the analysis as either being metabolically relevant or commonly affected by T2D as a complication of the disease.

3.2.1 Metabolic variables

Fasting glucose and HbA1c (glycosylated hemoglobin) levels were measured on a Roche Hitachi 912 instrument, with reagents from Roche Diagnostics. Fasting insulin levels were measured on a Roche Elecsys 2010 instrument with an electrochemiluminescence immunoassay, using two monoclonal antibodies and a sandwich principle. The first IRP WHO Reference Standard 66/304 (NIBSC) was used to standardize the method.

T2D was defined as self-reported doctor's diagnosis of diabetes, diabetic medication use or fasting blood glucose level ≥ 7.0 mmol/L at the AGES-Reykjavík examination. Homeostasis model assessment was used to determine HOMA-B and HOMA-IR as estimates for BCF and IR respectively [131, 132]. The following simplified equations of the model were used for the calculations:

$$HOMA\text{-}IR = (fasting\ insulin \times fasting\ glucose)/22.5$$

$$HOMA\text{-}B = (20 \times fasting\ insulin)/(fasting\ glucose - 3.5)$$

where fasting insulin was measured in mU/L and fasting glucose in mmol/L. BMI was calculated as weight/(height)^2 and midlife BMI was obtained from measurements from the Reykjavik Study. Waist circumference was measured in centimeters and weight trend calculated from a random effects model on weight measurements taken over time.

3.2.2 Lipids and cardiovascular variables

High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured in mmol/L and triglyceride levels in mg/dL from blood serum. MI was defined as having a MI either before or after the recruitment in AGES, according to hospital records as of 16.03.2011, data from the MONICA study [133] or electrocardiograph measurements at the AGES visit. Similarly, coronary heart disease (CHD) was defined as having any CHD event (MI, percutaneous coronary intervention or coronary artery bypass graft surgery) before or after the AGES recruitment. Blood pressure measurements were obtained and corrected for use of blood pressure medication by adding 10 to diastolic blood pressure and 15 to systolic blood pressure. Coronary calcium was measured from a CT scan in agatston scores and calculated as a sum of calcium scores for four major arteries. Plaque severity was determined from carotid ultrasound measurements on both left and right bifurcation and internal carotid artery, using the following scoring criteria.

1. None
2. Minimal
3. Moderate
4. Severe
5. Semi occlusion

Each individual was given the highest score of their four readings.

3.2.3 Renal variables

Serum creatinine was measured with the Roche Hitachi 912 instrument in µmol/L. GFR was estimated with the four-variable MDRD Study equation [134]. CKD was defined as GFR < 60 ml/min/1.73m^2 according to National Kidney Foundation guidelines [135]. Microalbuminuria was defined as urinary albumin excretion rate of 30 – 299 mg/L. Individuals with macroalbuminuria (n = 47, urinary albumin > 299 mg/L) were included in the microalbuminuria group for easier analysis. The albumin/creatinine ratio (ACR) was calculated as the ratio between urinary albumin and creatinine levels (mg/mmol).

3.2.4 Retinopathy

Retinopathy was defined as having any retinopathy on worse eye.

3.2.5 Brain volumes

Brain tissue and white matter lesion (WML) volumes were computed from high-resolution MRI scans, using an automatic algorithm referred to as the AGES-RS/MNI pipeline [136]. Grey and white matter volumes were corrected for head size of the individual and calculated as a percentage of intra-cranial volume. Similarly, total WML volume was given as a percentage of intra-cranial volume. Subcortical WMLs were measured on a semiquantitative volume scale (mm^3). The lesions largest diameter was used to categorize them as small (≤ 3 mm), medium (4 – 10 mm) or large (≥ 10 mm) and each size was given a weight to approximate volume. The number was multiplied with the number of lesions of the respective size and they all added together to create a rating. Periventricular WMLs were rated on a score scale based on lesion diameter size; 0 (absent), 1 (> 0 – 5), 2 (6 – 9 mm) or 3 (≥ 10 mm) and the scores added together to create a total periventricular WML score.

3.3 Genotypes

3.3.1 DNA extraction

DNA was extracted from whole blood that had been kept frozen at -20° C with a simple salting out method developed by Scotlab Bioscience, Kirshaws Road, Coatbridge, Strathclyde, ML5 8AD, UK. The method and the solutions used are described in Appendix 1.

3.3.2 SNP selection and genotyping

In 2008, all the established T2D susceptibility variants (then 21) were directly genotyped in the whole AGES cohort. The variants rs1111875, rs1801282, rs4402960, rs5219, rs564398, rs7756992, rs7903146, rs13266634 and rs3852876 were genotyped at the IHA using the MADGE protocol [137]. The variants rs10811661, rs9472138, rs8050136, rs5015480, rs864745, rs1153188, rs10490072, rs17036101, rs12779790, rs7578597, rs4607103, rs10923931 and rs7961581 were genotyped at KBioscience, Unit 7, Maple Park, Hoddesdon, Herts, England.

In the period since 2008 and to the start date of the current analysis, the number of identified variants had approximately doubled. Therefore, 26 more newly discovered SNPs were selected from the National Human Genome Research Institute (NHGRI) GWAS catalog [138] and added to the analysis. Genotype data for these variants was extracted from imputed data from Illumina 370CNV BeadChip array genotype information for the AGES-GWAS subcohort (n = 3,179).

All SNPs were in Hardy Weinberg equilibrium, except for *IGF2BP2* rs4402960 (P < 0.001). Therefore, imputed data was used for that particular variant. This resulted in a final number of 47 T2D susceptibility SNPs that were included in the analysis and are shown in Table 1. One SNP associated with HOMA-IR [76], *IGF1* rs35767, was added later into the GRS analysis.

3.4 Statistical analysis

All analyses were conducted in R [139] and PLINK [82].

3.4.1 Variable transformation and population characteristics

Variables were log transformed if needed to approximate a normal distribution (HbA1c, triglycerides, serum creatinine, ACR and total WML volume). For subcortical WMLs and coronary calcium the transformation log(variable+1) was used because of zero values. The fasting insulin levels showed a very skewed distribution, where log transformation was not sufficient to approximate a normal distribution. Therefore, the variables were ranked and then normalized with the probability quantile function, which is a transformation that compresses the outliers. The same was done for the HOMA-B and HOMA-IR variables, as they were derived from fasting insulin levels.

Variable means and standard deviations (SD) were calculated for continuous variables, but median and 25^{th} – 75^{th} percentile were calculated for the skewed variables that were transformed for the analysis. The demographic and clinical characteristics were compared by T2D status using a 2-sample t-test for continuous variables and by Pearson's Chi-squared test for dichotomous variables with a significance level at $\alpha = 0.05$.

3.4.2 Statistical power analysis and single SNP associations

The statistical power within the AGES-Reykjavík cohort to detect SNP associations with T2D was approximated, using the genetic power calculator created by Purcell [140]. Statistical power was calculated for a matrix of SNP risk allele frequencies (0.1 – 0.9) and ORs (1.05 – 1.30), but ORs usually resemble relative risks when the risk is low [141]. The prevalence of T2D was set to 0.081, which was the average prevalence in Europe in 2011. The power was calculated from a case-control model, with a general 2df test and $\alpha = 0.05$. Sample size was set as 5,640 as 63 individuals had missing data for the T2D variable and the number of cases was 721. The control:case ratio within the AGES-Reykjavík cohort was calculated to be 6.82 (4,919 controls : 721 cases). D-prime was set as 1 and marker allele frequency equal to high risk allele frequency, as instructed. The statistical power to detect an association for the average risk allele frequency and average reported OR was also calculated, both for genotyped and imputed variants. Only one variant from each locus was used to calculate the averages. Two variants (rs17584499 and rs391300) had unusually high reported ORs (1.57 and 1.28 respectively) that were found in an Asian population, and were thus excluded from the calculation. This resulted in a final number of 17 genotyped and 24 imputed variants for the statistical power calculations. The sample size and control:case ratio for the genotyped variants was the same as described above, but for the imputed variants the sample size was 3,179 with a calculated control:case ratio of 7.73 (2,815 controls : 364 cases).

The association of each of the 47 T2D susceptibility SNPs with the metabolic traits (T2D, fasting glucose, fasting insulin, HbA1c, HOMA-B, HOMA-IR) was examined. All associations were estimated with multi-variable logistic or linear regression analysis and adjusted for age and sex. For each association the β-value, standard error (SE) and *P*-value are presented in the results chapter, or OR with 95% CI. ORs per risk allele were derived from logistic regression β-values, calculated as exp(β). The association of each SNP with a set of 24 additional phenotypes, which are commonly affected by T2D, was determined. The $\alpha = 0.05$ significance level was divided by the number of SNPs to correct for multiple testing, hence a *P*-value < 0.001 was considered statistically significant. A Bonferroni

correction for the total number of statistical tests was considered to be too stringent; as the analysis had the predefined hypothesis of known T2D susceptibility SNPs affecting phenotypes related to the disease and were not randomly chosen variants. Furthermore, a strict multiple testing correction assumes that all variables are independent of each other, and as many of the traits examined in the study are interrelated it was not considered fully appropriate for this analysis.

3.4.3 *TCF7L2* association analysis

The rs7903146 variant in the *TCF7L2* gene was given special attention in the analysis, as it is the strongest T2D susceptibility SNP that has been discovered to date. The interaction effect between the genotype and BMI, triglycerides, HDL and LDL levels on the risk of both T2D and CHD was investigated, by adding an interaction term into the multi-variable logistic regression model. Furthermore, the effect size conferred by the variant was assessed in different BMI and lipid level groups to determine if these factors modulated the effect size. Individuals were divided into three categories by levels of BMI, lean (BMI < 25), overweight (BMI 25 – 30) or obese (BMI ≥ 30) in accordance with WHO definitions [142]. For each lipid class the cohort was divided into quartiles. The association of the variant with BMI and waist circumference was also evaluated.

This part of the analysis was repeated in a separate study sample, the REFINE-Reykjavík cohort.

3.4.4 Genetic risk scores

GRSs were created to reduce multiple testing issues and to potentially amplify the effect of the low-effect variants. Risk alleles for the T2D susceptibility SNPs were determined from the literature and the total number of risk alleles counted for each individual to generate a GRS. Each of the risk alleles exerts a very small effect on T2D risk but combining the variants adds to the power of the analysis and a greater effect can be observed. For genotyped SNPs, missing genotypes were set to imputed values when available, otherwise to the average frequency of the SNP. Only one SNP was chosen from each locus, to avoid double counting of its effect. The SNP within each gene or region showing the strongest association with T2D in the AGES-Reykjavik cohort was chosen (Table 3). Therefore the SNPs rs5015480 (*HHEX*), rs564398 (*CDKN2B*) and rs17036101 (*SYN2*,*PPARG*) were excluded from all of the GRSs. This resulted in a T2D-GRS constructed from 44 T2D susceptibility SNPs. The GRS was kept unweighted as the estimated effect sizes of the loci for T2D do not necessarily reflect their effect on the other variables that were examined in the study.

As the T2D susceptibility variants influence T2D pathogenesis through various physiologic mechanisms, the SNPs were partitioned by functional similarity to create genetic risk subscores. More specifically, they were divided by their association with either BCF (measured by HOMA-B) or IR (measured by HOMA-IR). The SNPs were partitioned into the two groups based on literature references, resulting in a score of 22 BCF associated SNPs and 6 IR associated SNPs.

Of the 16 remaining SNPs, with no reported association with any measurement of BCF or IR, six showed a trend towards decreased HOMA-B and/or lower insulin levels in the current study, although not statistically significant. These variants were added to the BCF group to create a second BCF-GRS from 28 SNPs. Similarly, three SNPs showed a trend towards increased HOMA-IR and insulin levels in

the study and were subsequently added to the group of SNPs associated with IR. The *IGF1* rs35767 SNP associated with HOMA-IR was also included in that group, to compensate for the low number of SNPs associated with IR in the initial set of 44 T2D SNPs, resulting in an IR-GRS of 10 SNPs. The final seven SNPs with unknown function did not show any trend towards HOMA-B or HOMA-IR in the analysis and hence, were not included in either of the two subscores. An overview of the division process is shown in Figure 8.

The association of the five GRSs with the same variables as before was determined. All associations were estimated with multi-variable logistic or linear regression analysis and adjusted for age and sex, and then additionally for T2D in a second statistical model. The observed associations were plotted on a bar graph, where the *y* axis showed the *P*-value for each association on a $-\log_{10}$ scale, to put emphasis on the smaller values. The direction of each bar was used to represent either a positive or a negative β-value and the variables were color coded, depending on their respective variable group.

A replication of the association trend of the T2D-GRS with anthropometric and lipid variables was attempted in the REFINE-Reykjavík cohort. Genotype data was available for the following variants in 2,375 individuals in REFINE-Reykjavík: rs7903146 (*TCF7L2*), rs7756992 (*CDKAL1*), rs13266634 (*SLC30A8*), rs1111875 (*HHEX*), rs10811661 (*CDKN2B*) and rs5219 (*KCNJ11*). A genetic score (REFINE-GRS) was constructed from these variants and the association with T2D, fasting glucose (log-transformed), BMI, waist circumference, triglycerides (log-transformed), HDL and LDL determined.

3.4.4.1 Modulation of anthropometric indices on genetic risk score effect

Interaction effects between each GRS and anthropometric indices on T2D risk and fasting glucose were investigated by adding an interaction term into the logistic and linear regression models respectively. Furthermore, the association of each GRS with T2D was assessed separately in obese (BMI ≥ 30) and non-obese (BMI < 30) individuals to determine if there was a difference in the effect size between the two groups. The same effect was investigated using the REFINE-GRS, where the interaction between the score and BMI or waist circumference on both T2D risk and glucose levels was examined.

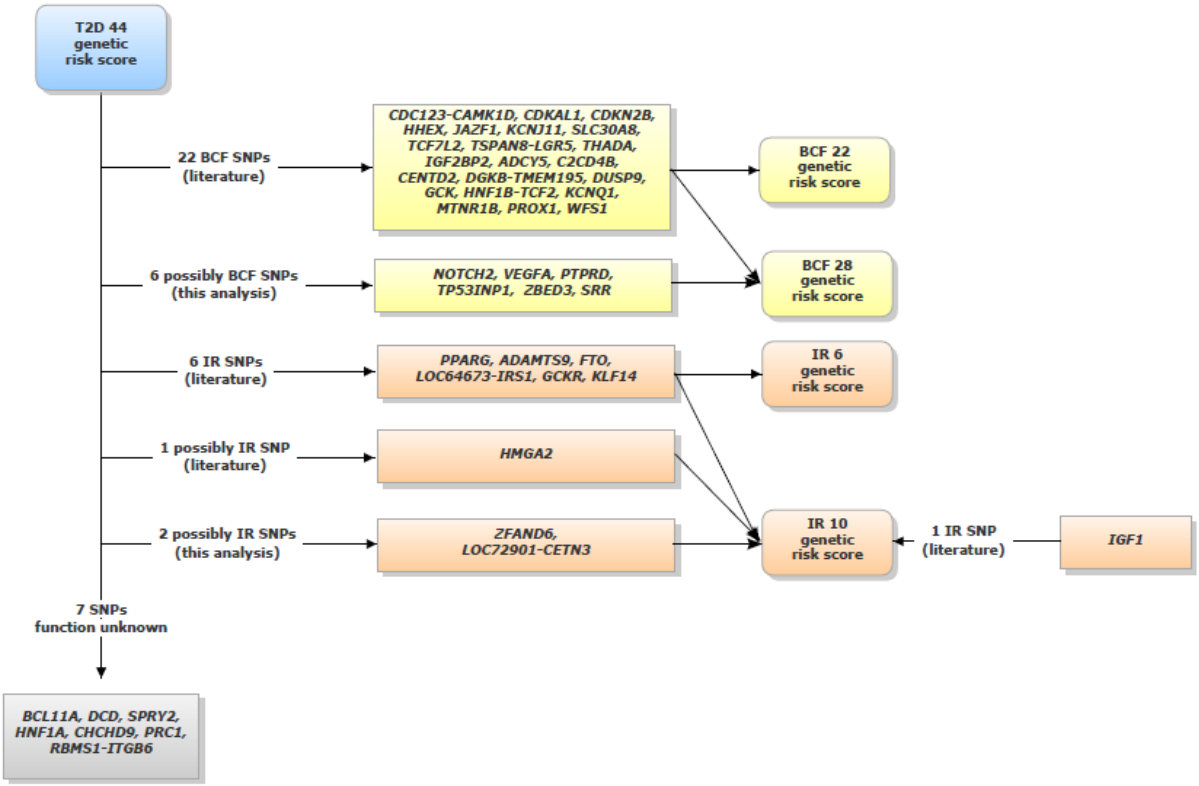

Figure 8 An overview of the SNP selection for each GRS. The T2D-GRS was constructed from 44 variants, which remained after the exclusion of 3 SNPs that showed a weaker association with T2D in AGES-Reykjavík than another variant in the same locus. The BCF-GRS (22) was constructed from the 22 SNPs with a reported association with BCF in the literature and the BCF-GRS (28) was created by adding six variants that showed a possible association with BCF in the present study. Similarly, the IR-GRS (6) was constructed from T2D SNPs with a reported association with IR and the IR-GRS (10) by adding a SNP which has been suggested, but not confirmed, to affect IR, two SNPs which were found to associate with IR in the present study and finally, a SNP which has been associated with IR, but not with T2D, in a previous study. The remaining 7 variants with no reported function in the literature and no evident associations with BCF or IR in the present study were not included in the subscores.

3.4.4.2 HOMA-IR and selected phenotypes

The association of HOMA-IR with periventricular and subcortical WMLs, total WML volume and white and grey matter volume in brain was determined with multi-variable linear regression and adjusted for age and sex. The association of HOMA-IR with the renal traits GFR, serum creatinine, CKD, microalbuminuria and ACR was assessed with multi-variable linear or logistic regressions in four different statistical models. Model A was adjusted for age and sex, model B was adjusted for age, sex and T2D, model C was adjusted for age, sex, T2D and BMI and finally model D was adjusted for age and sex, and diabetic individuals excluded. Additionally, the correlation between HOMA-IR and GFR was found using Pearson's product-moment correlation coefficient, as well as the correlation between the 10 SNP IR-GRS and GFR.

3.4.4.3 Automatic stepwise regression

Further statistical analysis was conducted for the IR-GRS to explore its relationship with kidney disease. The relative contribution of each SNP to the total variance of GFR explained by the score was estimated by automatic stepwise regression, using the "relaimpo" package for R. The GFR was first regressed on age and sex and the residuals from that model used for the automatic stepwise regression. The "lmg" metric was used for the estimation, which is the R^2 contribution averaged over orderings among regressors [143].

3.4.4.4 Bootstrap analysis

Bootstrap analysis was applied to further assess the association between the IR-GRS and GFR. The "boot" package for R was used for this purpose and the number of bootstrap replicates was set to 1000.

3.4.4.5 Instrumental variable regression

Mendelian randomization analysis is an increasingly popular method to investigate causality within a relationship between a non-genetic factor and a health related outcome. The method is an instrumental variable regression (commonly used in econometrics) that applies genetic variants as instruments. The method is based on the Mendelian principle that alleles are passed from parent to offspring independently of other factors, especially socioeconomic and lifestyle factors that may be confounding in epidemiologic association studies. The Mendelian randomization approach has gained increased recognition in the absence of randomized controlled trials and has been described in detail by Lawlor et al. [144].

Linear regression, fitted using the ordinary least squares (OLS) method, was used to explore the relationship between HOMA-IR and GFR in the study population. Two stage least squares instrumental variable analysis for the Mendelian randomization test was carried out using the "AER" package in R. GFR was transformed to a scale where one unit increase equaled one SD, to enable a more clear interpretation of the results. GFR was regressed on HOMA-IR and the 10 SNP IR-GRS used as an instrumental variable to evaluate the un-confounded relationship between the two, after adjusting for age and sex. The strength of the instrumental variable method was determined with the F-statistic from the first stage regression, where $F > 10$ is usually considered adequate [145]. Durbin-Wu-Hausman test was used to compare estimates from OLS and instrumental variable regression for endogeneity.

3.4.4.6 Longitudinal analysis

In this part of the analysis, data from AGESII was used to investigate if the HOMA-IR value at the AGES examination was predictive for renal impairment at the 5-year follow up visit. Data was available for 2,910 individuals. Variables were created which represented change in GFR and serum creatinine levels (log-transformed) and multi-variable linear regression used to assess the association with HOMA-IR, adjusting for age and sex. Only individuals who were free of CKD at baseline (n = 2,100) were included in the CKD analysis, and 250 of those had developed CKD 5 years later. Multi-variable logistic regression was used to estimate the predictive value of HOMA-IR for CKD, again adjusting for age and sex.

4 Results

4.1 Population characteristics

The descriptive statistics of the AGES-Reykjavík cohort are shown in Table 2. The age of the cohort ranged from 66 to 98 years (mean ± SD was 77.0 ± 5.9) and 42.5% were males. The prevalence of T2D was high, or 12.8%, and was more common among males (P = 7.1 x 10^{-13}). Diabetics had significantly higher fasting glucose (P = 6.8 x 10^{-125}), fasting insulin (P = 1.6 x 10^{-73}), HbA1c (P = 2.9 x 10^{-100}) and HOMA-IR (P = 5.4 x 10^{-137}) than non-diabetics, as expected. Diabetic individuals also had lower HOMA-B, although there was not as strong difference observed between the two groups (P = 1.2 x 10^{-4}). Diabetics had greater BMI (P = 7.7 x 10^{-39}) and waist circumference (P = 1.7 x 10^{-37}) than non-diabetics. The average weight trend in diabetic individuals was weight gain, compared to weight loss in non-diabetics. The diabetics had lower HDL (P = 5.1 x 10^{-45}), higher triglycerides (P = 1.7 x 10^{-39}), more plaque (P = 1.1 x 10^{-9}), increased coronary calcium (P = 2.5 x 10^{-13}) and higher prevalence of CHD (P = 2.0 x 10^{-12}) and MI (P = 1.4 x 10^{-5}). The diabetic individuals had lower LDL levels than non-diabetics (P = 2.6 x 10^{-24}), but were also more likely to be on cholesterol lowering statin drugs (P = 4.15 x 10^{-19}). They had higher systolic blood pressure (P = 2.1 x 10^{-6}) but there was no difference in diastolic blood pressure between the groups (P = 0.99). CKD and microalbuminuria were more common in diabetic individuals (P = 2.6 x 10^{-5} and P = 6.4 x 10^{-20} respectively), they had slightly worse GFR (P = 0.016), higher serum creatinine (P = 9.3 x 10^{-10}) and higher ACR (P = 3.7 x 10^{-19}). Of the 1,621 individuals with CKD who had data on urinary albumin, 20.3% had microalbuminuria. Retinopathy was much more common in diabetics than non-diabetics (P = 1.6 x 10^{-23}). There was no difference in periventricular WMLs between the groups (P = 0.48), whereas subcortical WMLs were more common in diabetic individuals (P = 6.5 x 10^{-4}), and total WML volume was greater (P = 1.1 x 10^{-4}). Finally, diabetic individuals had smaller white matter and grey matter volumes than non-diabetic individuals (P = 7.3 x 10^{-9} and P = 8.1 x 10^{-5} respectively). The population characteristics of the REFINE-Reykjavík cohort are shown in Table 1 – Appendix 2.

4.2 Statistical power and single SNP associations

The statistical power curves for different ORs across a range of risk allele frequencies are shown in Figure 9. More than half of the variants had a reported OR between 1.05 and 1.15, which can give a maximum power of 0.67 in this cohort when the allele frequencies are around 0.50.

The average risk allele frequency for the 17 genotyped variants used in the statistical power calculations was 0.52 and their average reported OR for T2D was 1.14. In AGES-Reykjavík (n = 5,640) the calculated statistical power was thus 0.61. The average risk allele frequency for the 24 imputed variants used in the statistical power calculations was 0.53 and the average reported T2D OR was 1.11. The calculated statistical power for this subgroup of 3,179 individuals was 0.23.

Table 2 Characteristics of the whole AGES-Reykjavík population and by T2D status.

