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Institut für Experimentelle Genetik

Genetic Analysis of Candidate Genes for the Metabolic Syndrome and Type 2 Diabetes

Harald H. Grallert

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1. apl. Prof. Dr. J. Adamski
2. Univ.-Prof. Dr. H. Daniel
3. Priv.-Doz. Dr. Th. Illig,
   Ludwig-Maximilians-Universität München

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Table of contents

Summary

The metabolic syndrome (MetS) is a cluster of cardiovascular risk factors, with controversial clinical relevance. MetS is closely connected to diabetes mellitus (DM), one of the most challenging diseases of the 21st century, which most common form, type 2 diabetes mellitus (T2DM), was another focus of this thesis. Due to the enormous increase of both diseases there is immediate need of action to develop new therapies and prediction methods for MetS and DM. Both diseases are influenced by a number of environmental factors, but influences of genetic components have also been demonstrated. This work addresses the genetic influences by investigating genetic variation in selected genes with a putative role in development of MetS or T2DM. In particular single nucleotide polymorphisms (SNPs) within these gene loci were analyzed for association with MetS, T2DM and related parameters. SNPs were genotyped using mainly Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI TOF MS) in different study populations depending on design of the specific analyis. The genes *IL-6*, *APOA5*, *MCP-1* and *RETN* were selected as candidate genes for central analyis. Furthermore, projects that should replicate and enhance association results for *TCF7L2*, *ACBP* and *PTGES2* were supported within this work.

For the first time a variant in the *APOA5* locus (c.56G>C) was associated with higher risk for MetS in Caucasians. However, this association might be driven by strong association with triglyceride and HDL cholesterol levels, two components of MetS. Evidence for associations with components of MetS was found for variants within *MCP-1* an *RETN* as well. Minor alleles of *IL-6* promoter polymorphism -174 G>C and variants in *ACBP* and *PTGES2* were associated with lower risk for T2DM. Association of *TCF7L2* variants with higher risk of T2DM could be confirmed. In addition trends for lower fasting insulin levels and basal insulin secretion were observed. Evidence for an influence on bone metabolism was found for two variants in *IL-6* and *MCP-1* in healthy men. Relevance of these findings in MetS development is yet unclear.

Associations between gene variants and complex diseases are hardly detectable. This is accomplished by manifold genetic and environmental factors. Nevertheless, associations with MetS, T2DM or related features were shown for several variants within this work. Thus, this work provides a basis for further functional studies that are necessary for understanding of the underlying mechanisms, which are still speculative after association analyses.

Zusammenfassung

Das Metabolische Syndrom (MetS) ist ein Cluster kardiovaskulärer Risikofaktoren, dessen klinische Relevanz umstritten ist. Das MetS ist eng verbunden mit Diabetes Mellitus (DM), einer der bedeutendsten Stoffwechselerkrankungen des 21. Jahrhunderts, deren häufigste Form Typ 2 Diabetes Mellitus (T2DM) darstellt. Beide Krankheiten nehmen in enormem Maß zu, so dass die Entwicklung neuer Therapie- und Prognosemethoden für das MetS und T2DM höchste Priorität hat. Bei beiden Krankheiten spielen verschiedene Umweltfaktoren, aber auch genetische Komponenten eine Rolle. Diese Arbeit befasst sich mit den genetischen Komponenten, indem die genetische Variation in Form einzelner Basenaustausche ausgewählter Gene, die an relevanten Prozessen, die zu MetS oder T2DM führen, beteiligt sind, auf Assoziationen mit MetS, T2DM und verwandter Parameter untersucht wurde. Die Genvarianten wurden hauptsächlich mittels MALDI TOF MS, je nach Design der spezifischen Analyse in verschiedenen Studienpopulationen genotypisiert. Als Kandidatengene für die Kernanalyse wurden *IL-6*, *APOA5*, *MCP-1* und *RETN* ausgewählt. Weitere Projekte, die Assoziationsergebnisse in den Genen *TCF7L2*, *ACBP* und *PTGES2* replizieren und erweitern sollten, wurden im Rahmen dieser Arbeit unterstützt.

Im *APOA5* Gen konnte erstmals in Kaukasiern ein SNP (c.56G>C) mit MetS assoziiert werden, was vermutlich auf dessen starke Assoziation mit erhöhten Triglyzerid und HDL-Cholesterolspiegeln, zweier MetS Komponenten, zurückzuführen ist. Hinweise auf Assoziationen mit MetS Komponenten waren auch für Genvarianten in *MCP-1* und *RETN* zu finden. Für SNPs wie den *IL-6* Promotor SNP -174 G>C und Genvarianten in *ACBP* und *PTGES2* wurden Assoziationen mit verringertem T2DM Risiko gefunden. Für *TCF7L2* konnte die starke Assoziation zweier Genvarianten mit erhöhtem T2DM Risiko bestätigt werden. Zusätzlich konnte ein Trend zu verringerten Nüchterninsulinspiegeln sowie verminderter basaler Insulin Sekretion bei Individuen, die das seltene Allel tragen, beobachtet werden. Zwei Genvarianten in den Genen *IL-6* und *MCP-1* zeigten in gesunden Männern Hinweise auf Einflüsse im Knochen Metabolismus. Ob diese Beobachtung ein Rolle bei MetS oder T2DM spielt, bleibt jedoch unklar.

Assoziationen zwischen Genvarianten und komplexen Erkrankungen sind durch die Vielfalt genetischer Suszeptibilität und unterschiedlicher Umweltfaktoren schwer detektierbar. Trotzdem konnten in dieser Arbeit Assoziationen mit MetS, T2DM oder einzelnen Komponenten dieser Krankheiten gezeigt werden. Die zugrunde liegenden Mechanismen, über die anhand von Assoziationsanalysen nur spekuliert werden kann, müssen auf Basis der in dieser Arbeit gewonnenen Erkenntnisse in zukünftigen Studien näher untersucht werden.