Variable	Total cohort	*n*	Diabetes	*n*	No diabetes	*n*	*P*
Age (years)	77.0 ± 5.9	5703	77.3 ± 5.8	721	76.9 ± 5.8	4919	0.084
Male sex	2418 (42.4)	5703	395 (54.8)	721	1999 (40.6)	4919	7.1×10^{-13}
T2D	721 (12.8)	5640					
Fasting glucose (mmol/L)	5.80 ± 1.2	5703	7.83 ± 2.1	721	5.50 ± 0.5	4919	6.8×10^{-125}
Fasting insulin (mU/L)	8.41 (5.6 – 13.0)	5701	13.5 (8.6 – 23.8)	720	7.86 (5.4 – 11.9)	4919	1.6×10^{-73}
HbA1c (%)	0.48 (0.43 – 0.52)	5252	0.58 (0.51 – 0.66)	660	0.47 (0.43 – 0.51)	4531	2.9×10^{-100}
HOMA-B	82.0 (56.3 – 117.3)	5697	74.0 (43.8 – 116.3)	719	82.5 (57.7 – 116.5)	4919	1.2×10^{-4}
HOMA-IR	2.11 (1.3 – 3.5)	5697	4.53 (2.8 – 8.5)	719	1.92 (1.3 – 3.0)	4916	5.4×10^{-137}
BMI (kg/m^2)	25.3 ± 3.6	5676	27.1 ± 3.8	719	25.0 ± 3.5	4894	7.7×10^{-39}
Midlife BMI (kg/m^2)	25.2 ± 3.6	5491	27.1 ± 3.8	704	24.9 ± 3.4	4742	2.1×10^{-40}
Waist circumference (cm)	100.8 ± 12.0	5649	106.3 ± 12.2	718	100.1 ± 11.8	4914	1.7×10^{-34}
Weight trend	-3.5×10^{-4} ± 0.2	5703	0.026 ± 0.3	721	-0.003 ± 0.2	4919	0.008
HDL (mmol/L)	1.58 ± 0.4	5703	1.38 ± 0.4	721	1.62 ± 0.4	4919	5.1×10^{-45}
LDL (mmol/L)	3.49 ± 1.0	5696	3.12 ± 1.0	720	3.55 ± 1.0	4914	2.6×10^{-24}
Statin drugs	1251 (21.9)	5703	252 (35.0)	721	988 (20.1)	4919	4.15×10^{-19}
Triglycerides (mg/dL)	93.8 (69.9 – 130.1)	5703	119.5 (86.7 – 165.5)	721	90.3 (68.1 – 123.9)	4919	1.7×10^{-39}
CHD	1262 (22.1)	5703	232 (32.2)	721	1012 (20.6)	4919	2.0×10^{-12}
MI	614 (10.8)	5703	111 (15.4)	721	495 (10.1)	4919	1.4×10^{-5}
Plaque category	2.84 ± 0.9	5044	3.05 ± 0.9	603	2.82 ± 0.9	4434	1.1×10^{-9}
Coronary calcium (agatston)	156.8 ± 10.1	5261	292.5 ± 7.7	631	145.6 ± 10.3	4623	2.5×10^{-13}
Systolic BP (mmHg)	154.6 ± 23.7	5652	158.1 ± 22.5	719	154.1 ± 23.8	4914	9.6×10^{-6}
Diastolic BP (mmHg)	81.9 ± 11.1	5650	81.9 ± 11.3	719	81.9 ± 11.1	4912	0.986
CKD	1794 (31.5)	5703	275 (38.1)	721	1490 (30.3)	4919	2.6×10^{-5}
GFR (mL/min/1.73 m^2)	68.1 ± 17.7	5703	66.5 ± 20.1	721	68.4 ± 17.3	4919	0.016
Serum creatinine (µmol/L)	87.0 (74.0 – 101.0)	5703	91.0 (78.0 – 109.0)	721	86.0 (74.0 – 100.0)	4919	9.3×10^{-10}
Microalbuminuria	638 (12.1)	5275	146 (23.2)	628	491 (10.6)	4640	6.4×10^{-20}
ACR (mg/mmol)	2.64 (1.28 – 6.93)	5275	4.55 (1.91 – 16.9)	628	2.54 (1.23 – 6.33)	4622	3.7×10^{-19}
Retinopathy	609 (12.4)	4915	147 (25.2)	584	461 (10.7)	4326	1.6×10^{-23}
Periventricular WML	6.10 ± 2.5	4582	6.17 ± 2.5	510	6.09 ± 2.4	4065	0.477
Subcortical WML (mm^3)	2.38 ± 1.3	4649	2.81 ± 1.3	519	2.33 ± 1.3	4123	6.5×10^{-4}
Total WML volume (%)	0.009 (0.005 – 0.018)	4612	0.011 (0.006 – 0.020)	514	0.009 (0.005 – 0.017)	4091	1.1×10^{-4}
White matter volume (%)	0.449 ± 0.04	4612	0.443 ± 0.04	514	0.450 ± 0.04	4091	7.3×10^{-9}
Grey matter volume (%)	0.256 ± 0.02	4612	0.250 ± 0.02	514	0.256 ± 0.02	4091	8.1×10^{-5}

Data are means ± SD or median value (25^{th} – 75^{th} percentile) for all continuous variables. For dichotomous variables the data are *n* (%).

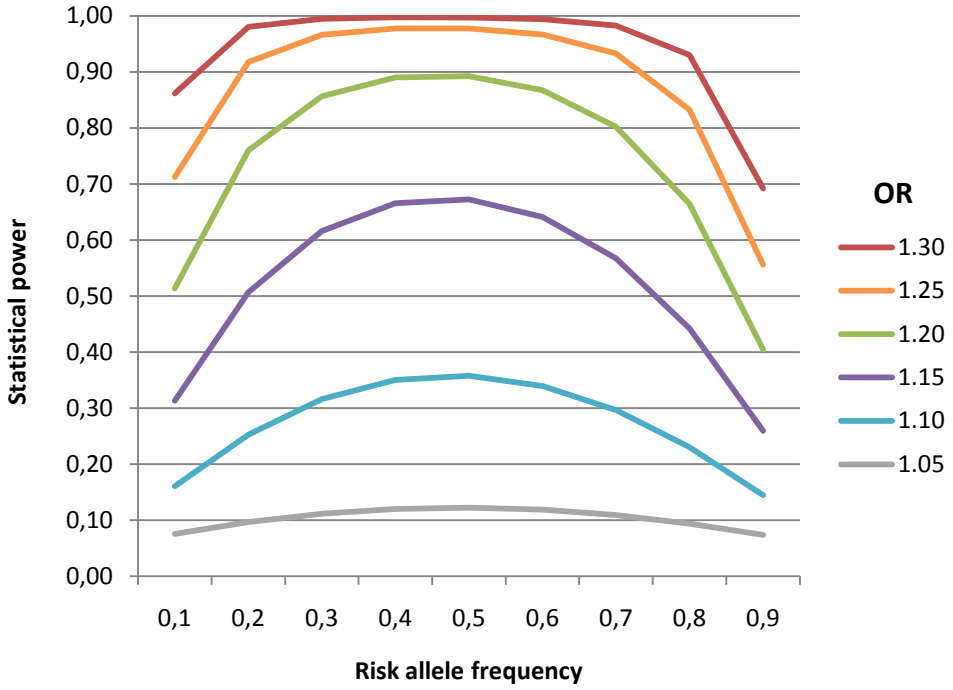

Figure 9 The statistical power for detecting an SNP association with T2D in the AGES-Reykjavík cohort (*n* = 5,640) for a range of risk allele frequencies and effect sizes (ORs).

Table 3 shows the association of the 47 SNPs with T2D in the cohort. Given the calculated statistical power, the expected number of loci showing a significant association with T2D would be approximately 10 of the 17 genotyped loci and 6 of the 27 imputed ones. The majority of the SNPs included in the analysis (38 of 47) showed the expected direction of effect and 12 variants in 11 loci showed an association where $P < 0.05$.

The association of each SNP with fasting glucose levels, fasting insulin levels, HOMA-B, HOMA-IR and HbA1c was examined and the results shown in Table 2 – Appendix 2. As for the T2D association, 40 SNPs of the 47 showed the expected association with increased glucose and 39 with increased HbA1c. Of the 24 SNPs that had a reported association with BCF in the literature, 20 showed the expected direction of effect for decreased HOMA-B but only 13 showed an association with lower insulin levels. All of the seven reported IR associated T2D SNPs showed the expected association trend with increased HOMA-IR, although the *FTO* and *KLF14* variants showed very small effect sizes. Six of the seven IR associated SNPs showed a trend with increased insulin levels. The *GCKR* associations showed the expected direction only after adjusting for triglyceride levels.

Table 3 Association of the 47 T2D susceptibility SNPs with T2D in the AGES-Reykjavík cohort. The variants highlighted in grey are variants which were excluded from the GRS analysis as there was another variant in the same region showing a stronger association with T2D.

SNP	Gene	Risk allele	Frequency	Genotyping	*n*	OR	95% CI	*P*
rs7903146	*TCF7L2*	T	0.31	genotyped	5620	1.29	(1.17 - 1.41)	2.2×10^{-5}
rs7756992	*CDKAL1*	G	0.25	genotyped	5613	1.26	(1.13 - 1.39)	2.5×10^{-4}
rs13266634	*SLC30A8*	C	0.65	genotyped	5680	1.21	(1.08 - 1.33)	0.002
rs1111875	*HHEX*	C	0.55	genotyped	5679	1.19	(1.07 - 1.31)	0.003
rs5015480	*HHEX*	C	0.56	genotyped	5546	1.18	(1.06 - 1.30)	0.004
rs5219	*KCNJ11*	T	0.40	genotyped	5609	1.18	(1.06 - 1.29)	0.005
rs12518099	*LOC729011,CETN3*	G	0.19	imputed	3179	1.27	(1.07 - 1.47)	0.012
rs896854	*TP53INP1*	T	0.55	imputed	3179	1.22	(1.06 - 1.38)	0.013
rs2943641	*LOC64673,IRS1*	C	0.60	imputed	3179	1.21	(1.05 - 1.38)	0.019
rs231362	*KCNQ1*	G	0.48	imputed	3179	1.22	(1.04 - 1.40)	0.023
rs972283	*KLF14*	G	0.45	imputed	3179	1.19	(1.03 - 1.36)	0.032
rs7957197	*HNF1A*	T	0.72	imputed	3179	1.20	(1.01 - 1.39)	0.044
rs10811661	*CDKN2B*	T	0.82	genotyped	5602	1.16	(1.00 - 1.32)	0.051
rs4607103	*ADAMTS9*	C	0.76	genotyped	5614	1.14	(1.00 - 1.28)	0.055
rs1387153	*MTNR1B*	T	0.26	imputed	3179	1.18	(1.00 - 1.36)	0.058
rs1436955	*C2CD4B*	C	0.71	imputed	3179	1.17	(0.98 - 1.35)	0.085
rs7961581	*TSPAN8,LGR5*	C	0.26	genotyped	5551	1.11	(0.98 - 1.25)	0.098
rs11634397	*ZFAND6*	G	0.62	imputed	3179	1.14	(0.96 - 1.32)	0.132
rs4607517	*GCK*	A	0.13	imputed	3179	1.17	(0.94 - 1.40)	0.150
rs1359790	*SPRY2*	G	0.71	imputed	3179	1.14	(0.95 - 1.32)	0.151
rs4402960	*IGF2BP2*	T	0.31	imputed	3179	1.12	(0.95 - 1.30)	0.180
rs391300	*SRR*	C	0.62	imputed	3179	1.10	(0.93 - 1.27)	0.250
rs9472138	*VEGFA*	T	0.29	genotyped	5553	1.07	(0.94 - 1.20)	0.282
rs1801214	*WFS1*	T	0.57	imputed	3179	1.09	(0.92 - 1.26)	0.298
rs4430796	*HNF1B,TCF2*	G	0.46	imputed	3179	1.08	(0.91 - 1.25)	0.347
rs564398	*CDKN2B*	T	0.58	genotyped	5569	1.06	(0.94 - 1.17)	0.356
rs1801282	*PPARG*	C	0.88	genotyped	5638	1.09	(0.90 - 1.27)	0.369
rs864745	*JAZF1*	T	0.53	genotyped	5603	1.05	(0.93 - 1.16)	0.397
rs17036101	*SYN2,PPARG*	G	0.94	genotyped	5616	0.91	(0.67 - 1.15)	0.414
rs2191349	*DGKB, TMEM195*	T	0.51	imputed	3179	1.05	(0.89 - 1.21)	0.530
rs10923931	*NOTCH2*	T	0.12	genotyped	5574	1.05	(0.87 - 1.23)	0.571
rs17584499	*PTPRD*	T	0.20	imputed	3179	1.07	(0.81 - 1.32)	0.609
rs7593730	*RBMS1, ITGB6*	C	0.81	imputed	3179	0.96	(0.75 - 1.16)	0.639
rs340874	*PROX1*	C	0.57	imputed	3179	1.04	(0.88 - 1.20)	0.641
rs1531343	*HMGA2*	C	0.07	imputed	3179	0.94	(0.59 - 1.28)	0.682
rs7578597	*THADA*	T	0.91	genotyped	5610	0.96	(0.76 - 1.16)	0.689
rs11708067	*ADCY5*	A	0.79	imputed	3179	1.04	(0.83 - 1.25)	0.704
rs1552224	*CENTD2*	A	0.82	imputed	3179	1.04	(0.82 - 1.25)	0.730
rs12779790	*CDC123,CAMKJD*	G	0.23	genotyped	5571	0.98	(0.84 - 1.12)	0.750
rs5945326	*DUSP9*	A	0.77	imputed	3179	0.98	(0.82 - 1.14)	0.836
rs1153188	*DCD*	A	0.73	genotyped	5541	0.99	(0.86 - 1.12)	0.853
rs8042680	*PRC1*	A	0.28	imputed	3179	0.99	(0.81 - 1.17)	0.879
rs4457053	*ZBED3*	G	0.27	imputed	3179	1.01	(0.82 - 1.20)	0.920
rs780094	*GCKR*	C	0.65	imputed	3179	1.00	(0.84 - 1.17)	0.965
rs8050136	*FTO*	A	0.40	genotyped	5551	1.00	(0.88 - 1.12)	0.973
rs13292136	*CHCHD9*	C	0.91	imputed	3179	1.00	(0.69 - 1.30)	0.983
rs10490072	*BCL11A*	T	0.74	genotyped	5611	1.00	(0.87 - 1.13)	0.989

Some of the SNPs with no established function in the literature in T2D pathogenesis showed a suggestive trend toward an association with either HOMA-B or HOMA-IR and insulin levels, although not statistically significant. Variants which showed a large effect size for these variables (β ≥ 0.030) and had a relatively low *P*-value (*P* < 0.25) are highlighted with grey in Table 2 – Appendix 2. The variants which showed a hint of influencing BCF and insulin secretion were in the *NOTCH2, VEGFA, PTPRD, TP53INP1, ZBED3* and *SRR* loci, whereas variants in the *HMGA2*, *ZFAND6* and *CETN3* regions might possibly be involved in IR.

The association of each SNP with the other 24 phenotypes was determined. All associations where *P* < 0.01 are shown in Table 4, but associations where *P* = 0.01 – 0.05 are shown in Table 3 – Appendix 2. Only a handful of SNPs showed associations that were statistically significant after the multiple testing correction (*P* < 0.001). The *FTO* rs8050136 variant showed an association with BMI (β = 0.295, $P = 2.3 \times 10^{-5}$), midlife BMI (β = 0.305, $P = 1.4 \times 10^{-5}$) and waist circumference (β = 0.781, $P = 8.6 \times 10^{-4}$). The *GCKR* rs780094 C allele, which is associated with increased risk of T2D, was inversely associated with triglyceride levels (β = -0.066, $P = 1.5 \times 10^{-8}$). A SNP in the *HNF1B,TCF2* locus, rs4430796, was associated with increased HDL (β = 0.049, $P = 1.4 \times 10^{-5}$) and lower triglyceride levels (β = 0.032, *P* = 0.007). A SNP close to the *CDKN2B* gene, rs564398, showed an association with increased coronary calcium (β = 0.197, $P = 4.7 \times 10^{-4}$) but also with lower risk of microalbuminuria (OR = 0.85, 95% confidence interval (CI) 0.72 – 0.97, *P* = 0.007).

Only one of the associations that were significant at the *P* < 0.001 level was novel. The variant rs4457053, upstream of the *ZBED3* gene, was associated with smaller waist circumference (β = -1.191, $P = 6.8 \times 10^{-4}$). It also showed an association with decreased BMI (β = -0.263, *P* = 0.010) and midlife BMI (β = -0.267, *P* = 0.009). The variant rs17036101, which is located between the genes *PPARG* and *SYN2,* showed an association with subcortical WMLs (β = 0.099, *P* = 0.005) and a similar direction of effect for periventricular WMLs, although only marginally statistically significant (β = 0.196, *P* = 0.059). Furthermore, another SNP in the same region, rs1801282 within the *PPARG* gene, showed an association with periventricular WMLs (β = 0.170, *P* = 0.028). The SNP rs12518099, downstream of the *CETN3* gene, showed an association with increased risk of retinopathy (OR = 1.35, 95% CI 1.15 – 1.54, *P* = 0.002). A few other associations of similar degree were observed and shown in Table 4.

Table 4 Association of T2D susceptibility variants with T2D related phenotypes where *P* < 0.01.

Variable	SNP	Gene	Allele	β	SE	*P*	Additional associations[a]
BMI	rs8050136	*FTO*	A	0.295	0.070	2.3×10^{-5}	**Midlife BMI, WC,** *MA, WMvol*
Midlife BMI	rs8050136	*FTO*	A	0.305	0.070	1.4×10^{-5}	**BMI, WC,** *MA, WMvol*
Midlife BMI	rs4607103	*ADAMTS9*	C	-0.211	0.080	0.008	**GFR, CRE,** *BMI, CKD, WMvol*
Midlife BMI	rs4457053	*ZBED3*	G	-0.267	0.103	0.009	**WC,** *BMI, SC-WML*
WC	rs4457053	*ZBED3*	G	-1.191	0.350	6.8×10^{-4}	**Midlife BMI,** *BMI, WMvol*
WC	rs8050136	*FTO*	A	0.781	0.234	8.6×10^{-4}	**BMI, Midlife BMI***, MA, WMvol*
TG	rs780094	*GCKR*	C	-0.066	0.012	1.5×10^{-8}	
TG	rs7961581	*TSPAN8,LGR5*	C	-0.027	0.010	0.006	*WTREND*
TG	rs4430796	*HNF1B,TCF2*	G	-0.032	0.012	0.007	**HDL**
HDL	rs4430796	*HNF1B,TCF2*	G	0.049	0.011	1.4×10^{-5}	**TG**
CORNCAL	rs564398	*CDKN2B*	T	0.197	0.056	4.7×10^{-4}	**MA,** *Plaque category*
CORNCAL	rs17036101	*SYN2,PPARG*	G	0.333	0.115	0.004	**SC-WML, WMLvol,** *WTREND, Plaque category, ACR*
CORNCAL	rs11708067	*ADCY5*	A	0.198	0.070	0.005	*HDL, GMvol*
CHD	rs7961581	*TSPAN8,LGR5*	C	0.180	0.065	0.006	*WTREND, TG*
SYS-BP	rs864745	*JAZF1*	T	1.598	0.568	0.005	
SYS-BP	rs1153188	*DCD*	A	1.803	0.646	0.005	*WC, WTREND, MA, ACR, GMvol*
GFR	rs4607103	*ADAMTS9*	C	-1.217	0.382	0.001	**Midlife BMI, CRE,** *BMI, CKD, WMvol*
CRE	rs4607103	*ADAMTS9*	C	0.016	0.005	0.003	**Midlife BMI, GFR,** *BMI, CKD, WMvol*
MA	rs564398	*CDKN2B*	T	-0.166[b]	0.062	0.007	**CORNCAL,** *Plaque category*
Retinopathy	rs12518099	*LOC72901, CETN3*	G	0.299[c]	0.095	0.002	
SC-WML	rs7756992	*CDKAL1*	G	0.058	0.020	0.004	
SC-WML	rs17036101	*SYN2,PPARG*	G	0.099	0.036	0.005	**CORNCAL, WMLvol,** *WTREND, Plaque category, ACR*
WMLvol	rs17036101	*SYN2,PPARG*	G	0.101	0.037	0.007	**CORNCAL, SC-WML,** *WTREND, Plaque category, ACR*
GMvol	rs7593730	*RBMS1, ITGB6*	C	-0.003	0.001	0.009	*Retinopathy, SC-WML*

BMI: Body mass index, WC: Waist circumference, TG: Triglycerides, HDL: High-density lipoprotein, CORNCAL: Coronary calcium, SYS-BP: Systolic blood pressure, GFR: Glomerular filtration rate, CRE: Serum creatinine, MA: Microalbuminuria, SC-WML: Subcortical white matter lesions, WMLvol: Total white matter lesion volume, GMvol: Grey matter volume, WMvol: White matter volume, CKD: Chronic kidney disease, WTREND: Weight trend, ACR: Albumin/creatinine ratio. [a]**Bold**: *P* < 0.01 (this table), *italics*: *P* = 0.01 – 0.05 (Table 3 Appendix 2), [b]OR = 0.85 (95% CI 0.72 – 0.97), [c]OR = 1.35 (95% CI 1.15 – 1.54).

4.3 *TCF7L2* association analysis

The rs7903146 variant showed an association with T2D in the AGES-Reykjavík cohort as expected but showed no association with CHD (Table 5). No statistically significant interactions were observed between the rs7903146 genotype and BMI, HDL or triglyceride levels on T2D risk although the interaction with LDL levels was marginally significant (*P* = 0.062). No statistically significant interactions were observed either on CHD risk, but the interaction with BMI could be considered borderline significant (*P* = 0.084). When the AGES-Reykjavík cohort was stratified by BMI categories it was clear that a larger effect size on T2D risk was observed in the obese group compared to the other two (Table 4 – Appendix 2). There was no association observed between the genotype and CHD in any BMI or lipid group.

The analysis was repeated in an independent study sample, the REFINE-Reykjavík cohort (Table 5). The frequency of the rs7903146 T allele was 0.30 compared to 0.31 in AGES-Reykjavík and there was no association between the genotype and age in the combined sample (β = 0.22, SE = 0.25, *P* = 0.39). In REFINE-Reykjavík, the variant was again associated with T2D and showed a greater effect than in AGES (OR = 1.69, $P = 9.9 \times 10^{-6}$). Interestingly, it was also significantly associated with greater risk of CHD (OR = 1.32, *P* = 0.007). No statistically significant interactions for either T2D or CHD risk were observed in the REFINE-Reykjavík cohort. The association with both T2D and CHD was only statistically significant in the obese group (Tables 4 – 5, Appendix 2) so effect sizes could not be directly compared between the three BMI groups. The lean group showed a greater OR for T2D than the obese group, although only marginally significant and the three groups did not show a linear change in effect size by BMI (Table 4 – Appendix 2). Triglycerides, HDL or LDL levels did not seem to affect the effect size conferred by the variant on either T2D (Table 4 – Appendix 2) or CHD (Table 5 – Appendix 2).

Table 5 Interaction between rs7903146 *TCF7L2* genotype and BMI, LDL, HDL and triglyceride levels on T2D and CHD risk, adjusted for age and sex. *P*-values < 0.05 are shown in bold.

	AGES				REFINE			
Statistical model	*n*	OR	95% CI	*P*	*n*	OR	95% CI	*P*
T2D ~ SNP	5620	1.29	(1.17 – 1.41)	**2.3×10^{-5}**	3994	1.69	(1.44 – 1.94)	**9.9×10^{-6}**
T2D ~ (SNP*BMI)	5601	1.02	(0.99 – 1.05)	0.206	3974	1.01	(0.97 – 1.06)	0.640
T2D ~ (SNP*LDL)	5614	1.12	(1.00 – 1.25)	0.062	3970	0.83	(0.52 – 1.15)	0.220
T2D ~ (SNP*LDL) [a]	5614	1.12	(1.00 – 1.24)	0.064	3970	0.83	(0.52 – 1.14)	0.193
T2D ~ (SNP*HDL)	5620	1.27	(0.92 – 1.61)	0.141	3994	0.99	(0.20 – 1.78)	0.975
T2D ~ (SNP*TG)	5620	1.06	(0.79 – 1.33)	0.639	3994	1.13	(0.63 – 1.63)	0.590
CHD ~ SNP	5684	0.99	(0.90 – 1.08)	0.814	3916	1.32	(1.11 – 1.54)	**0.007**
CHD ~ (SNP*BMI)	5658	1.02	(1.00 – 1.05)	0.084	3900	1.03	(0.99 – 1.08)	0.124
CHD ~ (SNP*LDL)	5676	1.02	(0.92 – 1.12)	0.677	3894	0.98	(0.69 – 1.27)	0.898
CHD ~ (SNP*LDL) [a]	5676	1.04	(0.94 – 1.14)	0.439	3894	0.94	(0.64 – 1.24)	0.656
CHD ~ (SNP*HDL)	5683	0.95	(0.72 – 1.18)	0.629	3916	0.73	(0.14 – 1.33)	0.243
CHD ~ (SNP*TG)	5684	1.09	(0.89 – 1.29)	0.376	3916	1.13	(0.70 – 1.57)	0.590

[a] Additionally adjusted for statin drugs.

To better understand the discrepancies between the cohorts in the association of the *TCF7L2* locus with CHD, the association was examined after stratification by T2D status. Again, there was no association observed in the AGES-Reykjavík cohort (diabetics: OR = 0.90 (95% CI 0.63 – 1.17), *P* = 0.38, non-diabetics: OR = 0.97 (95% CI 0.85 – 1.09), *P* = 0.56) and the association previously observed in the REFINE-Reykjavík cohort seized to remain statistically significant after the stratification (diabetics: OR = 1.38 (95% CI 0.83 – 1.91), *P* = 0.24, non-diabetics: OR = 1.24 (95% CI 1.00 – 1.48), *P* = 0.054).

The rs7903146 variant was not associated with BMI or waist circumference in either cohort, but when they were stratified by T2D status the association with smaller waist circumference became significant in non-diabetic individuals in AGES-Reykjavík, and the association with lower BMI was borderline significant (Table 6).

Table 6 Association of the *TCF7L2* rs7903146 variant with BMI and waist circumference in both AGES- and REFINE-Reykjavík cohorts, and when stratified for T2D status. *P*-values < 0.05 are shown in bold.