Abbreviations

AACE	the American College of Endocrinology
ABCC8	ATP-binding cassette, subfamily C, member 8
ACBP	acyl-CoA-binding protein
ADIPOQ	adipocyte, C1Q
ADIPOR	adiponectin receptor
ADRB3	beta-3-adrenergic receptor
ALX4	aristaless-like 4
AMELX	amelogenin
apo	apolipoprotein
ATP III	adult treatment panel III
BIA	bioelectric impedance analysis
BIR	alias for KCNJ11
BMI	body mass index
BPR	blood pressure
CAPN	calpain
CCL2	chemokine ligand 2
CCR2	chemokine receptor 2
CDK5	cyclin-dependent kinase 5
CDKAL1	CDK5 regulatory subunit-associated protein 1-like 1
CDKN2	cyclin-dependent kinase inhibitor 2A
CHOD-PAP	method for cholesterol measurement
Chr	chromosome
CVD	cardiovascular disease
CYP19	cytochrome P450, subfamily XIX
D´	Lewontin´s disequilibrium coefficient
ddNTP	dideoxynucleotide triphosphate
DM	diabetes mellitus
dNTP	deoxynucleotide Triphosphate
DPP	Diabetes Prevention Program
EGIR	European Group for the Study of Insulin Resistance
ELISA	enzyme-linked immunosorbent assay
EM	expectation-maximization
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
EPIC	European-Prospective-Investigation-into-Cancer-and-Nutrition-Study
ERK	extracellular signal-regulated kinases
ESR1	estrogen receptor 1
EXT	exostosin
F	female
FAM	6-carboxy fluorescein
FBG	fasting blood glucose
FTO	fat mass- and obesity-associated gene
GLUT	glucose transport protein
gp130	transducer chain of cytokines
GSR	genotyping success rate
GYG	glycogenin
HBA1	Haemoglobulin Adult 1c
HHEX	hematopoietically expressed homeobox
HMCS	human–mouse conserved sequences
hME	homogenous mass extend
HNF4A	hepatocyte nuclear factor 4 -alpha
HOMA- % B	homeostasis model assessment-B-cell function
HOMA-IR	homeostasis model assessment–insulin resistance
HSD11B1	11-beta-hydroxsteroid dehydrogenase, type 1
HWE	Hardy Weinberg equilibrium
I(KATP)	inwardly rectifying ATP-sensitive potassium channel
IDE	insulin-degrading enzyme
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGF2BP2	insulin-like growth factor 2 mRNA-binding protein 2
IGF-I	insulin-like growth factor 1
IGT	impaired glucose tolerance
IIPGA	Innate Immunity Program for Genomic Applications
IL-6	interleukin-6
INS	insulin
IR	insulin resistance
IRAS	Insulin Resistance Atherosclerosis Study
IRS	insulin receptor
KCNJ11	potassium channel, inwardly rectifying, subfamily J, member 11
KIF11	kinesin family member 11
KORA	Cooperative Health Research in the Region of Augsburg
LDL	low density lipoprotein
LEP	leptin
LEPR	leptin receptor
LOD	logarithmic odds ratio
M	male/men
MAF	minor allele frequency
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry
MCP-1	monocyte chemotactic protein 1
MetS	metabolic syndrome
MONICA	monitoring trends and determinants on cardiovascular diseases
mRNA	messenger ribonucleic acid
NCBI	National Center for Biotechnology
NCEP	National Cholesterol Education Panel
NFkB1	nuclear factor kappa-B subunit 1
NR3C1	glucocorticoid receptor
OD	optical density
OGTT	oral glucose tolerance test
OR	odds ratio
p	p-value
PC	principal component
PCGM	percent change of geometric mean
PCR	polymerase chain reaction
PF	principal factor
PGE2	prostaglandin E2
PGH	prostaglandin H
PPARA	peroxisome proliferator-activated receptor alpha
PPARG	peroxisome proliferator-activated receptor gamma
PPG	postprandial glucose
PTGES2	prostaglandin E synthase 2
QTL	quantitative trait loci
RANKL	receptor activator of NF-kappa-B ligand
RBC	red blood cell
RETN	resitin
rs	reference sequence
SAP	shrimp alkaline phosphatase
SAPHIR	Salzburg Atherosclerosis Prevention program in subjects at High Individual Risk
SCL2A2	solute carrier family 2 (glucose transporter), member 2
SDS	Sequence Detector Software
SE	saline EDTA
SEV	secondary electron multiplier
SLC30A8	solute carrier family 30 (zinc transporter), member 8
SNP	single nucleotide polymorphism
SUR	sulfonylurea receptor
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
Taq	thermus aquaticus
TBE	tris-borate electrophoresis
TCF7L2	transcription like factor 7 like 2
TFBS	transcription factor-binding sites
TG	triglyceride
Tm	melting temperature
TNF	tumor necrosis factror
UTR	untranslated region
UV	ultra violet
VLDL	very low density lipoprotein
W	women
WHO	world health organization
WHR	waist to hip ratio
Wnt	wingless Int-1

1. Introduction

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors characterized by visceral obesity, atherogenic dyslipidaemia, hyperglycaemia, hypertension, hyperuricaemia, a proinflammatory and a prothromobotic state (Eckel et al. 2005b). MetS is closely connected to diabetes mellitus (DM) one of the most challenging diseases of the 21st century (Zimmet 2000). Type 2 diabetes mellitus (T2DM) is discussed as sequela or a component of MetS. While definition and clinical relevance of MetS are controversially discussed, the prevalence is alarmingly increasing. This applies as well for DM, which is known to be increasing frequent through all age groups worldwide. Increase of both disorders is according to rising epidemic of obesity, which has a central role in development of both disorders. Thus, there is immediate need of action to develop new therapies and prediction methods for MetS and DM.