		All			Diabetes			No diabetes		
		β	SE	*P*	β	SE	*P*	β	SE	*P*
AGES	BMI	-0.048	0.072	0.507	0.079	0.219	0.719	-0.144	0.075	0.056
	WC[a]	-0.357	0.242	0.140	0.370	0.689	0.591	-0.647	0.256	**0.011**
REFINE	BMI	0.156	0.481	0.745	0.053	0.583	0.927	-0.045	0.115	0.692
	WC[a]	0.089	0.290	0.760	-0.342	1.474	0.817	-0.095	0.293	0.746

[a]WC: Waist circumference

4.4 Genetic risk scores

After the single SNP association analysis it was clear that few of the novel associations were strong enough to withstand multiple testing corrections. Hence, a T2D-GRS was constructed from the 44 T2D susceptibility variants to reduce multiple testing issues and for increased statistical power. Table 6 - Appendix 2 shows the association of the T2D-GRS with all 30 variables for two statistical models. Figure 10 gives a visualization of both direction of effect and the *P*-value for each association in statistical model A. The T2D-GRS showed a strong association with T2D status in the cohort (β = 0.104, $P = 1.6 \times 10^{-13}$). Figure 11 shows the increasing ORs for T2D risk by number of risk alleles (ORs were calculated for each group, relative to the median group of 44 – 47 risk alleles). Individuals in the highest T2D-GRS group (> 53 risk alleles) had an OR of 2.32 (95% CI 1.46 – 3.69) compared to the median group, whereas the lowest group (≤ 38 risk alleles) had an OR of 0.14 (95% CI 0.03 – 0.58). There was a strong association observed of the T2D-GRS with fasting glucose levels (β = 0.036, $P = 8.8 \times 10^{-14}$) and HbA1c (β = 0.004, $P = 1.3 \times 10^{-7}$), which were attenuated after T2D adjustment in the statistical model (β = 0.013, $P = 5.1 \times 10^{-4}$ and β = 0002, *P* = 0.018 respectively). Furthermore, there was a strong negative association between the score and HOMA-B (β = -0.021, $P = 4.1 \times 10^{-7}$) but not with fasting insulin levels (β = 5.8×10^{-4}, *P* = 0.888) or HOMA-IR (β = 0.008, *P* = 0.062).

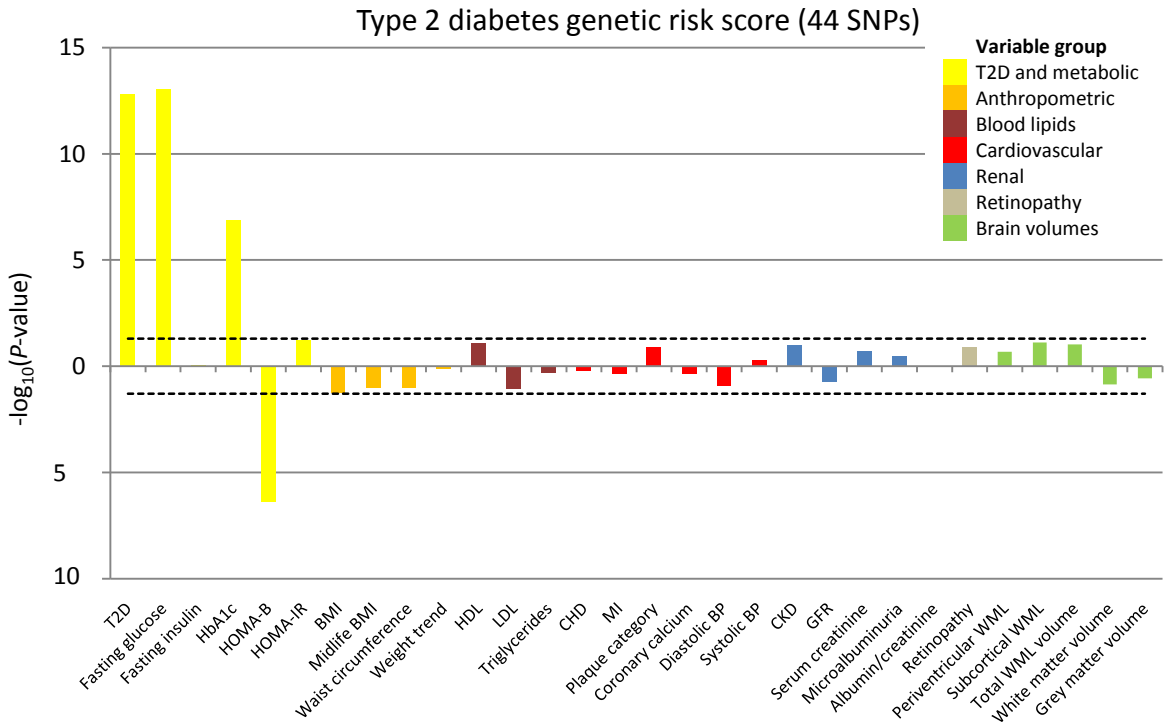

Figure 10 A visualization of the associations from Table 6 – Appendix 2, Model A. The *y* axis represents the *P*-value on a $-\log_{10}$ scale for each association and the dotted line denotes the $P = 0.05$ threshold. The direction of each bar represents the direction of the β-value for the respective association, where a bar pointing upwards represents a positive β-value and a bar pointing downwards represents a negative β-value. The variable categories are color coded. The T2D-GRS was significantly associated with increased risk of T2D, higher glucose and HbA1c and lower HOMA-B.

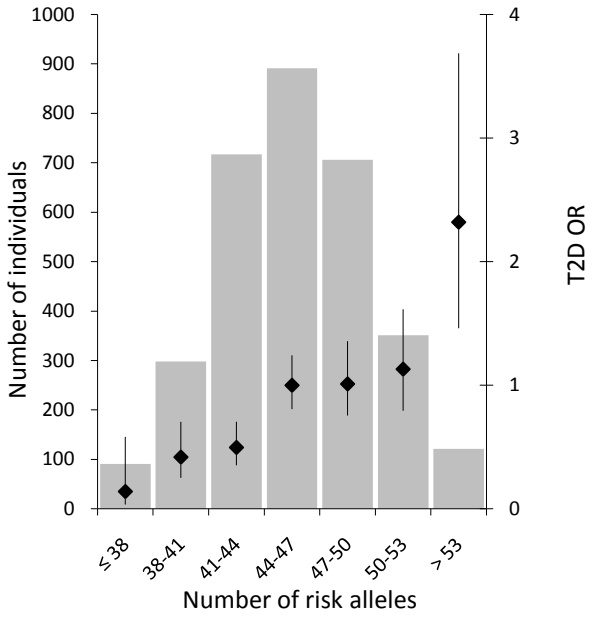

Figure 11 The combined impact of risk alleles on T2D risk. The bar plots represent ORs for T2D (adjusted for age and sex) with 95% CI for each group, relative to the median values of 44 – 47 risk alleles. The minimum number of risk alleles was 31.6 and the maximum number was 60.4 risk alleles.

After adjusting for T2D in the regression model the association of the T2D-GRS with fasting insulin levels became marginally significant (β = -0.007, *P* = 0.083) but not the association with HOMA-IR (β = -0.003, *P* = 0.46). The association of the score with reduced BMI and waist circumference became significant (β = -0.051, *P* = 8.2 x 10^{-4} and β = -0.148, *P* = 0.005 respectively). After the T2D adjustment there was also an association observed between the T2D-GRS and a favorable lipid profile, that is higher HDL (β = 0.005, *P* = 0.003) and lower triglycerides (β = -0.004, *P* = 0.028). Additional adjustment for statin use did not change those results, (β = 0.005, SE = 0.002, *P* = 0.003 and β = -0.004, SE = 0.002, *P* = 0.027 respectively). No statistically significant association was detected for any of the renal traits, retinopathy or brain variables (Table 6 – Appendix 2).

The relationship observed between the T2D-GRS and BMI and blood lipids was further assessed by comparing diabetic individuals from within the lowest or top quartile of the T2D-GRS (Table 7). There were 795 individuals in each T2D-GRS quartile. Within the lowest quartile there were 44 individuals with T2D, or 5.5%, whereas in the top quartile there were 132 diabetic individuals, or 16.6%. There was a significant difference in the mean BMI between the two groups as analyzed with Welch two sample t-test (*P* = 0.004) but there was not a significant difference in blood lipid levels. BMI was plotted against the T2D-GRS with a local regression trendline as shown in Figure 12. As seen on the plot, the trend was not very definite but reached statistical significance because of the large sample size.

Table 7 Comparison between the top and bottom quartiles of the T2D-GRS with regard to T2D, BMI and blood lipid variables. *P*-values < 0.05 are shown in bold.

	T2D-GRS quartile		
Variable	Q1 (*n* = 795)	Q4 (*n* = 795)	*P*
T2D (%)	5.5	16.6	**3×10^{-12}**
BMI (kg/m^2)	25.4 (± 3.5)	24.9 (± 3.4)	**0.004**
Triglycerides (mg/dL)	97.8 (± 1.6)	96.4 (± 1.6)	0.534
HDL (mmol/L)	1.55 (± 0.43)	1.58 (± 0.47)	0.180

For T2D and CHD the prevalence in each quartile is given in percentages. The mean (± SD) for each quartile is shown for BMI, triglyceride and HDL levels.

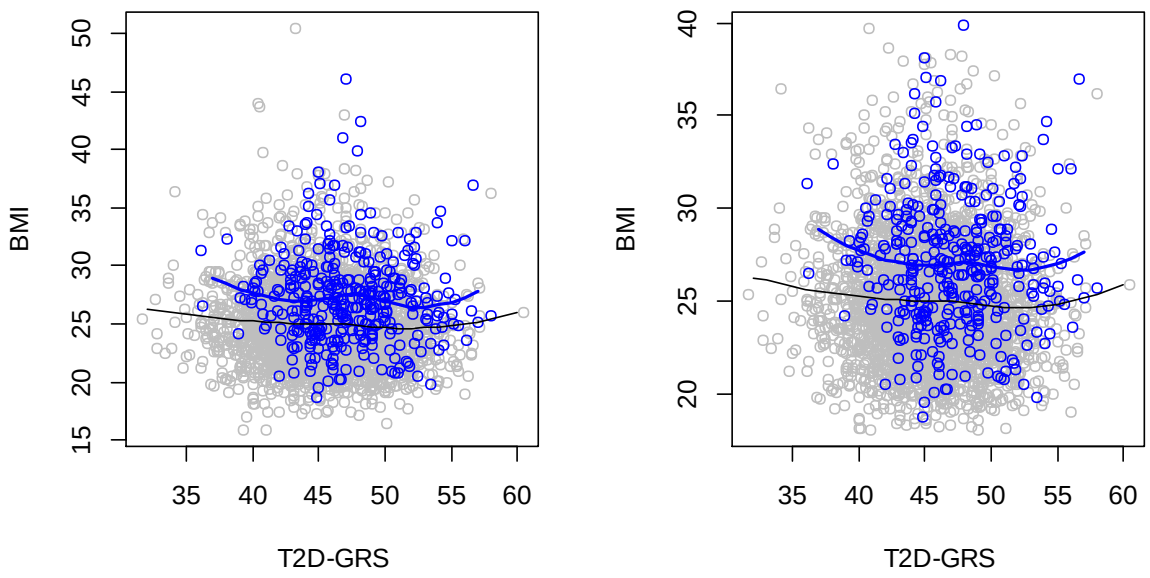

Figure 12 The relationship between the T2D-GRS and BMI in (A) the whole cohort, (B) individuals with BMI between 18 and 40. The blue points represent diabetic individuals whereas the grey points represent individuals without T2D.

A similar analysis was conducted in the REFINE-Reykjavík cohort, to investigate the validity of the results. Genotype data was available for six T2D SNPs in the REFINE-Reykjavík cohort, but only the *TCF7L2* rs7903146 variant showed a statistically significant association with T2D (Table 7 – Appendix 2). A score constructed from these variants did not show a statistically significant association with T2D (β = 0.106, *P* = 0.097) although the association with fasting glucose was evident (β = 0.006, *P* = 2.0 x 10^{-4}) (Table 8 – Appendix 2). The score was associated with both decreased HDL levels (β = -0.011, *P* = 0.005) and increased triglyceride levels (β = 0.012, *P* = 0.039) and did not show a statistically significant association with BMI, waist circumference or LDL (Table 8 – Appendix 2). These results were not consistent with what was observed for the T2D-GRS in the AGES-Reykjavík cohort.

4.4.1 Beta cell function genetic risk score (BCF-GRS)

Of the 44 T2D susceptibility SNPs used in the GRS construction, 22 SNPs had a reported association with BCF (Table 1). A GRS constructed from these SNPs (BCF-GRS) showed an enhanced association with HOMA-B compared to the T2D-GRS (β = -0.034, *P* = 4.6 x 10^{-9}) (Table 9 – Appendix 2).

The six SNPs with unknown function but which showed a suggestive trend towards lower HOMA-B and lower insulin levels in this analysis (Table 2 – Appendix 2) were added to the BCF-GRS, which resulted in an even stronger association with HOMA-B (β = -0.035, *P* = 1.4 x 10^{-11}) (Table 10 – Appendix 2). Figure 13A demonstrates the variation in the HOMA-B variable by number of BCF associated risk alleles. After T2D adjustment the score also showed a significant association with lower insulin levels (β = -0.017, *P* = 8.2 x 10^{-4}) (Table 10 – Appendix 2).

4.4.2 Insulin resistance genetic risk score (IR-GRS)

In the set of 44 T2D SNPs making up the T2D-GRS, there were six SNPs that have been shown to be associated with insulin sensitivity in GWAS (Table 1). A score was constructed from these six SNPs (IR-GRS), which showed an association with both T2D (β = 0.137, *P* = 0.002) and HOMA-IR (β = 0.024, *P* = 0.031) (Table 11 – Appendix 2).

As with the BCF-GRS analysis, SNPs which showed an association trend with HOMA-IR and/or insulin levels in this analysis (Table 2 – Appendix 2), and might thus possibly influence IR, were added to the score. The *IGF1* rs35767 SNP was also added to the group. This resulted in an IR-GRS of 10 SNPs which showed a significant association with T2D (β = 0.137, $P = 1.3 \times 10^{-5}$), increased HOMA-IR (β = 0.032, $P = 5.0 \times 10^{-4}$), fasting insulin (β = 0.026, *P* = 0.005) and glucose levels (β = 0.048, $P = 1.0 \times 10^{-5}$) (Table 12 – Appendix 2). The IR-GRS showed a stronger association with both HOMA-IR (β = 0.033, $P = 3.7 \times 10^{-5}$) and insulin levels (β = 0.027, $P = 7.3 \times 10^{-4}$) after additional adjustment for triglyceride levels. HOMA-IR variation by number of IR associated risk alleles carried is shown in Figure 13B. The association of the IR-GRS with glucose, insulin and HOMA-IR was somewhat attenuated after the adjustment for T2D (Table 12 – Appendix 2), although the association with glucose and HOMA-IR remained statistically significant (β = 0.017, *P* = 0.037 and β = 0.018, *P* = 0.039 respectively).

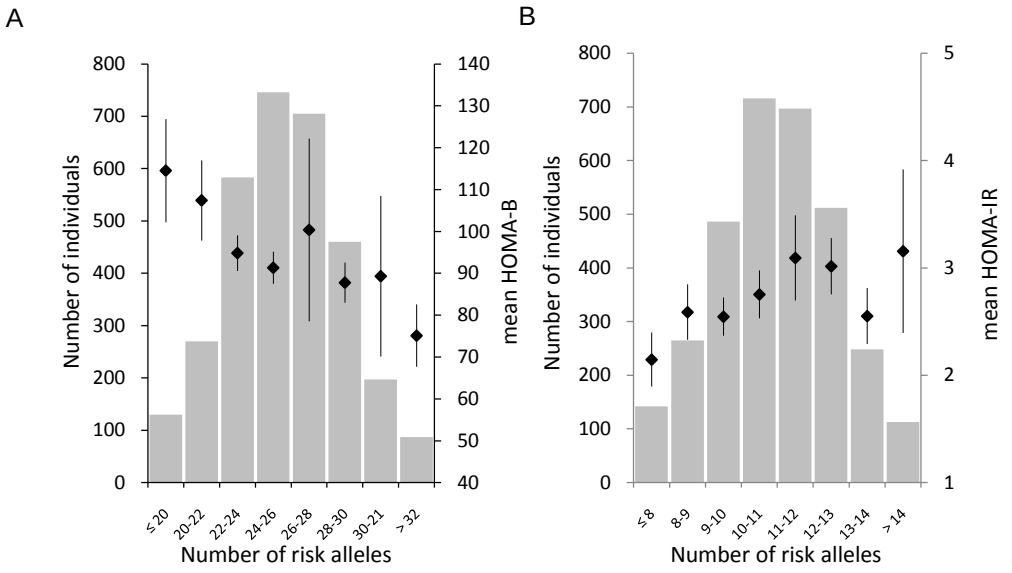

Figure 13 (A) The variation in HOMA-B depending on the number of risk alleles at the 28 BCF associated loci and (B) the variation in HOMA-IR depending on the number of risk alleles at the 10 IR associated loci. The histogram denotes the number of individuals in each genotype score category (left *y* axis) and the bar plots show the mean and the 95% CI (right *y* axis) for (A) HOMA-B and (B) HOMA-IR.

4.4.3 Modulation of anthropometric indices on genetic risk score effect

None of the five GRSs showed any significant interaction with BMI, midlife BMI, waist circumference or weight trend on T2D risk (Table 13 – Appendix 2). However, the T2D-GRS showed a significant interaction effect with BMI, midlife BMI and waist circumference on glucose levels (Table 14 – Appendix 2). The IR-GRS (10 SNPs) showed an interaction with BMI and midlife BMI on fasting glucose and the BCF-GRS (28 SNPs) only with midlife BMI (Table 15 – Appendix 2). As shown in Figure 14, a steeper increase in fasting glucose by T2D-GRS was seen in overweight and obese individuals compared to those with normal BMI. Similarly, the miniscore constructed from six T2D variants in the REFINE-Reykjavík cohort (Table 7 – Appendix 2) showed an interaction effect with BMI and waist circumference on glucose levels (Table 14 – Appendix 2) but not on T2D risk (Table 13 – Appendix 2).

A greater effect size on T2D risk was conferred by all of the GRSs in obese compared to non-obese individuals in the AGES-Reykjavík cohort (Table 8).

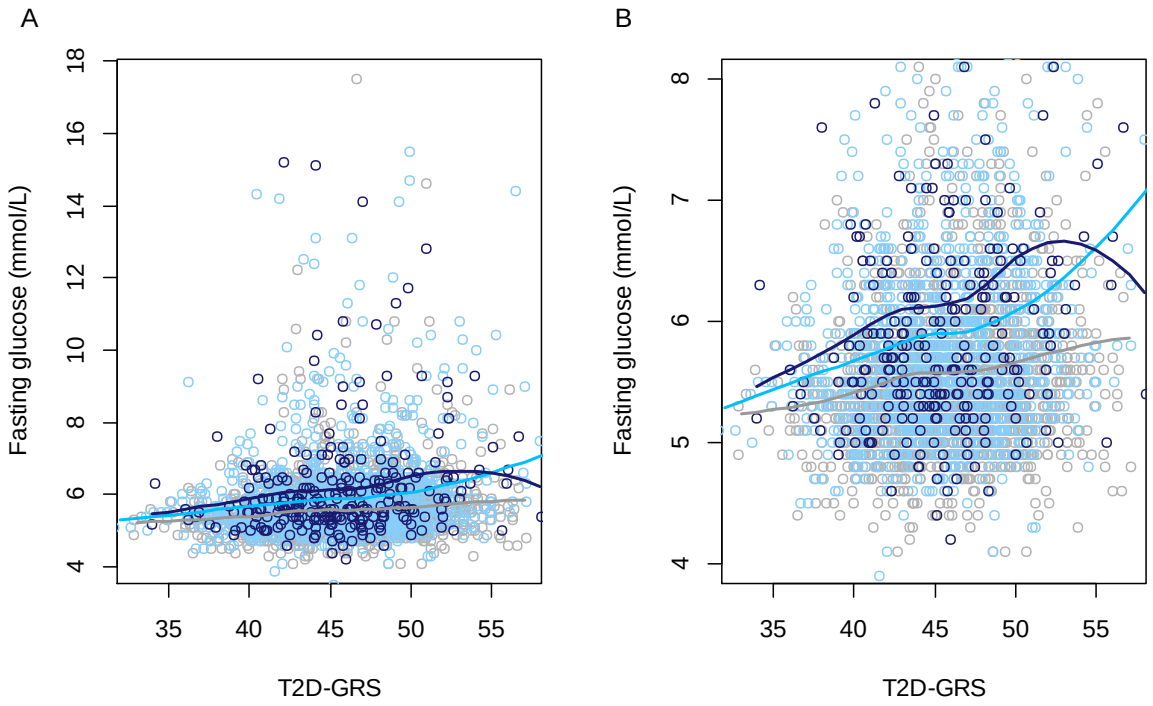

Figure 14 Grey points represent individuals with BMI < 25, light blue are individuals with BMI ≥ 25 & < 30 and dark blue have BMI ≥ 30. (A) The whole cohort, (B) The *y* axis limited to the fasting glucose range of 4 – 8 mmol/L to give a clearer picture of the slopes.

Table 8 Association of the five different GRSs with T2D by obesity status.

	Non-obese (BMI < 30, *n* = 2908)			Obese (BMI ≥ 30, *n* = 260)		
	OR	95% CI	*P*	OR	95% CI	*P*
T2D-GRS	1.11	(1.07 - 1.14)	6.1×10^{-11}	1.14	(1.06 - 1.23)	2.5×10^{-4}
BCF-GRS (22)	1.12	(1.07 - 1.17)	2.1×10^{-7}	1.21	(1.11 - 1.32)	2.9×10^{-4}
BCF-GRS (28)	1.12	(1.08 - 1.16)	1.0×10^{-8}	1.17	(1.07 - 1.28)	7.1×10^{-4}
IR-GRS (6)	1.11	(1.02 - 1.20)	0.015	1.19	(0.99 - 1.44)	0.069
IR-GRS (10)	1.14	(1.06 - 1.22)	1.7×10^{-4}	1.21	(1.02 - 1.43)	0.024

4.4.4 Comparison of the subscores and their association with type 2 diabetes complications

When the two subscores, the BCF-GRS (28 SNPs) and IR-GRS (10 SNPs), were compared (Figure 15, Tables 10 and 12 – Appendix 2) it was clear that the two set of SNPs showed clear differences in their effects on insulin levels. The BCF-GRS was associated with lower insulin levels and decreased HOMA-B, indicating a damaging effect on BCF and insulin secretion, whereas the IR-GRS was associated with higher insulin levels and HOMA-IR, representing IR and hyperinsulinemia.

The BCF-GRS showed the same direction of effect for BMI and other anthropometric measurements as the T2D-GRS and showed a statistically significant association with increased HDL (β = 0.005, *P* = 0.020) and decreased diastolic blood pressure (β = -0.130, *P* = 0.027). The IR-GRS on the other hand showed no significant associations with anthropometric variables and showed the opposite direction of effect for BMI and midlife BMI than the other two scores. The IR-GRS showed no significant associations with lipid or cardiovascular traits. However, a clear association was observed between the IR-GRS and the renal variables GFR (β = -0.476, *P* = 0.005), serum creatinine (β = 0.007, *P* = 0.003) and CKD (β = 0.061, *P* = 0.005), whereas the BCF-GRS showed no such association. There was no significant association of either score with retinopathy, although both showed a direction towards increased risk. The IR-GRS showed modest associations with subcortical WMLs (β = 0.018, *P* = 0.035), total WML volume (β = 0.020, *P* = 0.029) and white matter volume (β = -4.9×10^{-4}, *P* = 0.018), whereas the BCF-GRS did not.