1.1 Metabolic syndrome

The concept of metabolic syndrome has been evolving for years, whereas it was called syndrome X or reaven's syndrome (Reaven 1997), insulin resistance syndrome (DeFronzo et al. 1991), deadly quartet (Kaplan 1989), or polymetabolic syndrome (Crepaldi et al. 1995). A survey of the major definitions is given in Table 1. Originally, MetS was proposed as a set of clinical risk factors explainable by a common pathophysiologic link, insulin resistance (Reaven 1998). However, complex nature of insulin resistance resulted in controversy. Insulin resistance is defined as a decreased glucose lowering response to insulin and is usually associated with an abnormal insulin secretion pattern that results in increased plasma concentration of mature and immature forms of the hormone (Radziuk 2000). Multiple and diverse metabolic pathways regulated by insulin and selectivity of the defect to some tissues contribute to complexity. Insulin is the major determinant of protein and lipid synthesis in the liver and muscle. It is the main negative regulator of lipolysis in adipose tissue. In addition, it regulates synthesis of diverse compounds such as coagulation factors, sex hormones and apolipoproteins. Insulin coordinates global responses such as the lipoprotein sources during fasting or feeding, tissue growth or the endothelial function. In MetS, the severity of the defect varies between tissues and metabolic pathways. The liver, muscle, endothelial cells, and adipose tissue are the main affected organs (Legro et al. 2004). The most widely used criteria to identify these high risk individuals was defined by the National Cholesterol Education Panel (NCEP) in 2002 (NCEP 2002, Grundy et al. 2005). In summary, MetS can be named a multiplex MetS which is one of several patterns of risk for atherosclerotic cardiovascular disease (CVD). Despite the lack of a final definition there is agreement that two major disorders, obesity and T2DM, are relevant parts of MetS. Since the latest definition by the International Diabetes Federation (IDF) (Alberti et al. 2005) both disorders, which are closely related, are central constituents of MetS.

Table 1: Comparison of metabolic syndrome definitions

Guideline	Glucose/insulin abnormality	Obesity/central adiposity	Dyslipidaemia	Hypertension	Other	Minimum criteria for diagnosis
WHO (Alberti et al. 1998)	Type 2 diabetes, impaired fasting glucose (FBG ≥6.1 mmol/l), impaired glucose tolerance (2h PPG ≥7.8 mmol/l), or lowest 25% for hyperinsulinemic euglycemic clamp-glucose uptake	Waist-to-hip ratio >0.9 (M) or >0.85 (F) and/or BMI >30 kg/m²	Triglycerides ≥1.7mmol/l and/or HDL-C <0.9mmol/l (M) or <1.0mmol/l (F)	BPR ≥140/90mmHg (and/or medication)	Microalbuminuria (≥20µg/min albumin excretion rate or albumin:creatinine ratio ≥30mg/g)	Glucose intolerance/insulin resistance, plus two other features
EGIR[a] (Balkau et al. 1999)	Insulin resistance: hyperinsulinemia (nondiabetic fasting insulin in top 25%) and impaired fasting glucose (FBG ≥6.1 mmol/l	Waist circumference ≥94 cm (M) or ≥80 cm (F)	Triglycerides >2mmol/l and/or HDL-C <1.0mmol/l	BPR ≥140/90mmHg (and/or medication)	--	Insulin resistance, plus two other features
NCEP (NCEP 2001)	Impaired fasting glucose (FBG ≥6.1 mmol/l)	Waist circumference >102 cm (M) or >88 cm (F)	Triglycerides ≥1.69mmol/l, HDL-C <1.04mmol/l (M) or <1.29mmol/l (F)	BPR ≥130/85mmHg (and/or medication)	--	Any three features
NCEP med (Grundy et al. 2005)	Impaired fasting glucose (FBG ≥6.1 mmol/l) and/or medication	Waist circumference >102 cm (M) or >88 cm (F)	Triglycerides ≥1.69mmol/l, HDL-C <1.04mmol/l (M) or <1.29mmol/l (F); and/or medication	BPR ≥130/85mmHg and/or medication	--	Any three features
AACE (Einhorn et al. 2003)	Glucose intolerance (FBG ≥6.1 mmol/l or 2h PPG ≥7.8 mmol/l)	BMI ≥25 kg/m²	Triglycerides ≥1.69 mmol/l, HDL-C <1.04 mmol/l (M) or <1.29 mmol/l (F)	BPR ≥130/85mmHg	Family history of or high-risk ethnic group for type 2 diabetes, hypertension or CVD; polycystic ovarian syndrome; sedentary lifestyle; advancing age	Clinical judgment based on all features
IDF (Alberti et al. 2005)	Glucose intolerance (FBG ≥5.6 mmol/l) or pre-existing diabetes	Waist circumference: European ≥94 cm (M) or ≥80 cm (F); South Asian and Chinese ≥90 cm (M) or ≥80 cm (F); Japanese ≥85 cm (M) or ≥90 cm (F)	Triglycerides ≥1.7 mmol/l, HDL-C <1.0 mmol/l (M) or <1.3 mmol/l (F)	BPR ≥130/85mmHg and/or medication	--	Central adiposity, plus two other features