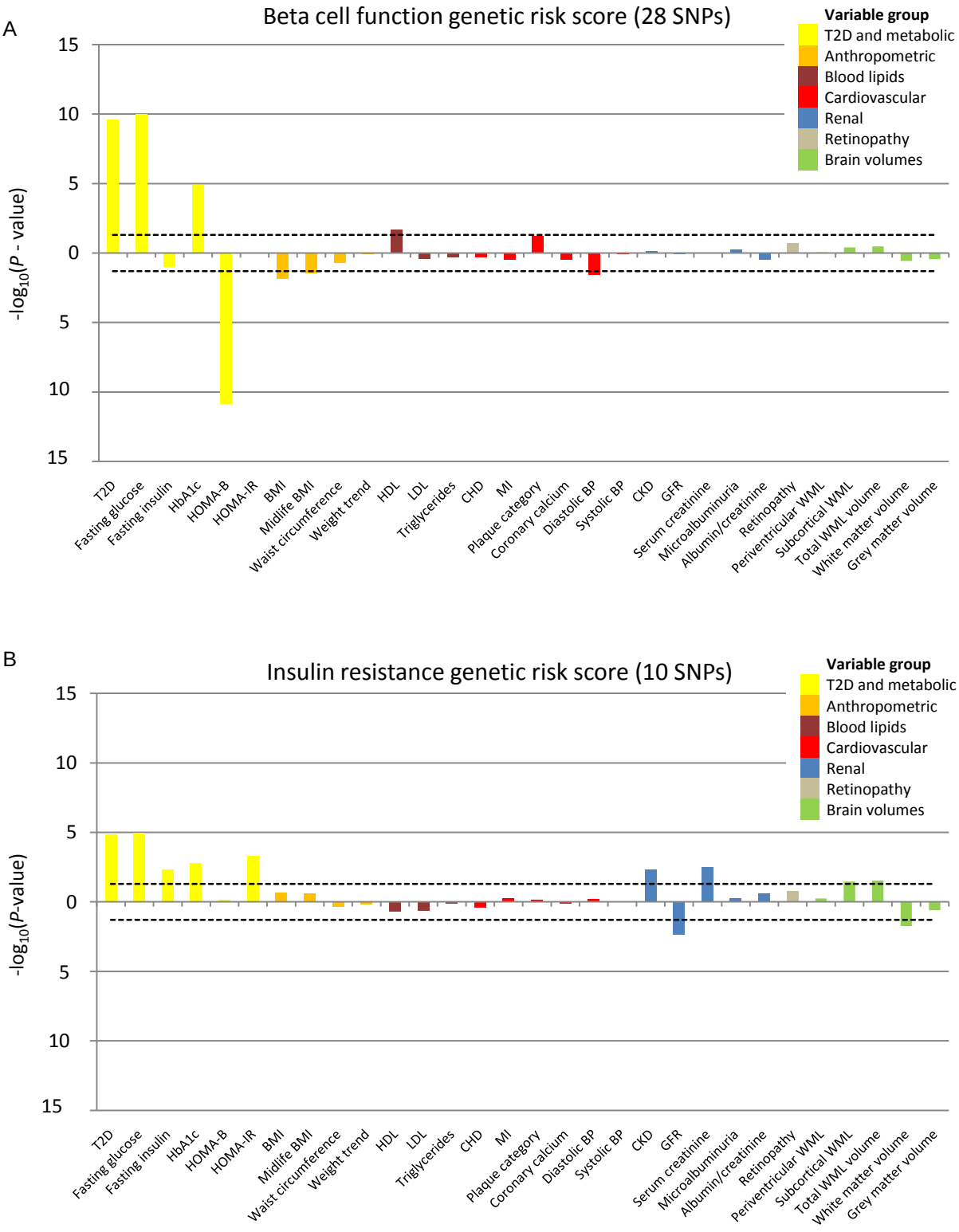

Figure 15 A visualization of the associations in (A) Table 10 – Appendix 2, Model A and (B) Table 12 – Appendix 2, Model A. The *y* axis represents the *P*-value on a $-\log_{10}$ scale for each association and the dotted line denotes the $P = 0.05$ threshold. The direction of each bar represents the direction of the β-value for the respective association, where a bar pointing upwards represents a positive β-value and a bar pointing downwards represents a negative β-value. The variable categories are color coded. The BCF-GRS was associated with decreased insulin levels and HOMA-B, indicating a damaging effect on BCF and insulin secretion, whereas the IR-GRS was associated with higher insulin levels and HOMA-IR, representing IR and hyperinsulinemia. The IR-GRS was associated with worse kidney function and brain volume measurements whereas the BCF-GRS was not.

4.5 The relationship between HOMA-IR and brain and renal phenotypes

The IR-GRS showed statistically significant associations with both some brain volume measurements and renal variables. The association between HOMA-IR and the same phenotypes was investigated to determine if the effect was mediated through increased IR, conferred by the increase in IR-GRS.

The IR-GRS was modestly associated with increased subcortical WMLs, increased total WML volume and decreased white matter volume, but was not associated with periventricular WMLs or grey matter volume changes. On the other hand, HOMA-IR was not associated with any measurement of WMLs, but was strongly associated with decreased white matter volume and modestly associated with a small increase in grey matter volume (Table 9). The association of white matter volume with HOMA-IR was attenuated after further adjustment for BMI but remained strong nonetheless (β = 0.003, SE = 4.3×10^{-4}, $P = 1.05 \times 10^{-10}$).

Table 9 Association of HOMA-IR with brain volume variables. *P*-values < 0.05 are shown in bold.

Variable	β	SE	*P*
Subcortical WMLs	0.006	0.013	0.621
Periventricular WMLs	0.005	0.037	0.885
Total WML volume	-0.004	0.013	0.786
White matter volume	-0.003	2.9×10^{-4}	**1.2×10^{-20}**
Grey matter volume	0.001	4.8×10^{-4}	**0.023**

The phenotypic association between HOMA-IR and GFR, serum creatinine and CKD was very strong (Table 10), consistent with the observed association between the IR-GRS and the same variables, even after the exclusion of diabetics. The correlation between HOMA-IR and GFR (r = -0.14, $P = 9.9 \times 10^{-27}$) compared to the correlation between the IR-GRS and GFR (r = -0.05, *P* = 0.006) is shown in Figure 16. The association of HOMA-IR with microalbuminuria and ACR was much weaker than with the other renal variables, and the association with microalbuminuria did not reach statistical significance in models C and D (Table 10). The association of HOMA-IR with GFR was only borderline significant in the diabetic individuals (β = -1.540, SE = 0.763, *P* = 0.044, *n* = 721), and there was not a significant association with CKD (β = 0.083, SE = 0.084, *P* = 0.32, *n* = 721). However, the CIs were very wide and overlapped with the ones for non-diabetics. Interaction analysis showed that diabetes status did not interact with HOMA-IR on the association with GFR (β = 1.38, SE = 1.04, *P* = 0.19).

Table 10 Association of HOMA-IR with renal variables in different statistical models. Model A: adjusted for age and sex, Model B: adjusted for age, sex and T2D, Model C: adjusted for age, sex, T2D and BMI, Model D: T2D individuals excluded, adjusted for age and sex.

	Model A			Model B		
Variable	β	SE	*P*	β	SE	*P*
CKD	0.298	0.030	4.0×10^{-23}	0.287	0.033	3.9×10^{-18}
GFR	-2.610	0.227	2.5×10^{-30}	-2.689	0.247	2.8×10^{-27}
Serum creatinine	0.036	0.003	5.1×10^{-31}	0.036	0.003	5.3×10^{-26}
Microalbuminuria	0.208	0.046	7.4×10^{-6}	0.093	0.049	0.059
ACR	0.156	0.023	1.5×10^{-11}	0.089	0.024	2.7×10^{-4}
	Model C			Model D		
Variable	β	SE	*P*	β	SE	*P*
CKD	0.287	0.034	8.3×10^{-17}	0.323	0.036	2.6×10^{-19}
GFR	-2.645	0.259	2.6×10^{-24}	-2.865	0.260	7.3×10^{-28}
Serum creatinine	0.035	0.004	3.9×10^{-22}	0.039	0.004	6.5×10^{-27}
Microalbuminuria	0.065	0.052	0.208	0.095	0.055	0.084
ACR	0.073	0.026	0.004	0.088	0.025	5.7×10^{-4}

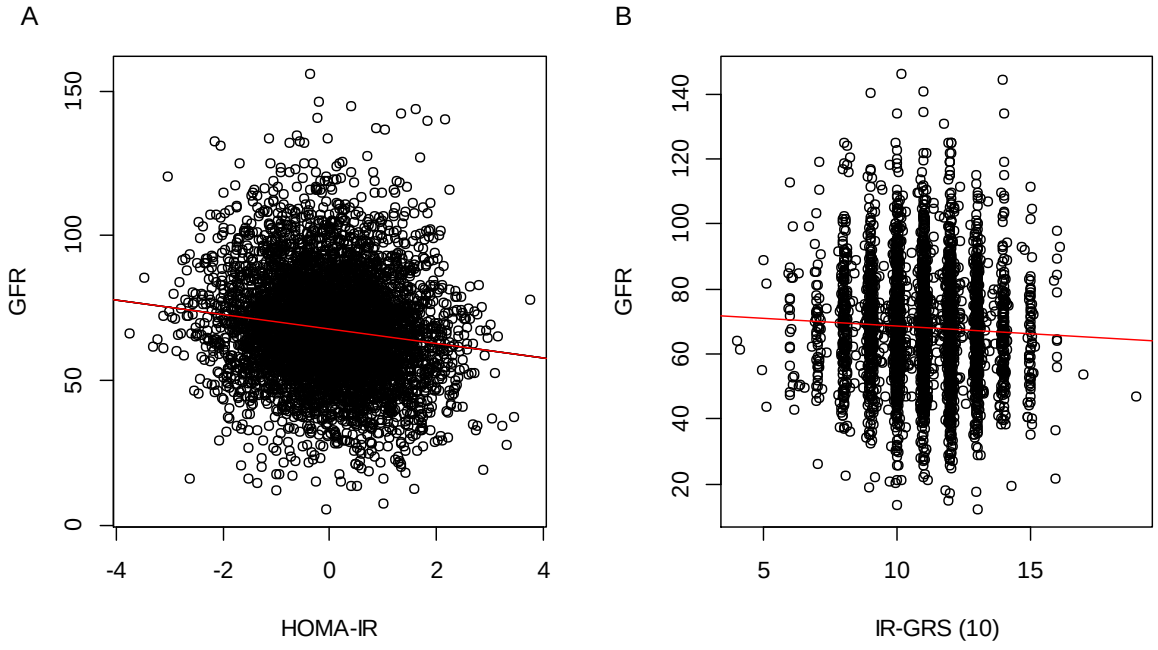

Figure 16 The correlation between (A) HOMA-IR and GFR, (B) IR-GRS (10 SNPs) and GFR.

4.6 Insulin resistance genetic risk score and renal phenotypes

The association of the three GRSs with the renal traits is highlighted in Table 11, where a clear association between the IR-GRS and CKD, GFR and serum creatinine was observed but no such relationship was detected for the other two scores. The cohort was divided into quartiles by the IR-GRS and ORs for CKD risk were calculated for each quartile, relative to the lowest one (Table 12). The OR for the top quartile relative to the lowest one was 1.30 (95% CI 1.05 – 1.62) after adjusting for age and sex.

Table 11 Association of the three GRSs with renal variables. *P*-values < 0.05 are shown in bold.

	T2D-GRS (44)			BCF-GRS (28)			IR-GRS (10)		
Trait	β	SE	*P*	β	SE	*P*	β	SE	*P*
CKD	0.016	0.010	0.10	0.004	0.012	0.73	0.061	0.022	**0.005**
GFR	-0.097	0.075	0.20	-0.018	0.094	0.85	-0.476	0.168	**0.005**
Serum creatinine	0.001	0.007	0.20	2.0×10^{-4}	0.001	0.88	0.007	0.002	**0.003**
Microalbuminuria	0.013	0.014	0.34	0.009	0.017	0.59	0.020	0.031	0.511
ACR	3.8×10^{-4}	0.007	0.96	-0.009	0.009	0.32	0.019	0.016	0.235

Table 12 ORs for CKD by IR-GRS (10 SNPs) quartiles relative to the lowest quartile. *P*-values < 0.05 are shown in bold.

	Model 1[a]			Model 2[b]		
IR-GRS (10) quartile	OR	95% CI	*P*	OR	95% CI	*P*
Q2	1.01	(0.81 - 1.25)	0.938	1.04	(0.83 - 1.29)	0.738
Q3	1.15	(0.93 - 1.42)	0.201	1.16	(0.94 - 1.45)	0.172
Q4	1.28	(1.04 - 1.59)	**0.021**	1.30	(1.05 - 1.62)	**0.016**

[a]unadjusted, [b]adjusted for age and sex.

Automatic stepwise regression was carried out to estimate the relative importance of each IR associated SNP within the relationship between the score and GFR (Figure 17). The total R^2 for the association was 0.46%, out of which the variants *FTO* and *ADAMTS9* explained the majority of the variance, or 47%. All of the SNPs however did contribute to the association, except for the one in the *HMGA2* locus. The bootstrap analysis of the association between the IR-GRS and GFR gave a normally distributed curve of the estimates (Figure 18). The β-value from the bootstrap analysis was -0.469 (95% CI -0.798 – -0.143).

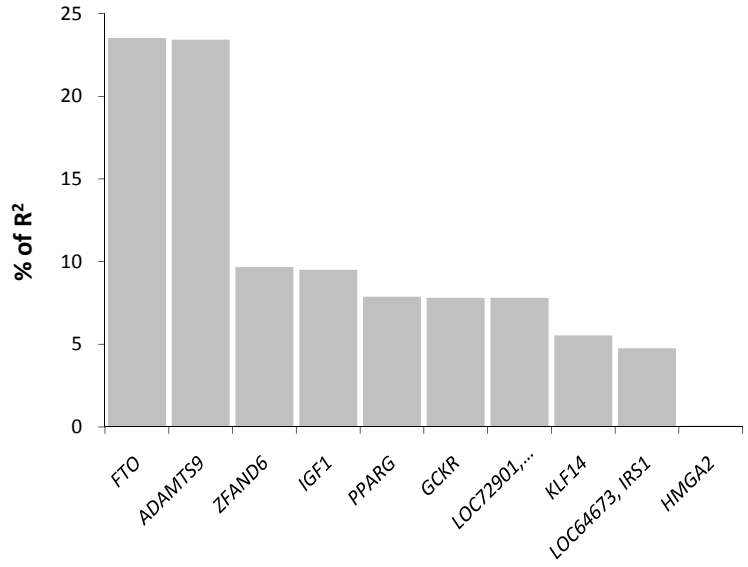

Figure 17 The relative importance of each SNP within the IR-GRS on GFR as estimated by automated stepwise regression and adjusted for age and sex. The total R^2 for the model was 0.46%.

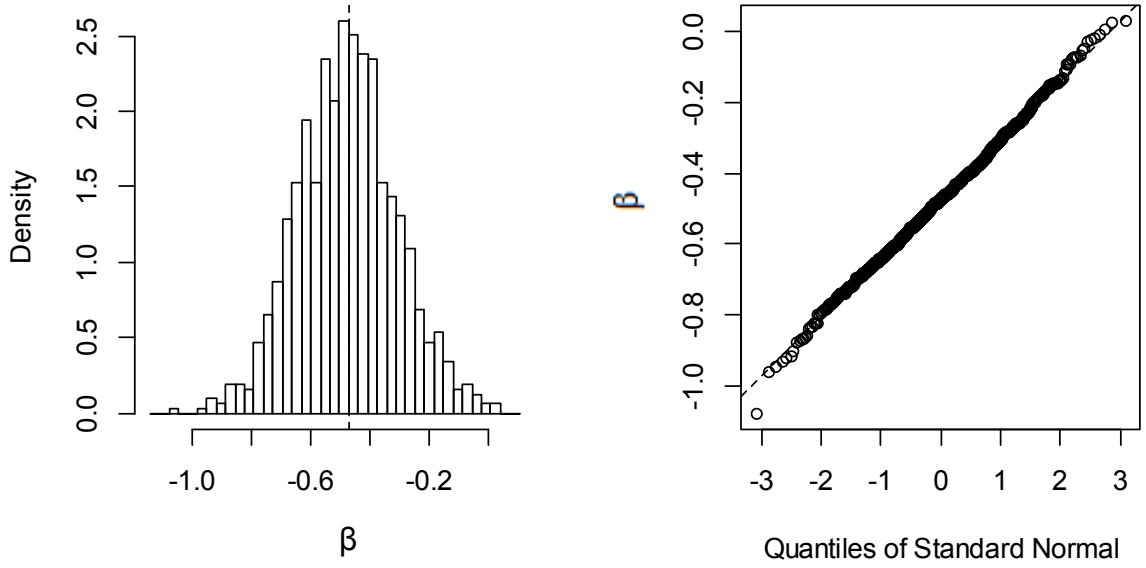

Figure 18 Bootstrapping of the estimate from the association between the IR-GRS (10 SNPs) and GFR, age and sex adjusted.

4.7 Mendelian randomization analysis

To assess the causal relationship between IR and renal function, an instrumental variable regression was carried out (Figure 19). The strength of the IR-GRS (10 SNPs) as an instrument was assessed by the F-statistic from the first stage regression, that is the association between the IR-GRS and HOMA-IR. The F-statistic from the first stage regression was 12.13, indicating sufficient strength of the instrumental variable for the analysis. The variance of HOMA-IR explained by the IR-GRS was 0.38%.

The instrumental variable regression results suggested a causal effect of IR on GFR (β = -0.854 SD GFR, SE = 0.358, *P* = 0.017) after adjusting for age and sex, and remained similar after additional adjustment for triglycerides (β = -0.842 SD GFR, SE = 0.339, *P* = 0.013). The Durbin-Wu-Hausman test showed that the instrumental variable regression model showed statistically significantly different estimates than the normal OLS model (*P* = 0.02). The instrumental variable regression association remained significant after further adjustment for BMI (β = -0.892 SD GFR, SE = 0.374 *P* = 0.017). After the exclusion of diabetic individuals the association was attenuated and did not reach statistical significance when adjusted for age and sex (β = -1.41, SE = 0.800, *P* = 0.091, *n* = 2811), but became significant when additionally adjusted for BMI and triglycerides (β = -1.093, SE = 0.540, *P* = 0.043).

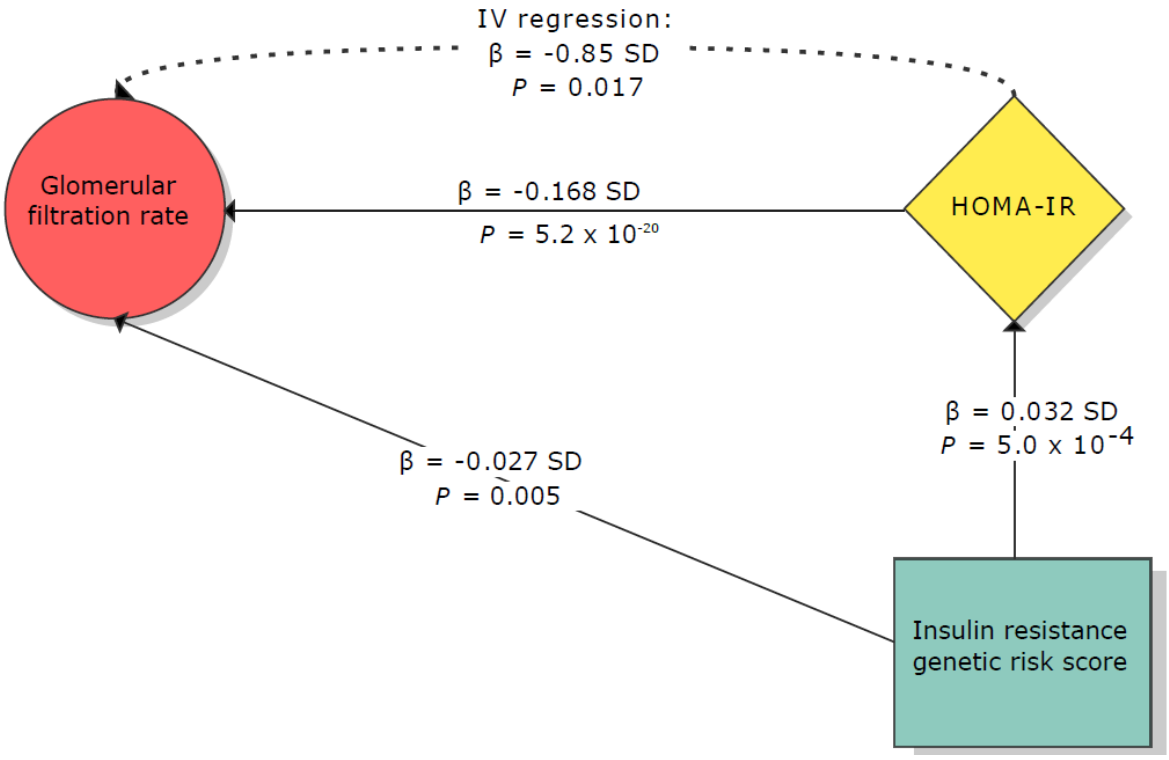

Figure 19 An overview of the Mendelian randomization analysis. The unbroken lines denote the normal OLS regression analyses, whereas the dotted line denotes the instrumental variable regression where the IR-GRS was used as an instrument to examine the unconfounded relationship between HOMA-IR and GFR. All models were adjusted for age and sex.

4.8 Longitudinal analysis

HOMA-IR was predictive for a decrease in GFR in the AGESII five year follow-up visit (β = -0.643, SE = 0.279, *P* = 0.021, *n* = 2,910) and was marginally significantly associated with serum creatinine increase (β = 0.007, SE = 0.003, *P* = 0.059, *n* = 2,910). Of the 2,100 individuals who did not have CKD at the AGES visit, there were 250 individuals who developed CKD in the five year follow-up period. HOMA-IR at the AGES visit was not significantly associated with having developed CKD at the AGESII visit (OR = 1.11, 95% CI 0.97 – 1.25, *P* = 0.12).

5 Discussion

5.1 Population characteristics

The AGES-Reykjavík population is a community based cohort of survivors from the Reykjavík Study. There were more female participants than male in the study (57.6%) as their life expectancy is greater and therefore more survivors, reflecting what is seen in the general population. The AGES-Reykjavík cohort is an elderly population, with mean age 77.0 ± 5.9 years, so the prevalence of T2D and other common chronic diseases, including CHD and CKD, was accordingly high. Almost 13% of the population suffered from diabetes, out of which one third had developed CHD, four out of ten had developed CKD and a quarter had developed some sort of retinopathy. Diabetes was more common in males than females, which is consistent with what has been observed elsewhere this age group [146, 147]. Diabetic individuals had significantly higher BMI, higher fasting glucose, fasting insulin and HbA1c as expected. They had higher triglyceride levels and lower HDL, but also lower LDL levels. This is likely explained by the significantly greater portion of diabetic individuals who are on statin drugs, which lower LDL cholesterol. As expected, the diabetic individuals had worse outcome for almost all of the complication phenotypes examined, except for diastolic blood pressure, which is a less important risk factor for CVD than systolic blood pressure [148], and periventricular WMLs. Studies on brain lesions in diabetic individuals have reported a significant difference in subcortical WML load in diabetic individuals compared to controls but no difference in periventricular WMLs [149, 150], which is consistent with the observation in this study.

The subpopulation of the REFINE-Reykjavík cohort with available genotype data was used in the analysis for replication and validation of results. The cohort was much younger than the AGES-Reykjavík population, with a mean age of 49.0 ± 12.1 years, and the prevalence of T2D and CHD was lower.

5.2 Single SNP associations

5.2.1 Metabolic phenotypes

A quarter of the SNPs in the analysis showed an association with T2D where $P < 0.05$. This was slightly less than anticipated given the statistical power calculations, however using the average SNP frequency and OR is a rather crude method to estimate an exact number of expected associations. The statistical power might also have been slightly overestimated by the use of ORs instead of genotype relative risks and some of the reported ORs might be inflated because of the effect known as the “winner’s curse”. However, the set of SNPs chosen for the analysis was clearly enriched for T2D associations, as one would only expect to see 2 – 3 associations where $P < 0.05$ by chance alone. Furthermore, the majority of the associations showed the expected direction of effect for T2D, glucose and HbA1c. The expected effects on insulin levels were not as clearly observed. Only half of the variants associated with reduced BCF, which should result in decreased insulin secretion, showed an association trend with lower insulin levels. Six of the seven reported IR associated SNPs showed an association trend with increased insulin level. Analysis on HOMA-B and HOMA-IR was more

consistent with the expected results, but the majority of the reported BCF SNPs showed a trend towards lower HOMA-B and all of the IR SNPs showed a trend toward increased HOMA-IR. The *GCKR* rs780094 variant showed a strong association with reduced triglycerides so its associations were adjusted for triglycerides to remove the negatively confounding effect. After the adjustment, the variant was significantly associated with HOMA-IR, fasting insulin and fasting glucose.

Six SNPs, from the *VEGFA, NOTCH2, ZBED3, TP52INP1* and *PTPRD* loci, were highlighted in this analysis as possibly having a role in BCF impairment and three, from the *HMGA2, ZFAND6* and *LOC729011-CETN3* loci, in insulin action. Although only few of these associations reached statistical significance, the trend may be considered as a suggestive involvement in the respective mechanism. *VEGFA* has been shown to possibly have a weak effect on insulin secretion [123] and *SRR* has been suggested to play a part in insulin secretion based on the biological function of the gene product [127]. *NOTCH2* has a biological link to BCF [151] but a couple of studies have failed to detect an association between the rs10923931 variant and measures of insulin secretion [107, 123]. Both of these studies used insulin secretion estimates from oral glucose tolerance tests (OGTT) instead of HOMA-B. Measures of insulin secretion using these two methods have been shown to correlate well, although the OGTT method is considered to be superior in accuracy [152]. It is difficult to say if the different methods are to blame for the discrepant findings, but the biological role of the gene supports the associations observed in this study. The product of *ZBED3* has a role in the Wnt signaling pathway [153], possibly indicating a biological function involved in insulin secretion [154], but *TCF7L2* is also a part of the same pathway [155]. In a recent report a molecular connection between *TCF7L2* and *TP53INP1* was shown, and furthermore, its effect on beta cell survival [156]. No convincing associations of *PTPRD* with metabolic traits have been reported in the literature to date.

The *HMGA2* gene has been suggested to have a role in fat-cell proliferation [126] and might thus influence insulin sensitivity. The function of *ZFAND6* and *LOC729011-CETN3* in T2D pathogenesis is completely unknown. Interestingly, the product of *ZFAND6* physically interacts with tumor-necrosis-factor receptor-associated-factor-2 (TRAF2) [157]. TRAF2 plays an important role in ER stress [158] which, as previously mentioned, is thought to underlay the development of IR [53].

5.2.2 Other phenotypes

The majority of the strongest associations of the T2D variants with other phenotypes had previously been reported in the literature. The association of the *FTO* loci with BMI is well established [78], and so is the relationship between *CDKN2B* and CAD [100, 159] and *GCKR* and triglyceride levels [160]. The association of *HNF1B* variants with lipoproteins was however only recently reported [161].

The only novel association observed in this analysis that was considered statistically significant was the one of the rs4457053 variant with decreased waist circumference. The SNP is located between the genes *ZBED3* and *PDE8B* on chromosome 5. It is presently unknown how this variant increases risk of T2D but, as previously mentioned, the gene product has recently been identified as an activating component of the Wnt signaling pathway, which *TCF7L2* is also involved in. Interestingly, *TCF7L2* has also been associated with decreased BMI. The Wnt signaling pathway is important for embryogenesis and cell proliferation, and has been extensively studied in regard to cancer mutations

[162]. It has a role in adipogenesis regulation and adipocyte differentiation [163] which may explain the association with smaller body mass. The Wnt signaling pathway also regulates bone mass [164], and it may also be possible that the association observed with reduced body mass is modulated to some extent through bone density. Finally, a SNP within the *PDE8B* gene, rs4704397, located 93 kb away from rs4457053 (although not in LD, $r^2 = 0.034$, D = 0.31) has been associated with thyroid function at a genome-wide significance level [165], which in turn may affect BMI [166]. If one was to follow up on the association between rs4457053 and smaller waist circumference and BMI it would be possible to look at the relationship between rs4457053 and CT scan measurements of regional specific fat distribution, bone density measurements and thyroid stimulating hormone to try to determine which of those three possible mechanisms might be the mediating one.

A number of other novel associations were observed in the analysis but none of those reached statistical significance when the multiple testing correction was considered. It is therefore unclear which of those are true signals, and biological evidence or a replication would be needed to confirm the significance of those findings. Below, the most interesting associations are discussed in the context of previously reported biological data which may give base to a possible true mechanism.

The rs17036101 variant, located between the genes *PPARG* and *SYN2*, showed an association with subcortical WMLs in brain, a marginally significant association with periventricular WMLs and the rs1801282 SNP (which is in LD with rs17036101, $r^2 = 0.49$, D = 0.98) was associated with periventricular WMLs. Both of the genes are possible culprits within this association. *SYN2* encodes for the protein synapsin II, which is expressed in brain and central nervous system and is involved synaptic development, transmission and plasticity of neurons [167]. *PPARG* has three different splicing isoforms, PPARγ2 being the one primarily expressed in adipocytes whereas PPARγ1 is expressed in most cells, including neurons and glia in the brain. PPARγ activation is known to be neuroprotective [168] and the expression of *PPARG* has been shown to change in response to ischemia in the brain [169]. The *SYN2,PPARG* rs17036101 variant was also associated with coronary calcium and plaque category in this analysis, which is consistent with previous association of the rs1801282 variant with carotid atherosclerosis [170].

A variant close to the gene *CETN3* was associated with increased risk of retinopathy in the study. The gene product, centrin-3, is a member of the centrin protein family which may have a role in retinal cell signaling [171], giving biological grounds for the observed association.

Some of the variants which showed associations with more than one phenotype in the analysis were surprisingly inconsistent with their directions. For example, the *TSPAN*,*LGR5* rs7961581 variant was associated with decreased triglyceride levels but at the same time with increased risk of CHD. Similarly, the *ADCY2* rs11708067 variant was associated with increased coronary calcium but also with higher HDL levels. This might question the validity of associations which were not statistically significant after adjusting for multiple testing. However, another example shows that some of the non-statistically significant associations may very well be true. The rs17584499 *PTPRD* variant was very modestly associated with plaque severity, MI and CHD ($P = 0.01 – 0.05$) in the analysis, but *PTPRD* has just recently been identified as a risk locus for CAD [172]. This supports the idea that there may

be real associations identified in this analysis even though they were not statistically significant when considering the multiple testing correction.

5.3 *TCF7L2* association analysis

The *TCF7L2* locus is the strongest locus that has been identified for T2D through GWAS and before its discovery had no anticipated biological connection to T2D pathogenesis. Therefore a lot of research has been focused on variants from this locus and possibly modulating factors of their effect. Since its discovery the locus has been associated with some complications of T2D.

TCF7L2 variants have been associated with nephropathy in T2D patients [173] and with reduced kidney function in the population based cohorts of the Atherosclerosis Risk in Communities Study (ARIC), Framingham Heart Offspring Cohort (FHS) and Heredity and Phenotype Intervention Heart Study (HAPI) [174]. No evidence for this association was observed in the present study.

In a recent study by Muendlein et al. [175] on 1,650 coronary angiography patients an association was detected between *TCF7L2* variants and CAD in T2D patients only. Conversely, a previous study by Sousa et al. [176] on 889 patients undergoing cardiac catheterization observed an association between the *TCF7L2* genotype and CAD, only in non-diabetic individuals. Buraczynska et al. [173] demonstrated a marginally significant association of the rs7903146 genotype with increased risk of cardiovascular events in a study on 980 Polish T2D patients and an association was reported between *TCF7L2* and increased risk of cardiovascular disease in the population based ARIC sample, but only in lean individuals [177]. Conversely, in the population based inCHIANTI study on the elderly a significant association was observed between the rs7903146 variant and decreased risk of MI and metabolic syndrome in individuals with T2D or IGT, and favorable lipid profile [178]. It is difficult to conclude anything about the association between *TCF7L2* and coronary and cardiovascular disease from the published data. As *TCF7L2* is thought to have a greater effect on T2D risk in lean individuals compared to obese, it is not inconceivable that a similar effect can take place for CHD risk, as demonstrated in the ARIC study. However, there are discrepancies between the studies in regard to T2D status, where Muendlein et al. and Buraczynska et al. seemed to observe an increased CAD or CVD risk in diabetic individuals, whereas Sousa et al. only found this association in non-diabetic individuals and Melzer et al. reported a decreased risk of MI in diabetic subjects associated with the same variant in the inCHIANTI study.

An attempt was made to determine the nature of the relationship between the *TCF7L2* rs7903146 variant and CHD in the AGES-Reykjavík study but no such association was observed in the cohort. Partition by BMI or T2D status did not change those results. However when the same analysis was performed in the REFINE-Reykjavík cohort, a statistically significant association of the rs7903146 variant with CHD was observed, with an OR of 1.32 per risk allele. The association was only significant in obese individuals, which is the opposite of what has previously been reported in the ARIC study [177], and ceased to remain statistically significant when the cohort was stratified by T2D status. Finally, the variant showed a significant association with decreased BMI and waist circumference in non-diabetic individuals in AGES-Reykjavík, but not in REFINE-Reykjavík.

If one was to assume that the association observed in REFINE-Reykjavík is a true one then the reason why it was not detected in the AGES-Reykjavík cohort is unclear. The main difference between the two cohorts lies in their age range, but the mean age in AGES-Reykjavík was 77 years, ranging from 66 to 98 years, whereas the REFINE-Reykjavík mean age was 49 years and ranged from 20 to 73 years old. As age is a strongly contributing factor to the development of CHD it may be more difficult to discriminate between those in AGES-Reykjavík who have developed atherosclerosis through lifestyle choices or the natural process of aging and those who have genetic susceptibility to CHD, whereas in younger individuals the genetic contribution may weigh more heavily on the CHD development, as fewer individuals have developed CHD through aging and environmental factors.

Melzer et al. reported a favorable effect of the rs7903146 T allele on lipid profile in a population based sample of a similar age as the AGES-Reykjavík cohort and a reduced risk of metabolic syndrome and MI associated with the T allele in those with T2D and IGT [178]. The protective effect of rs7903146 observed by Melzer et al. and its lack of association with CHD in AGES may possibly be modulated through its association with decreased waist circumference and BMI in elderly individuals.

5.4 Genetic risk scores

The T2D-GRS, constructed from 44 established T2D risk variants, showed a strong association with T2D, increased glucose and HbA1c levels as expected. The associations with glucose and HbA1c were largely attenuated after adjusting for T2D, indicating that they were mainly driven through T2D. The score showed a strong association with decreased HOMA-B, which is consistent with previous reports [84]. The association remained strongly significant after the adjustment for T2D, indicating that the association should be present in diabetics and non-diabetics alike. This consistently observed association has given rise to the hypothesis that the genetic architecture of T2D mainly affects BCF, making some individuals more susceptible than others to beta cell failure and the subsequent progression to T2D. There was no association of the score with insulin levels, but it became marginally significant after adjusting for T2D. Conversely, the borderline significant association with HOMA-IR disappeared after the T2D adjustment. This might indicate that the association of the score with HOMA-IR was mediated through its effect on T2D risk, but the correlation between T2D and high insulin levels was a negatively confounding factor on the association with decreased insulin levels, so it only became evident after adjusting for T2D.

The T2D-GRS was dissected into to two subscores as previously described, based on the variants independent associations with BCF or IR, in other studies and this analysis. The two subscores showed clear dissimilarities in their associations with the metabolic variables, which highlighted the different roles of the genetic variants in the pathogenesis of T2D and supported the validity of the score construction. The set of 22 SNP with an established association with beta cell impairment exhibited a strong effect on reduced HOMA-B, and furthermore, the addition of six SNPs whose function in the literature is largely unknown, considerably enhanced this signal. Even though these SNPs have not been individually associated with any quantitative metabolic traits, their combined effect seemed to add to BCF impairment in this sample.

Similarly a corresponding score for SNPs associated with insulin sensitivity was constructed. Only seven of the 44 selected T2D SNPs had been convincingly associated with increased IR in the literature, as shown in Table 1, out of which two were from the same loci. A score constructed from six of those variants showed an association with T2D and HOMA-IR, albeit not a very strong one. When four additional SNPs were included in the score, based on a reported association with HOMA-IR or associations with fasting insulin levels and HOMA-IR in this analysis, the association of the score with HOMA-IR became stronger, indicating that the combined effect of IR associated SNPs increases the risk of developing the IR phenotype. The association of the score with HOMA-IR and insulin levels became stronger after adjusting for triglyceride levels, probably because of the negative confounding effect of the *GCKR* rs780094 C allele, which was strongly associated with decreased triglycerides but also with increased HOMA-IR and insulin levels.

An interaction between the T2D-GRS and IR-GRS (10 SNPs) and BMI on fasting glucose levels was observed, but not on T2D risk which was probably a result of reduced statistical power for the dichotomous variable. A greater effect on glucose levels was observed in those with higher BMI. The BCF-GRS (28) showed a hint of a similar interaction for midlife BMI on fasting glucose, but did not show any evidence of a greater effect in lean individuals as has been suggested for variants affecting BCF [93].

The two subscores clearly and distinctly affect BCF and IR but it is impossible to determine if the variants within each score propagate their effects through similar or distinct biologic mechanisms. The genetics of complex disease produce alterations in the molecular interactions of cellular pathways and networks underlying pathophysiological states. Thus, the collective effect of many genetic alterations in disease may become clear through the organized structure of molecular networks. The two set of SNPs were therefore applied as seeds for construction of gene regulatory Bayesian networks in human adipose tissue, but the method has been previously described [179]. Eight of the 10 IR risk loci clustered together in a Bayesian subnetwork, along with the *ADCY5* BCF locus, while 14 of the 28 BCF associated loci clustered together as a separate subnetwork, along with *CETN3* and six other loci with unknown function in T2D pathogenesis, indicating a higher connectivity between these loci than would be expected from a randomly chosen set of SNPs (Valur Emilsson, personal communication). There was highly distinct functional category enrichment between the two sets of SNPs. The BCF network was enriched for processes essential for cell survival, such as cell cycle regulation, which is consistent with the findings of Voight et al. [73] and suggests that dysfunctional beta cell mass regulation is predisposing for impaired glucose homeostasis and T2D. On the other hand, the IR network was enriched for very different functional categories, mostly related to metabolism and energy pathways, as well as with aging, suggesting a very different mechanism for the T2D susceptibility exerted by those particular SNPs. The enriched categories for each subnetwork along with the respective *P*-values are shown in Table 15 – Appendix 2. The two subnetworks can be perceived as a validation of the SNP categorization into BCF and IR groups, which the GRSs were constructed from, and may provide insights into the selection of novel candidate genes for each group.

The T2D-GRS was associated with lower BMI and waist circumference and a favorable lipid profile of increased HDL and decreased triglycerides, after T2D adjustment. This was an unexpected finding.

T2D may be a negatively confounding factor as it is positively correlated with BMI and increases risk of dyslipidemia, whereas the score was inversely associated with BMI and less risk of atherogenic lipid profile, so the relationship was only evident after adjusting for T2D in the statistical model. A similar pattern was observed for the subset of BCF associated variants, but the IR-GRS showed no statistically significant association with anthropometric or lipid traits. The observed effect was thus likely driven by variants in the BCF associated group. A miniscore from six BCF associated SNPs in the REFINE-Reykjavík cohort showed a significant association with decreased HDL and increased triglyceride levels, the opposite of what was observed in AGES-Reykjavík, and no association with BMI or waist circumference. Therefore the unexpected associations seemed to be specific to the AGES-Reykjavík cohort, possibly because of the age of the population or even a sampling bias.

5.4.1 Insulin resistance genetic risk score and its associations

Unlike the T2D-GRS and BCF-GRS, the IR-GRS showed statistically significant associations with both brain volume measurements and renal function variables. To determine if these associations were driven through the intermediate phenotype IR or a different mechanism altogether, the phenotypic relationship between HOMA-IR and the respective variables was tested.

The IR-GRS was associated with decreased white matter volume, increased subcortical WML load and total WML volume, whereas the HOMA-IR phenotype was only strongly associated with decreased white matter volume but not with any other measurement of brain volume. The association of the score with white matter volume may therefore be modulated through IR but the association of the score with WMLs seems to be independent of the HOMA-IR phenotype. This is consistent with a recent report from the Framingham Offspring Study, where HOMA-IR was associated with total cerebral brain volume but not with white matter hyperintensity volume [180].

The phenotypic relationship between HOMA-IR and renal function and kidney disease was very strong, even after adjustment for confounding factors and the exclusion of diabetic individuals. None of the associations were statistically significant in the diabetic individuals alone, which is most likely a result of a reduced statistical power, as the 95% CI were very wide and overlapped with the ones for non-diabetics.

The relationship between IR and kidney function has been described in the literature for years but has not been investigated from a genetic point of view before and the causality within the relationship has not been firmly established. It was therefore of a great interest to see the association between the IR-GRS and renal variables, which suggested a causal effect of IR on renal impairment. The bootstrap analysis showed that the estimate from the association between the score and GFR was valid. The causality between IR and GFR was investigated in this study using instrumental variable regression with genetic instruments, based on the Mendelian randomization principle. The instrumental variable regression showed a significant association between the IR-GRS and GFR, supporting the former observation. The IR-GRS only explained a small percentage of the HOMA-IR variance but the first-stage regression F-statistic was high enough to enable the use of the IR-GRS as an instrumental variable. The Durbin-Wu-Hausman test showed that there was a statistically significant difference between the regression estimates from the OLS and the instrumental variable regression, and thus

further validated the use of the Mendelian randomization study in this analysis. Adjustment was made for triglyceride levels because of the pleiotropic, and possibly confounding, effects of the *GCKR* rs780094 variant in the score. HOMA-IR is highly correlated with BMI, but the association was still significant after additional BMI adjustment, indicating that the IR itself is the causal factor rather than obesity. This theory is supported by a recent study where obese insulin resistant subjects had significantly worse renal function than obese metabolically healthy individuals [181]. The instrumental variable regression association was attenuated when diabetic individuals were excluded from the cohort but became statistically significant when adjusted for BMI and triglycerides. This suggests that the association of IR with renal function is independent of both BMI and T2D, after adjusting for the confounding effects of lower triglyceride levels associated with the *GCKR* rs780094 variant. Interestingly, the effect size became greater when diabetic individuals had been excluded from the analysis.

The IR-GRS was not associated with microalbuminuria or ACR in the cohort. A correlation between IR and microalbuminuria has been reported [182] but the association did not reach statistical significance in the AGES-Reykjavík cohort after adjustment for age, sex, T2D and BMI. Microalbuminuria is often used as marker for kidney disease and the typical definition of diabetic nephropathy is the presence of micro- or macroalbuminuria [183]. However the fact that a significant portion of both diabetic and non-diabetic individuals with GFR < 60 ml/min/1.73m^2, and thus defined as having CKD, are normoalbuminuric, is increasingly gaining attention [184-186]. In fact, almost 80% of the individuals in this study who had renal impairment, as defined by impaired GFR, were free of microalbuminuria. This may indicate a very heterogenous disease, where different mechanisms affecting kidney function are present and not all may induce albuminuria or only at more advanced stages. IR might thus be a potential risk factor to consider for normoalbuminuric CKD, as it was strongly associated with reduced GFR, but not microalbuminuria, in this study.

5.4.2 Individual insulin resistance associated SNPs

The automatic stepwise regression showed that all of the IR associated SNPs contributed to the association of the IR-GRS with GFR, except for the *HMGA2* rs1531343 variant which might be attributed to the low frequency of its risk allele. This supports the idea that it was the combined impact of those variants, whose commonality lies in the involvement with IR, that drove the observed association with renal function, rather than individual variants. The majority of the association was carried out by the *FTO* rs8050136 and *ADAMTS9* rs4607103 variants. The *FTO* variant was not associated with insulin levels or HOMA-IR but was significantly associated with increased body mass and waist circumference, which are strong risk factors for developing IR. Variants in the *FTO* gene have repeatedly been associated with increased body mass in various populations and have been associated with IR phenotypes, although its effect is mediated through obesity [73, 187, 188]. A variant in the *FTO* gene was recently associated with end-stage renal disease [189]. The *ADAMTS9* SNP showed a marginally significant association with insulin levels and HOMA-IR in the cohort and was also associated with impaired renal function and CKD. The association of this variant with insulin

sensitivity is established in the literature [122] but there is no previous report of its association with renal function.

The *ZFAND6* variant was significantly associated with increased insulin levels and HOMA-IR in the AGES-Reykjavík cohort. Two previous studies have failed to detect any significant association of the variant with any quantitative metabolic traits, although Nielsen et al. observed the same directions of effect [73, 113]. However, it remains to be confirmed if this variant has a role in insulin sensitivity. The gene *IGF1* encodes for the protein insulin-like growth factor-1 (IGF-1). IGF-1 is a hormone that mediates growth hormone somatic growth, but its signaling pathway is also related to life span and various cancers [190, 191]. IGF-1 is similar in structure to insulin and can even bind to insulin receptors to some extent. Plasma IGF-1 levels are associated with insulin sensitivity [192] and liver specific *IGF1* knockout mice quickly develop IR [193]. A recent study has suggested that IGF-1 levels may be a modulating factor of kidney disease associated with IR [181].

PPARG is a long established insulin sensitivity locus, identified through candidate gene studies on T2D. The insulin sensitizing thiazolidinedione drugs, which bind to PPARγ, have been shown to have various beneficial effects on the kidneys in T2D subjects [194], supporting the pathogenic role of IR in kidney disease.

The *GCKR* locus is interesting as it seems to exert very pleiotropic effects. The rs780094 C allele is associated with a favorable decrease in triglycerides [116] but at the same time with increased HOMA-IR, blood sugar and T2D risk [29, 73], as was confirmed in this study. Another *GCKR* variant in strong LD with rs780094 was identified in a recent GWAS on GFR [195] and another study reported an association of rs780094 and other *GCKR* variants with reduced GFR in newly diagnosed T2D patients [196]. Furthermore, the rs780094 has been associated with C-reactive protein (CRP) levels [197], but CRP is a good marker of inflammation and is strongly associated with IR and metabolic syndrome [198]. It is thus possible that the risk exerted by *GCKR* variants on impaired glucose regulation and renal function is mediated through IR and inflammation pathways.

The rs12518099 variant is located in an intergenetic region, the closest gene being *CETN3*. It has not gained much attention since discovered in 2009 [125] but it was significantly associated with T2D in the AGES-Reykjavík cohort and marginally with increased insulin levels and HOMA-IR. There are no reports on its association with any quantitative metabolic traits or on biological mechanisms linking this locus to T2D. *KLF14* is a transcription factor which has been shown to trans regulate a number of genes in adipose tissue at the genome-wide significance level [199]. The expression of these genes was associated with various components of the metabolic syndrome, four of which were strongly associated with HOMA-IR. *IRS1*, which encodes for insulin receptor substrate 1 (IRS-1), has a very clear biological connection to IR, as any defects in IRS-1 will directly affect insulin signaling. Variants close to the *IRS1* gene have been associated with decreased body fat percentage, and more specifically less subcutaneous fat and higher visceral to subcutaneous ratio, indicating that its role in decreased insulin sensitivity may in part be mediated through changes in adiposity [81]. Finally, *HMGA2* is thought to possibly contribute to T2D through IR [73] which is consistent with the observed

trends in this study, although it did not seem to contribute to the impaired renal function associated with the IR-GRS.

Taken together, the majority of the SNPs included in the IR-GRS seem to have a clear role in IR, although a few need a further confirmation. Some of the variants have even been previously implicated in renal function, supporting the present finding of this score being associated with kidney disease. A fundamental presumption of the Mendelian randomization method is that the instrument should be associated with the independent variable, and only with the dependent variable through that effect. The independent associations of the IR SNPs with kidney function seem to be modulated through IR, which supports the use of the IR-GRS as an instrument in this analysis. The study was limited to variants discovered from GWAS but another possibility would have been to include variants from IR candidate genes, such as the adiponectin gene *ADIPOQ* [200] and *ENPP1* [201]. Interestingly, variants from these loci have also been shown to affect renal function [202, 203].

5.4.3 Insulin resistance and renal function

The HOMA-IR phenotype was predictive for a five year decrease in GFR in the longitudinal part of the analysis and was marginally significantly associated with increased serum creatinine. It was however not predictive for developing CKD. The statistical power is less for dichotomous variables, such as CKD, and the sample size was reduced, which might be the reason for the lack of significance.

CKD is a general term for disorders affecting kidney structure and function. Podocyte lesions in the glomeruli have been suggested to precede and cause glomerulosclerosis, or scarring of the glomerular tissue [204]. Glomerulosclerosis is often followed by interstitial fibrosis, when extracellular matrix proteins (such as collagen and fibronectin) accumulate in the renal interstitium [205], which in turn leads to tubular atrophy. A schematic figure of the renal corpuscle is shown in Figure 20.

Although it is not clear how IR initiates renal damage, a number of studies indicate that the podocytes are a target. The insulin resistant Zucker fatty rat shows prominent podocyte injury [206] and both insulin levels and HOMA-IR have been associated with podocyte number and density in humans [207]. Furthermore, it has been shown that podocytes show insulin-induced glucose uptake through the GLUT1 and GLUT4 receptors, whereas glomerular endothelial cells showed no such response to insulin [208]. A recent study demonstrated that insulin signaling was decreased in the glomeruli, but not tubules, of IR rats [209], further supporting the idea that the initial location of IR induced damage is in the glomeruli rather than in the kidney tubules. However, the exact mechanisms by which insulin sensitivity affects podocyte injury are still poorly understood.

A number of possible pathways have been suggested for mediating the pathogenic effects of IR on the kidney, as reviewed by Sarafidis and Ruilope [210]. Hyperinsulinemia, as a consequence of increased IR, can have pathogenic effects on tissues that are not resistant to the effect of insulin and insulin per se can affect mitogenic and fibrotic processes that are involved in renal injury by binding to IGF-1 receptors and stimulating the expression of TGF-β [210].

Insulin also promotes proliferation of mesangial cells and extracellular matrix protein production, which are important factors in the pathogenesis of CKD [194]. Furthermore, insulin stimulates both

expression and secretion of endothelin-1, which is known to play a part in many sorts of nephropathy. Oxidative stress may also increase as a result of IR and can further reduce the insulin signaling as well as contribute to the renal injury [210]. It is clear that further mechanistic studies are needed to shed light on the principal pathogenic pathways between IR and CKD, as this relationship continues to be detected in observational studies.

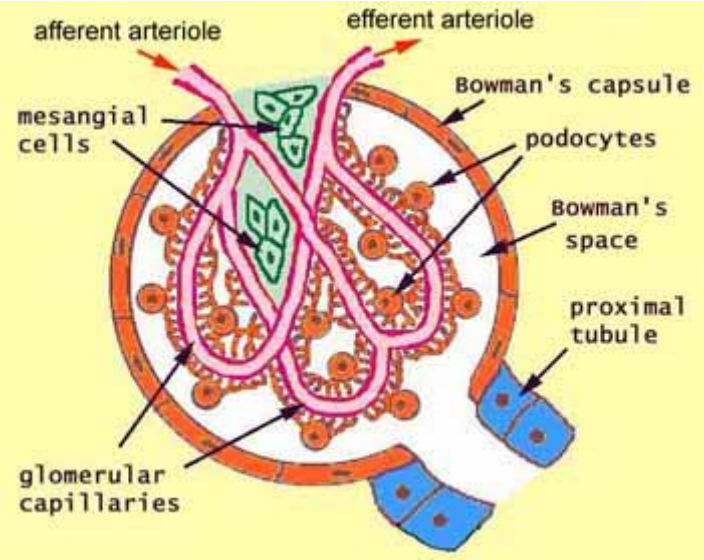

Figure 20 A schematic figure of the renal corpuscle and its various components [211].