[a] Individuals without diabetes only. [b] Without medication only. Abbreviations: AACE, the American College of Endocrinology; BPR, blood pressure, CVD, cardiovascular disease; EGIR, European Group for the Study of Insulin Resistance; F, female; FBG, fasting blood glucose; HDL-C, HDL cholesterol; IDF, international Diabetes Federation; M, male; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III; PPG, postprandial glucose

1.1.1 Pathophysiology of the metabolic syndrome

Understanding of pathophysiology is fundament for an efficient definition for MetS. However, disunity dominates view of biological processes in MetS development, too. Several models were proposed for the pathophysiology of MetS. One model included saturation of adipose tissue and subsequent ectopic fat storage leading to lipotoxicity (Yki-Jarvinen 2002). Another model proposed unbalanced secretion of adipokines and other active substances by adipose tissue as mechanism for pathophysiology of MetS (Hutley et al. 2005, Ronti et al. 2006, Trayhurn 2005). Laclaustra et al. hypothesized that MetS is an organ failure of adipose tissue derived from loss of functional activities of this tissue (Laclaustra et al. 2007). Unger et al. suggested leptin resistance, which is associated with triglyceride (TG) accumulation in non adipose organs as concept for MetS (Unger 2003). While leptin reduces insulin production (Cases et al. 2001) absence of down regulation of sterol response element binding protein 1c (Kakuma et al. 2000) and inability in activating AMP-kinase in muscle (Minokoshi et al. 2003) could relate to pathophysiology. However, leading model, still under scrutiny, regards insulin resistance as underlying mechanism of MetS. Thus, this model, which was reported by Eckel et al. (Eckel et al. 2005b) is addressed in detail below.

Insulin resistance is defined as a defect in insulin action resulting in fasting hyperinsulinemia to maintain euglycemia. Mainly derived from adipose tissue TG stores, but also from lipoprotein lipase mediated lipolysis of TG rich lipoproteins (Eckel 1989), free fatty acids substantially contribute to development of insulin resistance. As inhibition of lipolysis in adipose tissue is the most sensitive pathway of insulin action (Jensen et al. 1989), signaling abnormalities in adipocytes trigger lipolysis of TG stores and efflux of fatty acids into the bloodstream. Increasing fatty acids levels further inhibit antilipolytic effect of insulin. Furthermore, excessive free fatty acids create insulin resistance in other insulin sensitive tissues (Figure 1).

As criteria for obesity several MetS definitions include waist circumference (NCEP 2001, Alberti et al. 1998, Balkau et al. 1999) ignoring constitution of adipose tissue responsible for increased waist circumference. While increased intra-abdominal or visceral adipose tissue increases flux of splanchnic circulating fatty acids directly effecting hepatic metabolism, increases in abdominal subcutaneous fat increase systemic circulation of lipolysis products avoiding more direct effects on the liver. Gender, ethnic and even person to person differences in body fat distribution and fat type ratio must be noted.

Increased free fatty acid flux to the liver triggers increased production of apo B-containing TG-rich very low-density lipoproteins (VLDL) resulting in hypertriglyceridemia (Lewis et al. 1995). The complex effects of insulin process are not fully cleared as it inhibits secretion of VLDL (Lewis et al. 1996) due to an effect on degradation of apo B (Taghibiglou et al. 2002) whereas it increases transcription and enzyme activity of many genes related to TG synthesis. Lipoprotein lipase concentration reduction in peripheral tissues (Eckel et al. 1995) according to

insulin resistance seems to be less contributory to hypertriglyceridemia. Importance of hypertriglyceridemia as diagnosis criteria is mainly due to reflection of insulin resistant condition. However, hypertriglyceridemia is not the only lipoprotein disturbance within MetS as high density lipoprotein (HDL) cholesterol is reduced in consequence of decreased cholesteryl ester content of the lipoprotein core influenced from cholesteryl ester transfer protein (Murakami et al. 1995) in presence of hypertriglyceridemia. Thus, HDL is also reduced in circulation (Brinton et al. 1991).