5.5 Strengths and limitations

The main strength of the study was the large population-based AGES-Reykjavík cohort with its detailed phenotype information. The consistency of the measurements, which were almost all done at the IHA with standardized protocols, is very important in the epidemiologic setting to ensure the best possible results. The use of HOMA as an estimate of BCF and IR is more appropriate in large epidemiologic studies than the labor intensive euglycemic clamp technique, but it would be interesting to see if the relationship between IR genetic variants and renal function would be confirmed using both methods.

Many of the observed associations in this analysis were definitely suggestive but few were strong enough to fully rule out type I errors, or false positives. Therefore, a replication of the most notable results in another cohort would be appropriate to minimize the risk of chance findings. The REFINE-Reykjavík cohort was used for this purpose when possible. Unfortunately, data was not available to replicate the single SNP associations with various phenotypes, nor the association between the IR-GRS and renal variables. However, the IHA is currently in the process of acquiring data from a cohort comparable to AGES-Reykjavík with regard to the age of the participants and the prevalence of CKD, to attempt a validation of the observed association between the IR-GRS and renal function. Thus, the possibility of a real association between the IR associated variants and impaired renal function can neither be fully confirmed nor ruled out until the analysis has been repeated in an age appropriate sample.

6 Conclusions

In summary, the relationship between 47 T2D susceptibility variants and T2D associated phenotypes was examined in the population based AGES-Reykjavík cohort. The majority of the variants showed the expected direction of effect for T2D, HOMA-B and HOMA-IR, and 12 showed a statistically significant association with the disease. Six variants were found to possibly influence BCF and three were identified as possible IR risk variants. A handful of previously reported single SNP associations with BMI, triglycerides, HDL and coronary calcium were confirmed in this analysis and one novel association was observed with decreased waist circumference. A number of novel associations were observed that did not reach statistical significance after correcting for multiple testing, but the strongest ones might be of interest to follow up on, especially if biological data supports the observed association. Additional analysis on the effects of the *TCF7L2* locus showed that its effect on T2D and CHD risk did not seem to be modulated by BMI or blood lipid variables. The variant was associated with CHD risk in REFINE, but not in the AGES cohort.

Finally, a risk score consisting of an updated number of T2D susceptibility gene variants in 3,179 elderly Icelandic individuals showed a strong association with T2D and reduced HOMA-B. Its effect on fasting glucose levels seemed to be modulated by BMI, but the score was associated with greater increase in fasting glucose in obese individuals compared to normal or overweight individuals. The same effect was not seen for T2D risk, possibly because of the reduced statistical power in the logistic regression compared to the linear model. When the variants were partitioned by their proposed physiologic mechanism in the disease, a strong association was found with decreased HOMA-B for one set of SNPs and with increased HOMA-IR for another. The GRS associated with IR, but not the other two, showed a strong association with CKD. Mendelian randomization analysis suggested a causal effect of IR on impaired GFR and risk of CKD. The association was statistically significant after the exclusion of diabetic individuals when adjusted for BMI and triglyceride levels, indicating that the effect might be independent of T2D. These results suggest that IR is an important factor in the pathogenesis of CKD, even before the onset of T2D, and that individuals with genetic susceptibility for IR might be predisposed to renal damage. These findings await a replication for confirmation or refute.

References

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;94(3):311-21.
2. World Health Organization. Diabetes fact sheet N°312. World Health Organization. 2011 [cited 2011 1 September]. Available from: http://www.who.int/mediacentre/factsheets/fs312/en/
3. Þórsson B, Aspelund T, Harris TB, Launer LJ, Guðnason V. Þróun holdafars og sykursýki í 40 ár á Íslandi. Læknablaðið. 2009;95(4):259-66.
4. International Diabetes Federation. IDF Diabetes Atlas, 5th edn. International Diabetes Federation. 2011 [cited 2011 1 September]. Available from: www.idf.org/diabetesatlas
5. Lýðheilsustöð. Líkamsþyngd og holdafar fullorðinna Íslendinga 1990-2007. Reykjavík. 2009.
6. Alberti K, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med. 1998;15(7):539-53.
7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34(Supplement 1).
8. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344(18):1343-50.
9. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin; 2002.
10. American Diabetes A. Economic costs of diabetes in the U.S. in 2007. Diabetes Care. 2008;31(3):596-615.
11. Saczynski JS, Siggurdsson S, Jonsson PV, Eiriksdottir G, Olafsdottir E, Kjartansson O, et al. Glycemic status and brain injury in older individuals. Diabetes Care. 2009;32(9):1608.
12. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, et al. Retinopathy in diabetes. Diabetes Care. 2004;27(suppl 1):s84-s7.
13. International Diabetes Federation and International Society of Nephrology. Diabetes and kidney disease: time to act. Brussels. 2003.
14. Vilbergsson S, Sigurdsson G, Sigvaldason H, Sigfusson N. Coronary heart disease mortality amongst non-insulin-dependent diabetic subjects in Iceland: the independent effect of diabetes. The Reykjavik Study 17-year follow up. J Intern Med. 1998;244(4):309-16.
15. Tryggvason G, Indridason OS, Thorsson AV, Hreidarsson AB, Palsson R. Unchanged incidence of diabetic nephropathy in Type 1 diabetes: a nation-wide study in Iceland. Diabet Med. 2005;22(2):182-7.
16. Ólafsdóttir E, Stefánsson E. Biennial eye screening in patients with diabetes without retinopathy: 10-year experience. Br J Ophthalmol. 2007;91(12):1599-601.
17. Kristinsson JK, Stefánsson E, Jónasson F, Gislason I, Björnsson S. Screening for eye disease in type 2 diabetes mellitus. Acta Ophthalmol (Copenh). 1994;72(3):341-6.

18. Heimisdóttir F, Guðnason V, Sigurðsson G, Benediktsson R. Einkenni og teikn fótameins hjá íslenskum sjúklingum með sykursýki af tegund 2. Læknablaðið. 2008;94(2):109-14.
19. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet. 2011;378(9786):169-81.
20. O'Dea K. Westernisation, insulin resistance and diabetes in Australian aborigines. Med J Aust. 1991;155(4):258.
21. Østbye T, Welby TJ, Prior IAM, Salmond CE, Stokes YM. Type 2 (non-insulin-dependent) diabetes mellitus, migration and westernisation: the Tokelau Island Migrant Study. Diabetologia. 1989;32(8):585-90.
22. Herder C, Roden M. Genetics of type 2 diabetes: pathophysiologic and clinical relevance. Eur J Clin Invest. 2011.
23. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab. 2007;92(6):2017-29.
24. Yajnik C, Deshpande S, Jackson A, Refsum H, Rao S, Fisher D, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008;51(1):29-38.
25. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. Biochim Biophys Acta. 2009;1790(7):671-81.
26. Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol. 2011;73:135-62.
27. Arroyo C, Hu FB, Ryan LM, Kawachi I, Colditz GA, Speizer FE, et al. Depressive symptoms and risk of type 2 diabetes in women. Diabetes Care. 2004;27(1):129-33.
28. Williams E, Tapp R, Magliano D, Shaw J, Zimmet P, Oldenburg B. Health behaviours, socioeconomic status and diabetes incidence: the Australian Diabetes Obesity and Lifestyle Study (AusDiab). Diabetologia. 2010;53(12):2538-45.
29. Musso G, Gambino R, Cassader M. Obesity, Diabetes, and Gut Microbiota. Diabetes Care. 2010;33(10):2277-84.
30. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59-65.
31. Greenspan FS, Gardner DG. Basic & clinical endocrinology. 6 ed: McGraw-Hill; 2001.
32. Cartailler J-P. Insulin - from secretion to action. Beta Cell Biology Consortium. 2004 [cited 2012 25 January]. Available from: http://www.betacell.org/content/articleview/article_id/1/
33. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006;7(2):85-96.
34. Watanabe M, Hayasaki H, Tamayama T, Shimada M. Histologic distribution of insulin and glucagon receptors. Braz J Med Biol Res. 1998;31(2):243-56.
35. Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R. Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. The FASEB Journal. 2001;15(12):2099.

36. Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005;365(9467):1333-46.
37. Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(3):193-205.
38. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106(4):473-81.
39. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-6.
40. Hajer GR, Van Haeften TW, Visseren FLJ. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J. 2008;29(24):2959.
41. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000;404(6778):661-71.
42. Cheung CC, Clifton DK, Steiner RA. Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology. 1997;138(10):4489.
43. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest. 2006;116(7):1776.
44. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev. 2005;26(3):439-51.
45. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol. 2003;149(4):331.
46. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292-5.
47. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132(6):2169-80.
48. Wannamethee SG, Lowe GDO, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. Diabetes Care. 2007;30(5):1200-5.
49. Hotamisligil GS. The role of TNFα and TNF receptors in obesity and insulin resistance. J Intern Med. 1999;245(6):621-5.
50. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. Science. 1996;271(5249):665.
51. Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor-α suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes. Diabetes. 2002;51(5):1319.
52. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell. 2010;140(6):900-17.
53. Özcan U, Cao Q, Yilmaz E, Lee A-H, Iwakoshi NN, Özdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004;306(5695):457-61.

54. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes. 1997;46(1):3-10.
55. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest. 2000;106(2):171-6.
56. Poitout V, Robertson RP. Minireview: Secondary β-cell failure in type 2 diabetes: A convergence of glucotoxicity and lipotoxicity. Endocrinology. 2002;143(2):339-42.
57. Brownlee M. The pathobiology of diabetic complications. Diabetes. 2005;54(6):1615.
58. Turner RC, Holman RR, Cull CA, Stratton IM, Matthews DR, Frighi V, et al. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837-53.
59. Hanley AJG, Williams K, Stern MP, Haffner SM. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease. Diabetes Care. 2002;25(7):1177.
60. Cholerton B, Baker LD, Craft S. Insulin resistance and pathological brain ageing. Diabet Med. 2011;28(12):1463-75.
61. Chen J, Muntner P, Hamm LL, Fonseca V, Batuman V, Whelton PK, et al. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. J Am Soc Nephrol. 2003;14(2):469-77.
62. Kurella M, Lo JC, Chertow GM. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. J Am Soc Nephrol. 2005;16(7):2134-40.
63. Nerpin E, Risérus U, Ingelsson E, Sundström J, Jobs M, Larsson A, et al. Insulin sensitivity measured with euglycemic clamp is independently associated with glomerular filtration rate in a community-based cohort. Diabetes Care. 2008;31(8):1550.
64. Becker B, Kronenberg F, Kielstein JT, Haller H, Morath C, Ritz E, et al. Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. J Am Soc Nephrol. 2005;16(4):1091-8.
65. Fliser D, Pacini G, Engelleiter R, Kautzky-Willer A, Prager R, Franek E, et al. Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int. 1998;53(5):1343-7.
66. Levey AS, Coresh J. Chronic kidney disease. Lancet. 2012;379(9811):165-80.
67. Aksentijevic D, Bhandari S, Seymour A-ML. Insulin resistance and altered glucose transporter 4 expression in experimental uremia. Kidney Int. 2009;75(7):711-8.
68. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, et al. Large-scale association studies of variants in genes encoding the pancreatic β-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(2):568.
69. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl M-C, Nemesh J, et al. The common PPAR[gamma] Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000;26(1):76-80.

70. Nielsen E-MD, Hansen L, Carstensen B, Echwald SrM, Drivsholm T, Glümer C, 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(2):573-7.
71. Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, et al. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998;20(3):284-7.
72. McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med. 2010;363(24):2339-50.
73. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010;42(7):579-89.
74. Grarup N, Sparsø T, Hansen T. Physiologic characterization of type 2 diabetes-related loci. Curr Diabetes Rep. 2010;10(6):485-97.
75. Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, et al. Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. Diabetes. 2011;60(10):2624-34.
76. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42(2):105-16.
77. Panhuysen CIM, Cupples LA, Wilson PWF, Herbert AG, Myers RH, Meigs JB. A genome scan for loci linked to quantitative insulin traits in persons without diabetes: the Framingham Offspring Study. Diabetologia. 2003;46(4):579-87.
78. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, 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(5826):889-94.
79. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007;316(5829):1336.
80. Do R, Bailey SD, Desbiens K, Belisle A, Montpetit A, Bouchard C, et al. Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes. 2008;57(4):1147-50.
81. Kilpeläinen TO, Zillikens MC, Stančákova A, Finucane FM, Ried JS, Langenberg C, et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. Nat Genet. 2011;43(8):753-60.
82. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet. 2010;42(11):949-60.
83. The 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature. 2010;467(7319):1061-73.

84. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. New Engl J Med. 2008;359(21):2220-32.
85. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis Je, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359(21):2208-19.
86. Lango H, Palmer CNA, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, et al. Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes. 2008;57(11):3129-35.
87. Pascoe L, Frayling TM, Weedon MN, Mari A, Tura A, Ferrannini E, et al. Beta cell glucose sensitivity is decreased by 39% in non-diabetic individuals carrying multiple diabetes-risk alleles compared with those with no risk alleles. Diabetologia. 2008;51(11):1989-92.
88. Haupt A, Staiger H, Schäfer S, Kirchhoff K, Guthoff M, Machicao F, et al. The risk allele load accelerates the age-dependent decline in beta cell function. Diabetologia. 2009;52(3):457-62.
89. de Miguel-Yanes JM, Shrader P, Pencina MJ, Fox CS, Manning AK, Grant RW, et al. Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms. Diabetes Care. 2011;34(1):121-5.
90. Yan Y, North KE, Ballantyne CM, Brancati FL, Chambless LE, Franceschini N, et al. Transcription Factor 7-Like 2 (TCF7L2) polymorphism and context-specific risk of type 2 diabetes in African American and Caucasian Adults. Diabetes. 2009;58(1):285-9.
91. Heikkinen S, Argmann C, Feige JN, Koutnikova H, Champy M-F, Dali-Youcef N, et al. The Pro12Ala PPARγ2 variant determines metabolism at the gene-environment interface. Cell Metab. 2009;9(1):88-98.
92. Warodomwichit D, Arnett DK, Kabagambe EK, Tsai MY, Hixson JE, Straka RJ, et al. Polyunsaturated fatty acids modulate the effect of TCF7L2 gene variants on postprandial lipemia. J Nutr. 2009;139(3):439.
93. Cauchi S, Nead K, Choquet H, Horber F, Potoczna N, Balkau B, et al. The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies. BMC Med Genet. 2008;9(1):45.
94. Florez JC, Jablonski KA, Sun MW, Bayley N, Kahn SE, Shamoon H, et al. Effects of the type 2 diabetes-associated PPARG P12A polymorphism on progression to diabetes and response to troglitazone. J Clin Endocrinol Metab. 2007;92(4):1502-9.
95. Helgason A, Pálsson S, Thorleifsson G, Grant SFA, Emilsson V, Gunnarsdottir S, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet. 2007;39(2):218-25.
96. Doria A. Genetics of diabetes complications. Curr Diabetes Rep. 2010:1-9.
97. Grassi MA, Tikhomirov A, Ramalingam S, Below JE, Cox NJ, Nicolae DL. Genome-wide meta-analysis for severe diabetic retinopathy. Hum Mol Genet. 2011;20(12):2472.

98. Pezzolesi MG, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes. 2009;58(6):1403.
99. Maeda S, Araki S, Babazono T, Toyoda M, Umezono T, Kawai K, et al. Replication study for the association between four loci identified by a genome-wide association study on European American subjects with type 1 diabetes and susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes. Diabetes. 2010;59(8):2075.
100. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science. 2007;316(5830):1491.
101. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007;316(5830):1488.
102. Chen Y, Cunningham F, Rios D, McLaren W, Smith J, Pritchard B, et al. Ensembl variation resources. BMC genomics. 2010;11(1):293.
103. Doria A, Wojcik J, Xu R, Gervino EV, Hauser TH, Johnstone MT, et al. Interaction between poor glycemic control and 9p21 locus on risk of coronary artery disease in type 2 diabetes. JAMA: the journal of the American Medical Association. 2008;300(20):2389.
104. Sivakumaran S, Agakov F, Theodoratou E, Prendergast JG, Zgaga L, Manolio T, et al. Abundant pleiotropy in human complex diseases and traits. Am J Hum Genet. 2011;89(5):607-18.
105. Yamauchi T, Hara K, Maeda S, Yasuda K, Takahashi A, Horikoshi M, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet. 2010;42(10):864-8.
106. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, 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(5):638-45.
107. Grarup N, Andersen G, Krarup NT, Albrechtsen A, Schmitz O, Jørgensen T, et al. Association testing of novel type 2 diabetes risk alleles in the JAZF1, CDC123/CAMK1D, TSPAN8, THADA, ADAMTS9, and NOTCH2 loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. Diabetes. 2008;57(9):2534.
108. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet. 2007;39(6):770-5.
109. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, et al. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic β-cell function. Diabetes. 2007;56(12):3101.
110. Stančáková A, Kuulasmaa T, Paananen J, Jackson AU, Bonnycastle LL, Collins FS, et al. Association of 18 confirmed susceptibility loci for type 2 diabetes with indices of insulin

release, proinsulin conversion, and insulin sensitivity in 5,327 nondiabetic Finnish men. Diabetes. 2009;58(9):2129.

111. Ruchat SM, Elks CE, Loos RJF, Vohl MC, Weisnagel SJ, Rankinen T, et al. Association between insulin secretion, insulin sensitivity and type 2 diabetes susceptibility variants identified in genome-wide association studies. Acta Diabetol. 2009;46(3):217-26.
112. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, Albrechtsen A, et al. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects. Diabetes. 2007;56(12):3105-11.
113. Nielsen T, Sparsø T, Grarup N, Jørgensen T, Pisinger C, Witte DR, et al. Type 2 diabetes risk allele near CENTD2 is associated with decreased glucose-stimulated insulin release. Diabetologia. 2011;54(5):1052-6.
114. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445(7130):881-5.
115. Palmer ND, Goodarzi MO, Langefeld CD, Ziegler J, Norris JM, Haffner SM, et al. Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. Diabetes. 2008;57(4):1093.
116. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007;316(5829):1341.
117. Langenberg C, Pascoe L, Mari A, Tura A, Laakso M, Frayling TM, et al. Common genetic variation in the melatonin receptor 1B gene (MTNR1B) is associated with decreased early-phase insulin response. Diabetologia. 2009;52(8):1537-42.
118. Staiger H, Machicao F, Stefan N, Tschritter O, Thamer C, Kantartzis K, et al. Polymorphisms within novel risk loci for type 2 diabetes determine β-cell function. PLoS One. 2007;2(9):e832.
119. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjögren M, 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(10):2890.
120. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest. 2007;117(8):2155.
121. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, Daly A, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet. 2007;39(8):951-3.
122. Boesgaard TW, Gjesing AP, Grarup N, Rutanen J, Jansson PA, Hribal ML, et al. Variant near ADAMTS9 known to associate with type 2 diabetes is related to insulin resistance in offspring of type 2 diabetes patients - EUGENE2 study. PLoS One. 2009;4(9):e7236.
123. Staiger H, Machicao F, Kantartzis K, Schäfer SA, Kirchhoff K, Guthoff M, et al. Novel meta-analysis-derived type 2 diabetes risk loci do not determine prediabetic phenotypes. PLoS One. 2008;3(8):e3019.

124. Ingelsson E, Langenberg C, Hivert M-F, Prokopenko I, Lyssenko V, Dupuis J, et al. Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes. 2010;59(5):1266-75.
125. Rung J, Cauchi S, Albrechtsen A, Shen L, Rocheleau G, Cavalcanti-Proença C, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet. 2009;41(10):1110-5.
126. Anand A, Chada K. In vivo modulation of Hmgic reduces obesity. Nat Genet. 2000;24(4):377-80.
127. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet. 2010;6(2):e1000847.
128. Qi L, Cornelis MC, Kraft P, Stanya KJ, Linda Kao WH, Pankow JS, et al. Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. Hum Mol Genet. 2010;19(13):2706.
129. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, et al. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet. 2010;6(9):e1001127.
130. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, et al. Age, gene/environment susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol. 2007;165(9):1076.
131. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487-95.
132. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
133. Who Monica Project Principal Investigators. The World Health Organization MONICA project (monitoring trends and determinants in cardiovascular disease): A major international collaboration. J Clin Epidemiol. 1988;41(2):105-14.
134. Levey AS, Greene T, Kusek JW, Beck GJ, Group MS. A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. J Am Soc Nephrol. 2000;11(9):155A.
135. Eknoyan G, Levin NW. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1-266.
136. Sigurdsson S, Aspelund T, Forsberg L, Fredriksson J, Kjartansson O, Oskarsdottir B, et al. Brain tissue volumes in the general population of the elderly The AGES-Reykjavik Study. Neuroimage. 2012;59(4):3862-70.
137. Day INM, Humphries SE. Electrophoresis for genotyping: microtiter array diagonal gel electrophoresis on horizontal polyacrylamide gels, hydrolink, or agarose. Anal Biochem. 1994;222(2):389-95.
138. Hindorff L, MacArthur J, Wise A, Junkins H, Hall P, Klemm A, et al. A catalog of published Genome-Wide Association Studies. [Accessed Desember 2010]. Available from: www.genome.gov/gwastudies

139. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. 2011. Available from: www.R-project.org
140. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003;19(1):149-50.
141. Davies HTO, Crombie IK, Tavakoli M. When can odds ratios mislead? BMJ. 1998;316(7136):989.
142. World Health Organization. Obesity: preventing and managing the glopal epidemic. Report of a WHO Consultation (WHO Technical Report Series 894). Geneva. 2000.
143. Lindeman RH, Merenda PF, Gold RZ. Introduction to bivariate and multivariate analysis: Scott, Foresman Glenview, IL; 1980.
144. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27(8):1133-63.
145. National Bureau of Economic Research. Instrumental variables regression with weak instruments. 1994.
146. Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population. Diabetes Care. 2006;29(6):1263-8.
147. Carstensen B, Kristensen JK, Ottosen P, Borch-Johnsen K. The Danish National Diabetes Register: trends in incidence, prevalence and mortality. Diabetologia. 2008;51(12):2187-96.
148. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension. 2003;42(6):1206-52.
149. van Harten B, Oosterman JM, Potter van Loon BJ, Scheltens P, Weinstein HC. Brain lesions on MRI in elderly patients with type 2 diabetes mellitus. Eur Neurol. 2007;57(2):70-4.
150. Manschot SM, Brands AMA, van der Grond J, Kessels RPC, Algra A, Kappelle LJ, et al. Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. Diabetes. 2006;55(4):1106-13.
151. Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, et al. Notch signalling controls pancreatic cell differentiation. Nature. 1999;400(6747):877-81.
152. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care. 1997;20(7):1087.
153. Chen T, Li M, Ding Y, Zhang L, Xi Y, Pan W, et al. Identification of Zinc-finger BED domain-containing 3 (Zbed3) as a novel axin-interacting protein that activates Wnt/β-Catenin signaling. J Biol Chem. 2009;284(11):6683-9.
154. Welters HJ, Kulkarni RN. Wnt signaling: relevance to [beta]-cell biology and diabetes. Trends Endocrin Met. 2008;19(10):349-55.

155. Grant SFA, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006;38(3):320-3.
156. Zhou Y, Zhang E, Berggreen C, Jing X, Osmark P, Lang S, et al. Survival of pancreatic beta cells is partly controlled by a TCF7L2-p53-p53INP1-dependent pathway. Hum Mol Genet. 2012;21(1):196-207.
157. Chang E-J, Ha J, Kang S-S, Lee ZH, Kim H-H. AWP1 binds to tumor necrosis factor receptor-associated factor 2 (TRAF2) and is involved in TRAF2-mediated nuclear factor-kappaB signaling. The International Journal of Biochemistry And Cell Biology. 2011;43(11):1612-20.
158. Urano F, Wang XZ, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000;287(5453):664.
159. O'Donnell CJ, Kavousi M, Smith AV, Kardia SLR, Feitosa MF, Hwang S-J, et al. Genome-Wide Association Study for Coronary Artery Calcification With Follow-Up in Myocardial Infarction / Clinical Perspective. Circulation. 2011;124(25):2855-64.
160. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316(5829):1331.
161. Stancáková A, Paananen J, Soininen P, Kangas AJ, Bonnycastle LL, Morken MA, et al. Effects of 34 risk loci for type 2 diabetes or hyperglycemia on lipoprotein subclasses and their composition in 6,580 nondiabetic Finnish men. Diabetes. 2011;60(5):1608-16.
162. Polakis P. Wnt signaling and cancer. Genes Dev. 2000;14(15):1837-51.
163. Smith U. TCF7L2 and type 2 diabetes - we WNT to know. Diabetologia. 2007;50(1):5-7.
164. Krishnan V, Bryant HU, MacDougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest. 2006;116(5):1202.
165. Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, et al. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. Am J Hum Genet. 2008;82(6):1270-80.
166. Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, et al. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab. 2005;90(7):4019.
167. Cesca F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. Prog Neurobiol. 2010;91(4):313-48.
168. Culman J, Zhao Y, Gohlke P, Herdegen T. PPAR-γ: therapeutic target for ischemic stroke. Trends Pharmacol Sci. 2007;28(5):244-9.
169. Ou Z, Zhao X, Labiche LA, Strong R, Grotta JC, Herrmann O, et al. Neuronal expression of peroxisome proliferator-activated receptor-gamma (PPARγ) and 15d-prostaglandin J_2-mediated protection of brain after experimental cerebral ischemia in rat. Brain Res. 2006;1096(1):196-203.