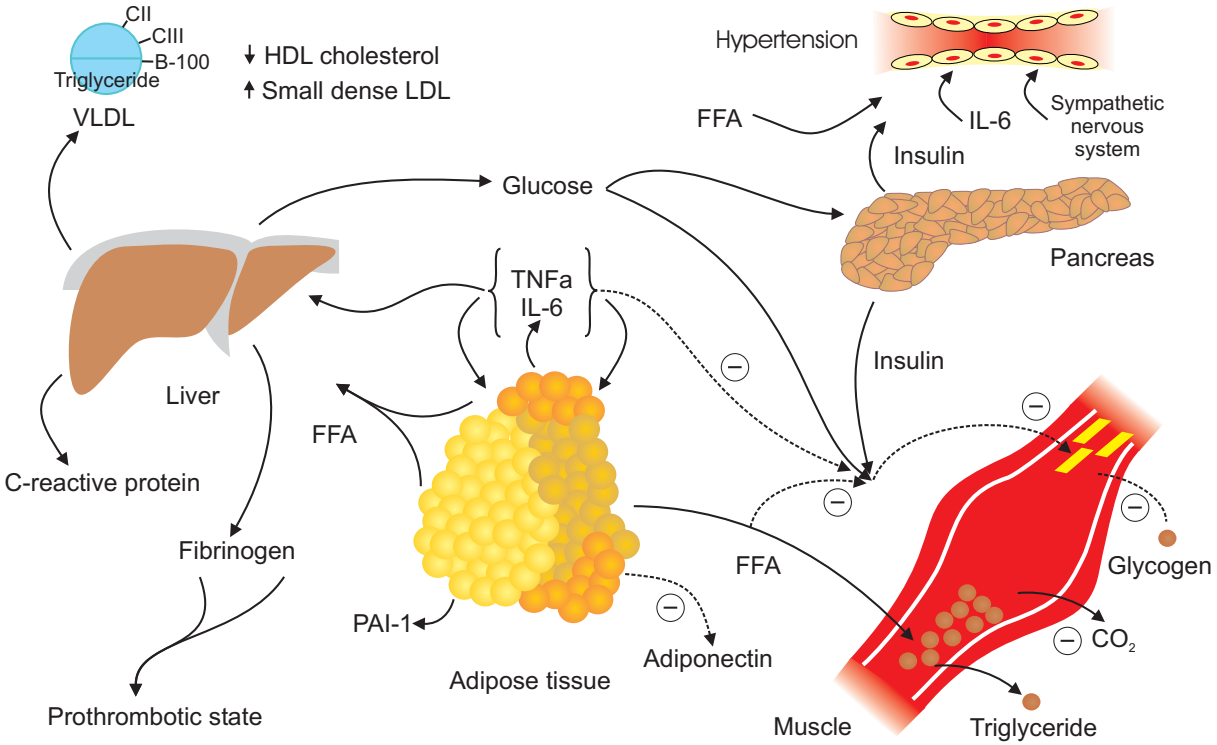

Figure 1: Pathophysiology of MetS. Adapted from Eckel et al. 2005 (Eckel et al. 2005b).
Free fatty acids (FFA) are released in abundance from an expanded adipose tissue mass. This results in increased production of glucose, TGs and secretion of VLDL in the liver. At the same time HDL cholesterol is reduced and density of LDL is increased. In muscle FFA reduce insulin sensitivity by inhibiting insulin mediated glucose uptake resulting in increased lipid accumulation in TG and reduction in glucose partitioning to glycogen affecting amino acid metabolism. Higher levels of circulating glucose increase pancreatic insulin secretion resulting in hyperinsulinemia, which may result in enhanced sodium reabsorption, and increased sympathetic nervous system (SNS) activity, and contribute to the hypertension as might increased levels of circulating FFA.
Paracrine and endocrine effects of the proinflammatory state contribute to insulin resistance produced by excessive FFA. Enhanced secretion of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) among others result in more insulin resistance, and lipolysis of adipose tissue TG stores to circulating FFA. Increased circulation of IL-6 and other cytokines may enhance hepatic glucose production, production of VLDL by the liver and insulin resistance in muscle. Fibrinogen and plasminogen activator inhibitor-1 (PAI-1) are overproduced resulting in a pro-thrombotic state. Reduction of the anti-inflammatory and insulin sensitizing cytokine adiponectin may further contribute to the pathophysiology of the syndrome.

Similar to HDL composition of low density lipoprotein (LDL) is also modified with a predominance of small dense LDL (de Graaf et al. 1993, Manzato et al. 1993) with either no change or an increase in LDL TG (Halle et al. 1999, Kwiterovich, Jr. 2002). However,

association with LDL composition changes is not independent from changes in other lipoproteins or other risk factors (Sacks et al. 2003).

Glucose intolerance mainly arises from free fatty acid induced loss of ability of insulin to suppress glucose production by the liver from lactate, amino acids, glycerol, and pyruvate. Insulin loses also ability to mediate glucose metabolism in insulin sensitive tissue like muscle and adipose tissue. Due to reduced glucose uptake in skeletal muscle and resulting reduction in partitioning to glycogen, pyruvate source for amino acid production is reduced. Thus, alanine and branched-chain α-ketoacids used for hepatic gluconeogenesis decrease and the liver loses its role as main regulator of blood glucose concentration.

In pancreatic islet beta cells fatty acids can interfere glucose-dependent insulin secretion by modifying signaling (Lee et al. 1994). Thus, insulin action defects can not be compensated.

Hypertension, which is related to insulin resistance (Ferrannini et al. 1987), can emerge from different mechanisms. Fatty acids mediate vasoconstriction as vasodilatory effect of insulin is lost (Tooke et al. 2000). However, insulin increases activity of the sympathetic nervous system (Anderson et al. 1991) and has effects on sodium reabsorption, which were only observed in white people, preserved in insulin resistant states (Egan 2003, Kuroda et al. 1999).

Proinflammatory state present in MetS is characterized by increases in proinflammatory cytokines reflecting enhanced adipose tissue mass overproduction (Trayhurn et al. 2004). Proinflammatory cytokines include interleukin-6 (IL-6), resistin, tumor necrosis factor alpha (TNFα) and C-reactive protein (Fernandez-Real et al. 2003) (Figure 1:). Source of these increases in local and systemic circulation could be monocyte-derived macrophages residing in adipose tissue (Weisberg et al. 2003, Xu et al. 2003). Abundance of proinflammatory cytokines seems to be directly involved in development of insulin resistance (Neuschwander-Tetri et al. 2003). Anti-inflammatory cytokine adiponectin concentration is simultaneously reduced in MetS patients (Medina et al. 2004, Yamauchi et al. 2003a). Adiponectin, which is produced exclusively in adipocytes, is able to enhance insulin sensitivity and to inhibit several steps of inflammatory processes (Nawrocki et al. 2004). Furthermore, adiponectin can inhibit hepatic rate of endogenous glucose production and expression of gluconeogenic enzymes (Combs et al. 2001).

1.2 Diabetes mellitus

The term diabetes mellitus combines several interferences in carbohydrate metabolism, characterized by increased blood glucose levels. DM has two major types. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in childhood characterized by acute onset, ketoacidosis and insulin dependency. T2DM is a metabolic disorder of middle-life, slow in onset and non insulin dependent. T2DM is characterized by insulin resistance and/or abnormal insulin secretion. People with T2DM are not dependent on exogenous insulin, but

may require it if diet and oral hypoglycaemic agents insufficiently control blood glucose levels. T2DM is diagnosed when subjects show random blood glucose levels of more than 200mg/dl repeatedly or fasting blood glucose levels of more than 110 mg/dl or 2-h glucose levels of more than 200mg/dl after an oral glucose tolerance test (OGTT) without any symptomes. Despite the two major types existence of several rare types of DM was reported, yet only marginally important, but problematic in diagnosis and therapy (Alberti et al. 1998).