170. Al-Shali KZ, House AA, Hanley AJG, Khan HMR, Harris SB, Zinman B, et al. Genetic variation in PPARG encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis. Stroke. 2004;35(9):2036-40.
171. Trojan P, Krauss N, Choe HW, Giessl A, Pulvermüller A, Wolfrum U. Centrins in retinal photoreceptor cells: regulators in the connecting cilium. Prog Retin Eye Res. 2008;27(3):237-59.
172. Saade S, Cazier JB, Ghassibe-Sabbagh M, Youhanna S, Badro DA, Kamatani Y, et al. Large scale association analysis identifies three susceptibility loci for coronary artery disease. PLoS One. 2011;6(12):e29427.
173. Buraczynska M, Swatowski A, Markowska-Gosik D, Kuczmaszewska A, Ksiazek A. Transcription factor 7-like 2 (TCF7L2) gene polymorphism and complication/comorbidity profile in type 2 diabetes patients. Diabetes Res Clin Pract. 2011;93(3):390-5.
174. Köttgen A, Hwang S-J, Rampersaud E, Coresh J, North KE, Pankow JS, et al. TCF7L2 variants associate with CKD progression and renal function in population-based cohorts. J Am Soc Nephrol. 2008;19(10):1989-99.
175. Muendlein A, Saely CH, Geller-Rhomberg S, Sonderegger G, Rein P, Winder T, et al. Single nucleotide polymorphisms of TCF7L2 are linked to diabetic coronary atherosclerosis. PLoS One. 2011;6(3):e17978.
176. Sousa AGP, Marquezine GF, Lemos PA, Martinez E, Lopes N, Hueb WA, et al. TCF7L2 polymorphism rs7903146 is associated with coronary artery disease severity and mortality. PLoS One. 2009;4(11):e7697.
177. Keri L, Suzette J, Eric B, Thomas D, Wayne D, James S, et al. Role of BMI in the association of the TCF7L2 rs7903146 variant with coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) Study. J Obes. 2010;2010.
178. Melzer D, Murray A, Hurst A, Weedon M, Bandinelli S, Corsi A, et al. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. BMC Med. 2006;4(1):34.
179. Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, et al. Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. Genome Res. 2010;20(8):1020-36.
180. Tan ZS, Beiser AS, Fox CS, Au R, Himali JJ, Debette S, et al. Association of metabolic dysregulation with volumetric brain magnetic resonance imaging and cognitive markers of subclinical brain aging in middle-aged adults. Diabetes Care. 2011;34(8):1766-70.
181. Sesti G, Succurro E, Arturi F, Andreozzi F, Laino I, Perticone M, et al. IGF-1 levels link estimated glomerular filtration rate to insulin resistance in obesity: A study in obese, but metabolically healthy, subjects and obese, insulin-resistant subjects. Nutr Metab Cardiovas. 2011;21(12):933-40.
182. Mykkänen L, Zaccaro DJ, Wagenknecht LE, Robbins DC, Gabriel M, Haffner SM. Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the insulin resistance atherosclerosis study. Diabetes. 1998;47(5):793-800.

183. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28(1):164-76.
184. Thomas MC, MacIsaac RJ, Jerums G, Weekes A, Moran J, Shaw JE, et al. Nonalbuminuric renal impairment in type 2 diabetic patients and in the general population (National Evaluation of the Frequency of Renal Impairment cO-existing with NIDDM [NEFRON] 11). Diabetes Care. 2009;32(8):1497-502.
185. Kramer HJ, Nguyen QD, Curhan G, Hsu C-y. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. JAMA. 2003;289(24):3273-7.
186. Dwyer JP, Parving HH, Hunsicker LG, Ravid M, Remuzzi G, Lewis JB. Renal dysfunction in the presence of normoalbuminuria in type 2 diabetes: results from the DEMAND study. Cardiorenal Medicine. 2012;2(1):1-10.
187. Jacobsson JA, Klovins J, Kapa I, Danielsson P, Svensson V, Ridderstrale M, et al. Novel genetic variant in FTO influences insulin levels and insulin resistance in severely obese children and adolescents. Int J Obes. 2008;32(11):1730-5.
188. Kring SII, Holst C, Zimmermann E, Jess T, Berentzen T, Toubro S, et al. FTO gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. PLoS One. 2008;3(8):e2958.
189. Hubacek JA, Viklicky O, Dlouha D, Bloudickova S, Kubinova R, Peasey A, et al. The FTO gene polymorphism is associated with end-stage renal disease: two large independent case-control studies in a general population [Abstract]. Nephrol Dial Transplant. 2011.
190. Barbieri M, Bonafè M, Franceschi C, Paolisso G. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. Am J Physiol Enoc M. 2003;285(5):E1064-E71.
191. Smith GD, Gunnell D, Holly J. Cancer and insulin-like growth factor-I. BMJ. 2000;321(7265):847-8.
192. Sesti G, Sciacqua A, Cardellini M, Marini MA, Maio R, Vatrano M, et al. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care. 2005;28(1):120-5.
193. Yakar S, Liu J-L, Fernandez AM, Wu Y, Schally AV, Frystyk J, et al. Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. Diabetes. 2001;50(5):1110-8.
194. Sarafidis PA, Bakris GL. Protection of the kidney by thiazolidinediones: An assessment from bench to bedside. Kidney Int. 2006;70(7):1223-33.
195. Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010;42(5):376-84.
196. Bonetti S, Trombetta M, Boselli ML, Turrini F, Malerba G, Trabetti E, et al. Variants of GCKR affect both β-cell and kidney function in patients with newly diagnosed type 2 diabetes. Diabetes Care. 2011;34(5):1205-10.
197. Ridker PM, Pare G, Parker A, Zee RYL, Danik JS, Buring JE, et al. Loci related to metabolic-syndrome pathways including LEPR, HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: The Women's Genome Health Study. Am J Hum Genet. 2008;82(5):1185-92.

198. Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: The Insulin Resistance Atherosclerosis Study (IRAS). Circulation. 2000;102(1):42-7.
199. Small KS, Hedman ÅK, Grundberg E, Nica AC, al. e. Identification of an imprinted master trans regulator at the KLF14 locus related to multiple metabolic phenotypes. Nat Genet. 2011;43(6):561-4.
200. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes. 2007;56(5):1198-209.
201. Bacci S, Ludovico O, Prudente S, Zhang YY, Di Paola R, Mangiacotti D, et al. The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. Diabetes. 2005;54(10):3021.
202. De Cosmo S, Minenna A, Zhang Y-Y, Thompson R, Miscio G, Vedovato M, et al. Association of the Q121 variant of ENPP1 gene with decreased kidney function among patients with type 2 diabetes. Am J Kidney Dis. 2009;53(2):273-80.
203. Jaziri R, Aubert R, Roussel R, Emery N, Maimaitiming S, Bellili N, et al. Association of ADIPOQ genetic variants and plasma adiponectin isoforms with the risk of incident renal events in type 2 diabetes. Nephrol Dial Transplant. 2010;25(7):2231-7.
204. Kriz W, Gretz N, Lemley KV. Progression of glomerular diseases: Is the podocyte the culprit? Kidney Int. 1998;54(3):687-97.
205. Eddy AA. Molecular insights into renal interstitial fibrosis. J Am Soc Nephrol. 1996;7(12):2495-508.
206. Hoshi S, Shu YJ, Yoshida F, Inagaki T, Sonoda J, Watanabe T, et al. Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats. Lab Invest. 2002;82(1):25-35.
207. Chen H-M, Liu Z-H, Zeng C-H, Li S-J, Wang Q-W, Li L-S. Podocyte lesions in patients with obesity-related glomerulopathy. Am J Kidney Dis. 2006;48(5):772-9.
208. Coward RJM, Welsh GI, Yang J, Tasman C, Lennon R, Koziell A, et al. The human glomerular podocyte is a novel target for insulin action. Diabetes. 2005;54(11):3095-102.
209. Mima A, Ohshiro Y, Kitada M, Matsumoto M, Geraldes P, Li C, et al. Glomerular-specific protein kinase C-[beta]-induced insulin receptor substrate-1 dysfunction and insulin resistance in rat models of diabetes and obesity. Kidney Int. 2011;79(8):883-96.
210. Sarafidis PA, Ruilope LM. Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. Am J Nephrol. 2006;26(3):232-44.
211. King D. The renal corpuscle. SIU School of Medicine. 2011 [cited 2012 25 January]. Available from: http://www.siumed.edu/~dking2/crr/rnguide.htm#glomerulus

Appendix 1

DNA extraction protocol

1. 5-10 mL EDTA blood is used, fresh or frozen.
2. Mix 5-10 mL EDTA blood with 30-40 mL reagent A.
3. Centrifuge at 1430 rpm for 10 min.
4. Decant the supernatant, leaving the pellet undisturbed.
5. Add 2 mL reagent B to the tube and vortex.
6. Transfer the solution to another tube and add 500 µL 70% natriumperchlorat and rotate for 15 min at 20°C.
7. Incubate at 65°C for 25 min.
8. Cool down at 4°C for 10 min and add 2 mL chloroform and rotate for 10 min at 20°C.
9. Centrifuge at 1430 rpm for 10 min.
10. Transfer the solution in the upper layer to another tube containing 5 mL 96% ethanol. Rotate carefully to precipitate the DNA.
11. Transfer the DNA pellet into a tube containing 70-80% ethanol and wash it. Transfer the pellet to a tube containing TE. The DNA is dissolved in the TE by rotating the tube at 20°C for 24 hours and stored at 4°C for 4 weeks before analysis. Permanent storage at -20°C.

Solutions

Tris buffer pH = 8.0

1. 88.8 g Trisma HCl. Dissolve in dH_2O
2. 53.0 g Tris base. Dissolve in dH_2O
3. 6 M HCl until pH = 8.0
4. dH_2O to make 1 L

0.6 M EDTA

1. 223.3 g EDTA
2. 900 mL dH_2O
3. NaOH until pH = 8.0
4. dH_2O to make 1 L

10x TE buffer

1. 100 mL 1 M Tris buffer, pH = 8.0
2. 16.7 mL 0.6 EDTA
3. dH_2O to make 1 L

Reagent A

1. 219.1 g sucrose, dissolve in dH_2O
2. 2.0 g $MgCl_2$
3. 20 mL 1 M Tris buffer, pH = 8.0
4. 20 mL Triton X
5. dH_2O to make 2 L

Reagent B

1. 200 mL 1 M Tris buffer, pH = 8.0
2. 50 mL 0.6 M EDTA
3. 50 mL 1.5 M NaCl
4. dH_2O to make 450 mL
5. Autoclave
6. 50 mL 10% SDS

Appendix 2

Table 1 Population characteristics for the REFINE-Reykjavík cohort.

Variable	Mean ± SD or n (%)	*n*
Age (years)	49.0 ± 12.1	4617
Male sex	2267 (49.0)	4617
T2D	176 (3.8)	4617
Fasting glucose (mmol/L)	5.4 ± 1.1	4617
BMI (kg/m^2)	27.3 ± 4.8	4597
Waist circumference (cm)	97.1 ± 13.3	4611
HDL (mmol/L)	1.50 ± 0.4	4617
LDL (mmol/L)	3.15 ± 0.9	4589
Triglycerides (mmol/L)	1.04 ± 1.7	4617
CHD	246 (5.4)	4526

Table 2 Association of the 47 T2D susceptibility variants with quantitative metabolic traits.

				HOMA-IR			Insulin			HOMA-B			Glucose			HbA1C		
SNP	Gene	Risk allele	Function	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
rs4607517	*GCK*	A	B	0.080	0.035	0.021	0.062	0.035	0.075	-0.013	0.035	0.708	0.112	0.041	0.006	0.019	0.006	0.004
rs7903146	*TCF7L2*	T	B	-0.057	0.020	0.005	-0.084	0.020	3.1E-05	-0.147	0.020	2.6E-13	0.097	0.024	5.5E-05	0.006	0.004	0.086
rs231362	*KCNQ1*	G	B	0.063	0.026	0.016	0.054	0.026	0.041	0.017	0.026	0.522	0.096	0.031	0.002	0.013	0.005	0.006
rs1387153	*MTNR1B*	T	B	0.033	0.027	0.224	0.011	0.027	0.687	-0.061	0.027	0.023	0.095	0.032	0.003	0.005	0.005	0.305
rs10811661	*CDKN2B*	T	B	0.055	0.024	0.023	0.046	0.024	0.061	-0.002	0.024	0.948	0.081	0.029	0.005	0.012	0.004	0.007
rs1801214	*WFS1*	T	B	0.011	0.024	0.655	-0.002	0.024	0.945	-0.037	0.024	0.128	0.076	0.029	0.009	0.002	0.005	0.598
rs864745	*JAZF1*	T	B	0.027	0.019	0.151	0.015	0.019	0.429	-0.018	0.019	0.323	0.066	0.022	0.003	0.007	0.003	0.044
rs1436955	*C2CD4B*	C	B	0.024	0.026	0.366	0.012	0.026	0.651	-0.034	0.026	0.195	0.064	0.031	0.040	0.003	0.005	0.527
rs7756992	*CDKAL1*	G	B	0.027	0.022	0.207	0.017	0.022	0.435	-0.013	0.022	0.552	0.059	0.026	0.022	0.006	0.004	0.125
rs1552224	*CENTD2*	A	B	0.034	0.031	0.273	0.019	0.031	0.540	-0.037	0.031	0.241	0.050	0.037	0.172	-0.001	0.006	0.878
rs5015480	*HHEX*	C	B	0.002	0.019	0.930	-0.005	0.019	0.776	-0.033	0.019	0.080	0.048	0.023	0.036	0.006	0.003	0.074
rs4402960	*IGF2BP2*	T	B	-0.024	0.026	0.344	-0.036	0.026	0.159	-0.060	0.026	0.021	0.046	0.030	0.134	0.003	0.005	0.550
rs1111875	*HHEX*	C	B	0.001	0.019	0.942	-0.006	0.019	0.768	-0.033	0.019	0.080	0.045	0.022	0.046	0.006	0.003	0.089
rs13266634	*SLC30A8*	C	B	0.010	0.020	0.596	-0.003	0.020	0.873	-0.041	0.020	0.036	0.037	0.023	0.117	0.010	0.003	0.004
rs5219	*KCNJ11*	T	B	0.001	0.019	0.949	-0.006	0.019	0.751	-0.014	0.019	0.473	0.033	0.023	0.147	0.004	0.003	0.187
rs11708067	*ADCY5*	A	B	-0.052	0.030	0.079	-0.066	0.030	0.025	-0.102	0.030	5.9E-04	0.025	0.035	0.477	0.009	0.005	0.095
rs2191349	*DGKB-TMEM195*	T	B	0.036	0.024	0.128	0.032	0.024	0.178	0.012	0.024	0.620	0.023	0.028	0.416	0.003	0.004	0.495
rs12779790	*CDC123,CAMK1D*	G	B	-0.022	0.022	0.336	-0.026	0.022	0.250	-0.035	0.022	0.124	0.018	0.027	0.510	0.000	0.004	0.902
rs7578597	*THADA*	T	B	0.063	0.033	0.057	0.064	0.033	0.052	0.056	0.033	0.086	0.006	0.039	0.886	0.008	0.006	0.158
rs564398	*CDKN2B*	T	B	-0.029	0.019	0.128	-0.035	0.019	0.067	-0.043	0.019	0.024	0.004	0.023	0.861	0.003	0.003	0.375
rs340874	*PROX1*	C	B	-0.020	0.024	0.410	-0.024	0.024	0.303	-0.032	0.024	0.178	0.000	0.028	0.993	0.005	0.004	0.294
rs4430796	*HNF1B,TCF2*	G	B	-0.027	0.025	0.278	-0.029	0.025	0.257	-0.032	0.025	0.211	0.000	0.030	0.999	0.001	0.005	0.813
rs7961581	*TSPAN8,LGR5*	C	B	-0.032	0.022	0.137	-0.030	0.022	0.164	-0.012	0.022	0.571	-0.006	0.026	0.811	-0.002	0.004	0.612
rs5945326	*DUSP9*	G	B	0.016	0.023	0.503	0.024	0.023	0.311	0.043	0.023	0.070	-0.015	0.028	0.591	0.000	0.004	0.966
rs972283	*KLF14*	G	IR	0.004	0.025	0.860	-0.006	0.025	0.824	-0.025	0.025	0.309	0.057	0.029	0.052	0.003	0.005	0.522
rs4607103	*ADAMTS9*	C	IR	0.040	0.022	0.071	0.034	0.022	0.125	0.005	0.022	0.819	0.054	0.026	0.039	0.011	0.004	0.006
rs2943641	*LOC64673, IRS1*	C	IR	0.049	0.024	0.042	0.048	0.024	0.047	0.040	0.024	0.095	0.053	0.028	0.063	0.012	0.004	0.009
rs1801282	*PPARG*	C	IR	0.020	0.029	0.490	0.015	0.029	0.602	-0.003	0.029	0.905	0.039	0.035	0.258	0.006	0.005	0.255
rs780094	*GCKR*	C	IR	-0.002	0.024	0.920	-0.011	0.024	0.661	-0.031	0.025	0.201	0.023	0.029	0.427	-0.003	0.005	0.441
rs780094	*GCKR*[a]	C	IR	0.066	0.021	0.002	0.056	0.022	0.009	0.017	0.023	0.455	0.068	0.028	0.015	0.003	0.004	0.484
rs17036101	*SYN2,PPARG*	G	IR	0.018	0.039	0.641	0.022	0.039	0.575	0.031	0.039	0.428	-0.003	0.046	0.943	0.002	0.007	0.756

rs8050136	*FTO*	A	IR	0.001	0.019	0.945	0.005	0.019	0.785	0.015	0.019	0.426	-0.027	0.023	0.248	0.000	0.003	0.913
rs1531343	*HMGA2*	C	IR?	**0.071**	**0.048**	**0.136**	**0.072**	**0.048**	**0.132**	0.073	0.048	0.125	0.005	0.056	0.935	-0.011	0.009	0.222
rs11634397	*ZFAND6*	G	U	**0.069**	**0.026**	**0.007**	**0.060**	**0.026**	**0.018**	0.019	0.026	0.452	0.087	0.030	0.004	0.009	0.005	0.053
rs12518099	*LOC729011, CETN3*	G	U	**0.055**	**0.030**	**0.066**	**0.046**	**0.030**	**0.127**	0.008	0.030	0.802	0.083	0.035	0.018	0.010	0.006	0.079
rs391300	*SRR*	C	U	0.011	0.025	0.663	-0.001	0.025	0.955	**-0.030**	**0.025**	**0.228**	0.059	0.029	0.041	0.007	0.005	0.149
rs1359790	*SPRY2*	G	U	0.009	0.026	0.733	0.002	0.026	0.953	-0.023	0.026	0.372	0.042	0.030	0.172	0.001	0.005	0.865
rs7957197	*HNF1A*	T	U	0.010	0.026	0.695	0.006	0.026	0.812	-0.004	0.026	0.889	0.030	0.031	0.342	0.000	0.005	0.932
rs4457053	*ZBED3*	G	U	-0.028	0.027	0.307	**-0.036**	**0.027**	**0.182**	**-0.050**	**0.027**	**0.070**	0.029	0.032	0.377	0.000	0.005	0.946
rs896854	*TP53INP1*	T	U	-0.008	0.023	0.717	-0.013	0.023	0.581	**-0.031**	**0.023**	**0.186**	0.025	0.027	0.349	0.004	0.004	0.396
rs17584499	*PTPRD*	T	U	-0.049	0.037	0.187	**-0.054**	**0.037**	**0.147**	**-0.056**	**0.037**	**0.130**	0.025	0.044	0.563	0.001	0.007	0.860
rs10923931	*NOTCH2*	T	U	-0.048	0.029	0.101	**-0.055**	**0.029**	**0.062**	**-0.075**	**0.029**	**0.011**	0.025	0.035	0.479	0.001	0.005	0.803
rs2641348	*ADAM3O*	G	U	-0.046	0.029	0.117	**-0.052**	**0.029**	**0.072**	**-0.072**	**0.029**	**0.013**	0.024	0.035	0.492	0.001	0.005	0.800
rs9472138	*VEGFA*	T	U	-0.042	0.021	0.047	**-0.049**	**0.021**	**0.021**	**-0.059**	**0.021**	**0.005**	0.013	0.025	0.617	-0.003	0.004	0.467
rs8042680	*PRC1*	A	U	-0.003	0.026	0.907	-0.006	0.026	0.829	-0.012	0.026	0.637	0.008	0.031	0.792	0.004	0.005	0.444
rs1153188	*DCD*	A	U	0.015	0.021	0.472	0.014	0.021	0.494	0.011	0.021	0.611	0.004	0.025	0.863	0.002	0.004	0.628
rs7593730	*RBMS1, ITGB6*	C	U	0.004	0.030	0.883	0.011	0.030	0.722	0.023	0.030	0.436	-0.005	0.035	0.890	-0.001	0.006	0.870
rs13292136	*CHCHD9*	C	U	-0.031	0.043	0.465	-0.030	0.043	0.487	-0.019	0.043	0.658	-0.033	0.050	0.517	-0.006	0.008	0.458
rs10490072	*BCL11A*	T	U	-0.021	0.022	0.324	-0.017	0.022	0.428	0.006	0.022	0.798	-0.036	0.026	0.167	0.002	0.004	0.597

For imputed SNPs: *n* = 3,179. For genotyped SNPs: HOMA-IR and HOMA-B: *n* = 5,536 – 5,678, insulin: *n* = 5,540 – 5,682, glucose: *n* = 5,541 – 5,683, HbA1c: *n* = 5,103 – 2,239. [a]Additionally adjusted for triglyceride levels.

Table 3 Association of T2D susceptibility variants with T2D related phenotypes where *P* = 0.1 – 0.5.

Variable	SNP	Gene	Allele	β	SE	*P*
BMI	rs4457053	*ZBED3*	G	-0.263	0.102	0.010
	rs4607103	*ADAMTS9*	C	-0.198	0.079	0.012
	rs391300	*SRR*	C	-0.217	0.092	0.018
	rs340874	*PROX1*	C	-0.185	0.089	0.037
Midlife BMI	rs391300	*SRR*	C	-0.229	0.092	0.013
	rs340874	*PROX1*	C	-0.204	0.089	0.022
WC	rs1153188	*DCD*	A	0.639	0.253	0.012
	rs2191349	*DGKB-TMEM195*	T	0.673	0.303	0.026
	rs972283	*KLF14*	G	-0.674	0.316	0.033
	rs391300	*SRR*	C	-0.647	0.314	0.039
WTREND	rs7961581	*TSPAN8,LGR5*	C	-0.012	0.005	0.016
	rs17036101	*SYN2,PPARG*	G	-0.019	0.009	0.028
	rs1153188	*DCD*	A	0.009	0.005	0.050
TG	rs11634397	*ZFAND6*	G	0.028	0.012	0.024
	rs10490072	*BCL11A*	T	-0.020	0.010	0.045
	rs1387153	*MTNR1B*	T	0.025	0.013	0.048
HDL	rs11708067	*ADCY5*	A	0.033	0.013	0.012
	rs972283	*KLF14*	G	-0.027	0.011	0.014
	rs10490072	*BCL11A*	T	0.018	0.009	0.044
LDL	rs231362	*KCNQ1*	G	-0.063	0.028	0.026
	rs972283	*KLF14*	G	-0.058	0.027	0.028
CORNCAL	rs1359790	*SPRY2*	G	-0.125	0.061	0.041
	rs7957197	*HNF1A*	T	-0.126	0.062	0.043
Plaque category	rs17036101	*SYN2,PPARG*	G	0.093	0.037	0.011
	rs564398	*CDKN2B*	T	0.042	0.018	0.020
	rs17584499	*PTPRD*	T	-0.082	0.036	0.024
	rs1436955	*C2CD4B*	C	0.054	0.026	0.036
	rs391300	*SRR*	C	0.049	0.024	0.039
	rs7578597	*THADA*	T	0.064	0.031	0.040
MI	rs1801214	*WFS1*	T	0.190	0.082	0.020
	rs17584499	*PTPRD*	T	-0.268	0.130	0.039
	rs1552224	*CENTD2*	A	-0.203	0.099	0.040
CHD	rs17584499	*PTPRD*	T	-0.216	0.093	0.020
DIAT-BP	rs10923931	*NOTCH2*	T	-0.821	0.323	0.011
	rs10811661	*CDKN2B*	T	-0.578	0.268	0.031
	rs1436955	*C2CD4B*	C	-0.605	0.300	0.044
	rs4607517	*GCK*	A	-0.783	0.397	0.049
SYS-BP	rs4607517	*GCK*	A	-1.909	0.847	0.024
GFR	rs9472138	*VEGFA*	T	0.854	0.367	0.020
	rs5015480	*HHEX*	C	-0.697	0.329	0.034
	rs1111875	*HHEX*	C	-0.686	0.326	0.035
CRE	rs1111875	*HHEX*	C	0.010	0.005	0.032

	rs5015480	*HHEX*	C	0.010	0.005	0.033
CKD	rs4607103	*ADAMTS9*	C	0.112	0.050	0.024
	rs4402960	*IGF2BP2*	T	0.128	0.062	0.038
MA	rs1153188	*DCD*	A	0.154	0.071	0.031
	rs8050136	*FTO*	A	0.132	0.064	0.038
ACR	rs9472138	*VEGFA*	T	0.083	0.035	0.019
	rs1153188	*DCD*	A	0.076	0.035	0.029
	rs972283	*KLF14*	G	0.091	0.042	0.032
	rs17036101	*SYN2,PPARG*	G	0.136	0.064	0.034
Retinopathy	rs7903146	*TCF7L2*	T	0.157	0.065	0.015
	rs7593730	*RBMS1, ITGB6*	C	-0.212	0.095	0.025
	rs7578597	*THADA*	T	-0.214	0.101	0.035
SC-WML	rs231362	*KCNQ1*	G	0.060	0.025	0.014
	rs8042680	*PRC1*	A	-0.056	0.024	0.023
	rs4457053	*ZBED3*	G	-0.057	0.026	0.026
	rs7593730	*RBMS1, ITGB6*	C	0.061	0.028	0.028
	rs1801282	*P*	C	0.053	0.027	0.046
PV-WML	rs1801282	*PPARG*	C	0.170	0.078	0.028
WMvol	rs4607103	*ADAMTS9*	C	-0.001	4.7×10^{-4}	0.013
	rs8050136	*FTO*	A	-9.2×10^{-4}	4.1×10^{-4}	0.027
	rs231362	*KCNQ1*	G	0.001	5.9×10^{-4}	0.045
GMvol	rs1153188	*DCD*	A	0.002	7.3×10^{-4}	0.018
	rs11708067	*ADCY5*	A	-0.002	0.001	0.028

BMI: Body mass index, WC: Waist circumference, TG: Triglycerides, HDL: High-density lipoprotein, CORNCAL: Coronary calcium, MI: Myocardial infarction, SYS-BP: Systolic blood pressure, GFR: Glomerular filtration rate, CRE: Serum creatinine, MA: Microalbuminuria, SC-WML: Subcortical white matter lesions, WMLvol: Total white matter lesion volume, GMvol: Grey matter volume, WMvol: White matter volume, CKD: Chronic kidney disease, WTREND: Weight trend, ACR: Albumin/creatinine ratio.

Table 4 Effects of the *TCF7L2* rs7903146 variant on T2D risk when partitioned by BMI group and LDL, HDL or triglyceride levels

		AGES			REFINE		
		n	OR	*P*	*n*	OR	*P*
BMI	<25	2943	1.24	0.045	1327	2.07	0.086
	25-30	2219	1.27	0.005	1667	1.44	0.099
	>30	532	1.69	8.2×10^{-4}	980	1.89	4.3×10^{-5}
LDL quartile	1	1400	1.31	0.010	1006	1.71	0.004
	2	1419	1.14	0.261	998	1.47	0.194
	3	1403	1.29	0.047	959	2.19	0.006
	4	1392	1.56	0.002	1007	1.20	0.531
HDL quartile	1	1383	1.12	0.216	1041	1.58	0.009
	2	1389	1.44	0.003	987	2.28	9.0×10^{-4}
	3	1437	1.48	0.004	999	1.63	0.088
	4	1411	1.32	0.077	967	1.32	0.450
Triglyceride quartile	1	1399	1.10	0.581	1015	1.09	0.820
	2	1403	1.51	0.002	961	1.97	0.035
	3	1428	1.20	0.138	1023	1.81	0.027
	4	1390	1.35	0.002	995	1.67	0.001

Table 5 Effects of the *TCF7L2* rs7903146 variant on T2D risk when partitioned by BMI group and LDL, HDL or triglyceride levels

		AGES			REFINE		
		n	OR	*P*	*n*	OR	*P*
BMI	<25	2934	0.94	0.368	1307	0.99	0.969
	25-30	2222	1.02	0.738	1637	1.11	0.533
	>30	514	1.13	0.440	956	1.95	1.1×10^{-4}
LDL quartile	1	1423	0.95	0.559	993	1.32	0.079
	2	1435	1.02	0.846	975	0.96	0.861
	3	1420	0.96	0.699	936	1.47	0.131
	4	1409	1.08	0.472	990	1.24	0.448
HDL quartile	1	1465	0.94	0.459	1022	1.47	0.025
	2	1388	1.10	0.292	967	1.22	0.395
	3	1460	1.07	0.449	976	1.46	0.055
	4	1381	0.84	0.107	951	0.99	0.979
Triglyceride quartile	1	1408	0.97	0.705	999	0.96	0.895
	2	1413	0.99	0.878	940	1.89	0.003
	3	1444	1.09	0.329	1002	0.90	0.640
	4	1429	0.93	0.393	975	1.43	0.030

Table 6 T2D-GRS (44 SNPs) association with T2D and related phenotypes (*n* = 3,179).

Variable group	Variable	Model A			Model B		
		β	SE	*P*	β	SE	*P*
	T2D	0.104	0.014	**1.6×10^{-13}**			
Metabolic	Fasting glucose	0.036	0.005	**8.8×10^{-14}**	0.013	0.004	**5.1×10^{-4}**
	Fasting insulin	5.8×10^{-4}	0.004	0.888	-0.007	0.004	0.083
	HbA1c	0.004	0.001	**1.3×10^{-7}**	0.002	0.001	**0.018**
	HOMA-B	-0.021	0.004	**4.1×10^{-7}**	-0.018	0.004	**9.6×10^{-6}**
	HOMA-IR	0.008	0.004	0.062	-0.003	0.004	0.462
Anthropometric	BMI	-0.029	0.015	0.056	-0.051	0.015	**8.2×10^{-4}**
	Midlife BMI	-0.025	0.015	0.102	-0.047	0.015	**0.002**
	Waist circumference	-0.086	0.053	0.103	-0.148	0.052	**0.005**
	Weight trend	-2.9×10^{-4}	0.001	0.765	-0.001	0.001	0.424
Blood lipids	Triglycerides	-0.001	0.002	0.487	-0.004	0.002	**0.028**
	HDL	0.003	0.002	0.086	0.005	0.002	**0.003**
	LDL	-0.007	0.004	0.092	-0.003	0.004	0.532
Cardiovascular	CHD	-0.005	0.010	0.613	-0.011	0.010	0.259
	MI	-0.010	0.014	0.475	-0.017	0.014	0.218
	Plaque category	0.006	0.004	0.122	0.004	0.004	0.281
	Coronary calcium	-0.010	0.010	0.302	-0.015	0.010	0.125
	Diastolic BP	-0.074	0.047	0.118	-0.078	0.047	0.101
	Systolic BP	0.079	0.100	0.431	0.021	0.101	0.835
Renal	CKD	0.016	0.010	0.101	0.011	0.010	0.260
	GFR	-0.097	0.075	0.196	-0.067	0.076	0.374
	Serum creatinine	0.001	0.001	0.201	0.001	0.001	0.435
	Microalbuminuria	0.013	0.014	0.339	0.002	0.014	0.869
	ACR	3.8×10^{-4}	0.007	0.957	-0.007	0.007	0.349
Retinal	Retinopathy	0.021	0.014	0.134	0.004	0.014	0.773
Brain	Periventricular WMLs	0.014	0.011	0.210	0.011	0.011	0.319
	Subcortical WMLs	0.007	0.004	0.075	0.005	0.004	0.215
	Total WML volume	0.007	0.004	0.094	0.005	0.004	0.266
	White matter volume	-1.4×10^{-4}	9.2×10^{-5}	0.138	-5.6×10^{-5}	9.2×10^{-5}	0.544
	Grey matter volume	-1.6×10^{-4}	1.5×10^{-4}	0.269	-1.2×10^{-4}	1.5×10^{-4}	0.433

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 7 Association of six T2D susceptibility SNPs with T2D in REFINE.

SNP	Gene	Risk allele	*n*	OR	95% CI	*P*
rs7903146	*TCF7L2*	T	3,994	1.02	(1.01 - 1.03)	**4.9×10^{-6}**
rs7756992	*CDKAL1*	G	4,326	1.01	(1.00 - 1.02)	0.227
rs13266634	*SLC30A8*	C	4,392	1.00	(1.00 - 1.01)	0.245
rs1111875	*HHEX*	G	4,559	1.00	(1.00 - 1.01)	0.353
rs10811661	*CDKN2B*	T	4,244	1.00	(0.99 - 1.01)	0.778
rs5219	*KCNJ11*	T	3,513	1.00	(0.99 - 1.01)	0.928

Table 8 Association of a GRS constructed from the SNPs in Table 7 – Appendix 2 with metabolic and lipid traits in REFINE (*n* = 2,735).

	Model A			Model B		
Variable	β	SE	*P*	β	SE	*P*
T2D	0.106	0.064	0.097			
Fasting glucose (log)	0.006	0.002	**2.0×10^{-4}**	0.004	0.001	**8.9×10^{-4}**
BMI	0.005	0.056	0.934	-0.011	0.055	0.836
Waist circumference	0.124	0.143	0.387	0.081	0.141	0.567
Triglycerides (log)	0.012	0.006	**0.039**	0.011	0.006	0.061
HDL	-0.011	0.005	**0.017**	-0.010	0.004	**0.025**
LDL	-0.002	0.010	0.862	0.001	0.010	0.947

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 9 BCF-GRS (22 SNPs) association with T2D and related phenotypes (*n* = 3,179).

Variable group	Variable	Model A			Model B		
		β	SE	*P*	β	SE	*P*
	T2D	0.118	0.020	**3.0×10^{-9}**			
Metabolic	Fasting glucose	0.042	0.007	**6.4×10^{-10}**	0.016	0.005	**2.1×10^{-3}**
	Fasting insulin	-0.005	0.006	0.390	-0.014	0.006	**0.017**
	HbA1c	0.005	0.001	**2.6×10^{-5}**	0.002	0.001	0.051
	HOMA-B	-0.034	0.006	**4.6×10^{-9}**	-0.031	0.006	**8.9×10^{-8}**
	HOMA-IR	0.005	0.006	0.408	-0.007	0.005	0.199
Anthropometric	BMI	-0.037	0.022	0.087	-0.062	0.022	**4.2×10^{-3}**
	Midlife BMI	-0.030	0.022	0.170	-0.054	0.022	**0.012**
	Waist circumference	-0.017	0.075	0.816	-0.085	0.074	0.251
	Weight trend	5.0×10^{-4}	0.001	0.714	-2.8×10^{-5}	0.001	0.984
Blood lipids	Triglycerides	-0.002	0.003	0.560	-0.005	0.003	0.076
	HDL	0.005	0.003	0.082	0.007	0.003	**0.006**
	LDL	-0.006	0.006	0.376	-1.9×10^{-4}	0.006	0.975
Cardiovascular	CHD	-0.007	0.014	0.639	-0.014	0.014	0.341
	MI	-0.015	0.019	0.449	-0.023	0.019	0.246
	Plaque category	0.012	0.006	**0.031**	0.010	0.006	0.073
	Coronary calcium	-1.2×10^{-4}	0.014	0.993	-0.005	0.014	0.697
	Diastolic BP	-0.109	0.067	0.103	-0.114	0.067	0.090
	Systolic BP	-0.029	0.142	0.838	-0.097	0.143	0.498
Renal	CKD	0.010	0.014	0.446	0.005	0.014	0.741
	GFR	-0.117	0.106	0.273	-0.082	0.107	0.445
	Serum creatinine	0.001	0.001	0.307	0.001	0.001	0.544
	Microalbuminuria	0.010	0.020	0.599	-0.003	0.020	0.878
	ACR	-0.012	0.010	0.216	-0.020	0.010	**0.041**
Retinal	Retinopathy	0.037	0.020	0.060	0.019	0.020	0.346
Brain	Periventricular WMLs	0.010	0.016	0.509	0.007	0.016	0.650
	Subcortical WMLs	0.008	0.005	0.125	0.006	0.005	0.272
	Total WML volume	0.008	0.006	0.153	0.006	0.006	0.330
	White matter volume	-1.7×10^{-4}	1.3×10^{-4}	0.193	-7.8×10^{-5}	1.3×10^{-4}	0.551
	Grey matter volume	-1.7×10^{-4}	2.1×10^{-4}	0.417	-1.2×10^{-4}	2.1×10^{-4}	0.583

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 10 BCF-GRS (28 SNPs) association with T2D and related phenotypes (*n* = 3,179).

Variable group	Variable	Model A			Model B		
		β	SE	*P*	β	SE	*P*
	T2D	0.111	0.018	**2.4×10^{-10}**			
Metabolic	Fasting glucose	0.039	0.006	**9.3×10^{-11}**	0.015	0.005	**1.8×10^{-3}**
	Fasting insulin	-0.009	0.005	0.095	-0.017	0.005	**8.2×10^{-4}**
	HbA1c	0.004	0.001	**1.3×10^{-5}**	0.002	0.001	**0.049**
	HOMA-B	-0.035	0.005	**1.4×10^{-11}**	-0.032	0.005	**4.6×10^{-10}**
	HOMA-IR	5.0×10^{-4}	0.005	0.923	-0.011	0.005	**0.025**
Anthropometric	BMI	-0.048	0.019	**0.013**	-0.071	0.019	**2.0×10^{-4}**
	Midlife BMI	-0.041	0.019	**0.034**	-0.064	0.019	**7.5×10^{-4}**
	Waist circumference	-0.088	0.066	0.180	-0.154	0.065	**0.019**
	Weight trend	-2.9×10^{-4}	0.001	0.810	-8.1×10^{-4}	0.001	0.502
Blood lipids	Triglycerides	-0.002	0.002	0.483	-0.005	0.002	**0.047**
	HDL	0.005	0.002	**0.020**	0.008	0.002	**6.6×10^{-4}**
	LDL	-0.005	0.006	0.388	2.5×10^{-4}	0.006	0.964
Cardiovascular	CHD	-0.009	0.012	0.494	-0.016	0.013	0.219
	MI	-0.016	0.017	0.342	-0.024	0.017	0.159
	Plaque category	0.010	0.005	0.056	0.008	0.005	0.134
	Coronary calcium	-0.009	0.012	0.469	-0.014	0.012	0.252
	Diastolic BP	-0.130	0.059	**0.027**	-0.135	0.059	**0.022**
	Systolic BP	-0.006	0.126	0.964	-0.069	0.126	0.586
Renal	CKD	0.004	0.012	0.732	-0.001	0.012	0.909
	GFR	-0.018	0.094	0.846	0.015	0.094	0.874
	Serum creatinine	0.000	0.001	0.878	0.000	0.001	0.781
	Microalbuminuria	0.009	0.017	0.590	-0.002	0.018	0.889
	ACR	-0.009	0.009	0.325	-0.016	0.009	0.066
Retinal	Retinopathy	0.023	0.017	0.193	0.004	0.018	0.807
Brain	Periventricular WMLs	0.002	0.014	0.896	-0.001	0.014	0.937
	Subcortical WMLs	0.004	0.005	0.389	0.002	0.005	0.685
	Total WML volume	0.005	0.005	0.334	0.002	0.005	0.626
	White matter volume	-1.3×10^{-4}	1.2×10^{-4}	0.263	-4.2×10^{-5}	1.2×10^{-4}	0.716
	Grey matter volume	-1.7×10^{-4}	1.8×10^{-4}	0.368	-1.2×10^{-4}	1.9×10^{-4}	0.529

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 11 IR-GRS (6 SNPs) association with T2D and related phenotypes (*n* = 3,179).

Variable group	Variable	Model A			Model B		
		β	SE	*P*	β	SE	*P*
	T2D	0.115	0.037	**0.002**			
Metabolic	Fasting glucose	0.033	0.013	**0.010**	0.008	0.010	0.425
	Fasting insulin	0.019	0.011	0.078	0.011	0.011	0.308
	HbA1c	0.005	0.002	**0.013**	0.002	0.002	0.256
	HOMA-B	0.004	0.011	0.728	0.007	0.011	0.547
	HOMA-IR	0.024	0.011	**0.031**	0.012	0.010	0.252
Anthropometric	BMI	0.025	0.041	0.546	0.001	0.040	0.972
	Midlife BMI	0.022	0.041	0.600	-0.006	0.040	0.891
	Waist circumference	-0.074	0.141	0.597	-0.145	0.139	0.299
	Weight trend	-5.2×10^{-4}	0.003	0.838	-0.001	0.003	0.683
Blood lipids	Triglycerides	-0.008	0.005	0.149	-0.011	0.005	**0.034**
	HDL	-0.005	0.005	0.350	-0.002	0.005	0.657
	LDL	-0.017	0.012	0.141	-0.012	0.012	0.312
Cardiovascular	CHD	-0.015	0.027	0.577	-0.021	0.027	0.444
	MI	0.043	0.037	0.243	0.038	0.037	0.307
	Plaque category	-0.004	0.011	0.733	-0.006	0.011	0.597
	Coronary calcium	0.009	0.026	0.718	0.004	0.026	0.873
	Diastolic BP	0.045	0.126	0.721	0.044	0.126	0.728
	Systolic BP	3.9×10^{-4}	0.268	0.999	-0.067	0.268	0.803
Renal	CKD	0.051	0.026	0.050	0.047	0.026	0.072
	GFR	-0.424	0.200	**0.035**	-0.400	0.201	**0.046**
	Serum creatinine	0.006	0.003	**0.031**	0.005	0.003	**0.046**
	Microalbuminuria	0.040	0.037	0.287	0.030	0.038	0.433
	ACR	0.008	0.019	0.657	0.001	0.019	0.945
Retinal	Retinopathy	0.012	0.037	0.755	-0.005	0.038	0.893
Brain	Periventricular WMLs	0.037	0.030	0.223	0.032	0.030	0.286
	Subcortical WMLs	0.028	0.010	**0.007**	0.025	0.010	**0.015**
	Total WML volume	0.023	0.011	**0.033**	0.020	0.011	0.063
	White matter volume	-4.2×10^{-4}	2.5×10^{-4}	0.090	-3.2×10^{-4}	2.4×10^{-4}	0.197
	Grey matter volume	-4.9×10^{-4}	3.9×10^{-4}	0.216	-4.2×10^{-4}	3.9×10^{-4}	0.288

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 12 IR-GRS (10 SNPs) association with T2D and related phenotypes (*n* = 3,179).

Variable group	Variable	Model A			Model B		
		β	SE	*P*	β	SE	*P*
	T2D	0.137	0.031	**1.3×10^{-5}**			
Metabolic	Fasting glucose	0.048	0.011	**1.0×10^{-5}**	0.017	0.008	**0.037**
	Fasting insulin	0.026	0.009	**4.9×10^{-3}**	0.016	0.009	0.077
	HbA1c	0.005	0.002	**0.002**	0.002	0.002	0.156
	HOMA-B	0.004	0.009	0.685	0.007	0.009	0.442
	HOMA-IR	0.032	0.009	**5.0×10^{-4}**	0.018	0.009	**0.039**
Anthropometric	BMI	0.042	0.034	0.226	0.012	0.034	0.714
	Midlife BMI	0.041	0.034	0.232	0.009	0.034	0.788
	Waist circumference	-0.093	0.118	0.431	-0.178	0.117	0.129
	Weight trend	-0.001	0.002	0.708	-0.001	0.002	0.500
Blood lipids	Triglycerides	-0.001	0.004	0.750	-0.005	0.004	0.212
	HDL	-0.005	0.004	0.206	-0.002	0.004	0.557
	LDL	-0.012	0.010	0.241	-0.005	0.010	0.588
Cardiovascular	CHD	-0.019	0.022	0.389	-0.027	0.022	0.227
	MI	0.017	0.030	0.567	0.010	0.031	0.753
	Plaque category	0.004	0.009	0.693	0.001	0.009	0.916
	Coronary calcium	0.003	0.022	0.877	-0.003	0.022	0.897
	Diastolic BP	0.056	0.105	0.597	0.053	0.106	0.614
	Systolic BP	0.046	0.225	0.838	-0.032	0.225	0.887
Renal	CKD	0.061	0.022	**0.005**	0.056	0.022	**0.010**
	GFR	-0.476	0.168	**0.005**	-0.450	0.168	**0.007**
	Serum creatinine	0.007	0.002	**0.003**	0.006	0.002	**0.006**
	Microalbuminuria	0.020	0.031	0.511	0.007	0.031	0.817
	ACR	0.019	0.016	0.235	0.011	0.016	0.499
Retinal	Retinopathy	0.044	0.031	0.159	0.024	0.032	0.442
Brain	Periventricular WMLs	0.014	0.025	0.571	0.010	0.025	0.690
	Subcortical WMLs	0.018	0.009	**0.035**	0.015	0.009	0.080
	Total WML volume	0.020	0.009	**0.029**	0.017	0.009	0.063
	White matter volume	-4.9×10^{-4}	2.1×10^{-4}	**0.018**	-3.8×10^{-4}	2.0×10^{-4}	0.064
	Grey matter volume	-3.8×10^{-4}	3.3×10^{-4}	0.255	-3.2×10^{-4}	3.3×10^{-4}	0.341

Model A: Adjusted for age and sex, Model B: Adjusted for age, sex and T2D.

Table 13 Interaction between GRSs and anthropometric variables on T2D risk.

	OR	(95% CI)	*P*
T2D-GRS (44)			
BMI	1.00	(0.99 – 1.01)	0.537
Midlife BMI	1.00	(1.00 – 1.01)	0.366
Waist circumference	1.00	(1.00 – 1.00)	0.923
Weight trend	0.94	(0.84 – 1.05)	0.260
BCF-GRS (22)			
BMI	1.00	(0.99 – 1.01)	0.886
Midlife BMI	1.00	(0.99 – 1.01)	0.496
Waist circumference	1.00	(1.00 – 1.00)	0.543
Weight trend	0.91	(0.78 – 1.07)	0.242
BCF-GRS (28)			
BMI	1.00	(0.99 – 1.01)	0.833
Midlife BMI	1.00	(0.99 – 1.01)	0.494
Waist circumference	1.00	(1.00 – 1.00)	0.777
Weight trend	0.97	(0.84 – 1.11)	0.629
IR-GRS (6)			
BMI	1.01	(0.99 – 1.03)	0.346
Midlife BMI	1.01	(0.99 – 1.03)	0.566
Waist circumference	1.00	(1.00 – 1.01)	0.723
Weight trend	0.86	(0.63 – 1.18)	0.348
IR-GRS (10)			
BMI	1.01	(0.99 – 1.03)	0.363
Midlife BMI	1.00	(0.99 – 1.02)	0.683
Waist circumference	1.00	(0.99 – 1.00)	0.754
Weight trend	0.80	(0.62 – 1.04)	0.095
REFINE-GRS (6)			
BMI	1.01	(0.98 – 1.03)	0.605
Waist circumference	1.00	(0.99 – 1.01)	0.574

Table 14 Interaction between GRSs and anthropometric variables on fasting glucose levels

	β	SE	*P*
T2D-GRS (44)			
BMI	0.004	0.001	**0.009**
Midlife BMI	0.004	0.001	**0.003**
Waist circumference	7.4×10^{-4}	3.8×10^{-4}	**0.049**
Weight trend	-0.021	0.020	0.305
BCF-GRS (22)			
BMI	0.002	0.002	0.340
Midlife BMI	0.003	0.002	0.153
Waist circumference	2.1×10^{-4}	5.5×10^{-4}	0.699
Weight trend	-0.013	0.028	0.635
BCF-GRS (28)			
BMI	0.003	0.002	0.120
Midlife BMI	0.003	0.002	**0.048**
Waist circumference	6.6×10^{-4}	4.8×10^{-4}	0.168
Weight trend	-0.010	0.025	0.698
IR-GRS (6)			
BMI	0.004	0.004	0.247
Midlife BMI	0.003	0.004	0.368
Waist circumference	0.001	0.001	0.213
Weight trend	-0.031	0.056	0.575
IR-GRS (10)			
BMI	0.008	0.003	**0.016**
Midlife BMI	0.007	0.003	**0.039**
Waist circumference	0.001	8.8×10^{-4}	0.140
Weight trend	-0.066	0.046	0.154
REFINE-GRS (6)			
BMI	6.8×10^{-4}	3.1×10^{-4}	**0.027**
Waist circumference	2.8×10^{-4}	1.1×10^{-4}	**0.013**

Table 15 List of enriched functional categories for the Bayesian subnetworks created from the IR and BCF associated seed SNPs.

BCF network		IR network	
Enriched categories	*P**	Enriched categories	*P**
Mitosis	1.4×10^{-6}	Carboxylic acid metabolism	1.0×10^{-10}
Cell cycle	1.7×10^{-5}	Energy pathways	4.8×10^{-7}
Cell adhesion	5.7×10^{-5}	Glucose metabolism	8.4×10^{-6}
Spindle checkpoint	6.0×10^{-5}	Coenzyme metabolism	6.0×10^{-5}
		Aging	0.0002
		Hexose metabolism	0.0007
		Lipid metabolism	0.0010

* Bonferroni adjusted