In contrast to the applied definition T1DM is present in adulthood. In these patients onset is slow and many do not develop acidosis or require insulin for a long time (Molbak et al. 1994). For T2DM observations in teenagers exist (Rosenbloom et al. 1999) sometimes with keto-acidosis (Aizawa et al. 1997). Insulin-dependency frequently ensues given time in those patients. Thus, from this point of view two types of diabetes seem to be distinguishable only by tempo, clinically. The ‘Accelerator Hypothesis’ addresses this issue by regarding T1DM and T2DM as one and the same differing only in their rate of beta cell loss and the responsible accelerators (Wilkin 2001).

1.3 Inflammation in type 2 diabetes mellitus and metabolic syndrome

Besides known involvement of inflammatory processes in pathogenesis of T1DM, increasing evidence suggests that individuals who progress to T2DM display features of inflammation years before onset of disease and low-grade inflammation has been proposed to be involved in the pathogenetic processes causing T2DM. Thus, during the past decade, it became clear that inflammation is a key feature of obesity and T2DM (Wellen et al. 2005).

Presence of chronic low-grade inflammation in obese states (Yudkin et al. 2000, Bays et al. 2004, Hak et al. 1999, Yudkin et al. 1999), in insulin resistance/T2DM (Pickup 2004, Festa et al. 2000, Hak et al. 1999, Hansson et al. 1989, Pickup et al. 1997, Yudkin et al. 1999), in the early stages of atherogenesis (Festa et al. 2000, Hak et al. 1999), and in MetS (Sutherland et al. 2004) support the notion that inflammation may be the putative link that connects adipose tissue dysfunction with metabolic and vascular pathologies (Schmidt et al. 2003, Duncan et al. 2001). The architectural organization of adipose tissue and liver in which metabolic cells (adipocytes and hepatocytes) are in close proximity to immune cells (macrophages) and have immediate access at vast network of blood vessels. Due to this configuration, both tissues form a suitable environment for continuous and dynamic interactions between immune and metabolic responses and establish communications with other sites such as pancreatic islets and muscle (Hotamisligil 2006). This interface might contribute to the emerging importance of these two organs in the initiation and development of metabolic diseases, particularly in context of obesity and T2DM (Shoelson et al. 2006, Wellen et al. 2005).

1.4 Epidemiology of type 2 diabetes mellitus and metabolic syndrome

1.4.1 Type 2 diabetes mellitus

Type 2 diabetes as a common, growing, serious, costly and potentially preventable public health problem (Narayan et al. 2001) represents about 97% of diabetes cases. In 2000 estimated number of adults ≥20 years with diabetes was ~171 million (Wild et al. 2004), 11% higher than estimates from 1998 (King et al. 1998). Estimates for 2030 predict a total number of 366 million diabetes cases (Wild et al. 2004). The IDF released estimates for 2003 of 194 million to 334 million diabetes cases in 2025 (IDF 2003). Actually in 2007 about 264 million people have diabetes (IDF 2003). Thus, number of diabetes cases is anticipated to double until 2030 worldwide. Figure 2 displays diabetes situation in 2003 and estimated expansion up to 2025.

1.4.2 Metabolic syndrome

The striking increase of the worldwide number of people with MetS is associated with the global epidemic of obesity and diabetes (Zimmet et al. 2001). However, comparisons of published prevalences of different populations are not easy despite efforts to reach agreement in the definition of MetS (Cameron et al. 2004). Despite different study designs and other variables certain inferences can be made. There is wide variation in prevalence of both sexes but there is a consistent high age dependence. Prevalences in studies including people 20-25 years and older defining MetS with NCEP:ATP-III criteria vary in urban populations from 8% (India) (Gupta et al. 2003) to 24% (USA) (Ford et al. 2002) in men, and from 7% (France) (Balkau et al. 2003) to 42% (Iran) (Azizi et al. 2003) in women. Until recently MetS as well as T2DM have been regarded as diseases of adults (Zimmet et al. 2001). Increasing rates of obesity in young people argue for a start of the disease even in early ages over all ethnic groups. Thus, T2DM and MetS can be evident in childhood (Sinha et al. 2002, Sung et al. 2003, Wei et al. 2003, Weiss et al. 2004). However, prevalence of MetS in children and adolescents (3% to 4%) (Ornstein et al. 2006) is lower than that reported by several studies in adults (Ford et al. 2002, Isomaa et al. 2001, Laaksonen et al. 2002, Meigs et al. 2003).

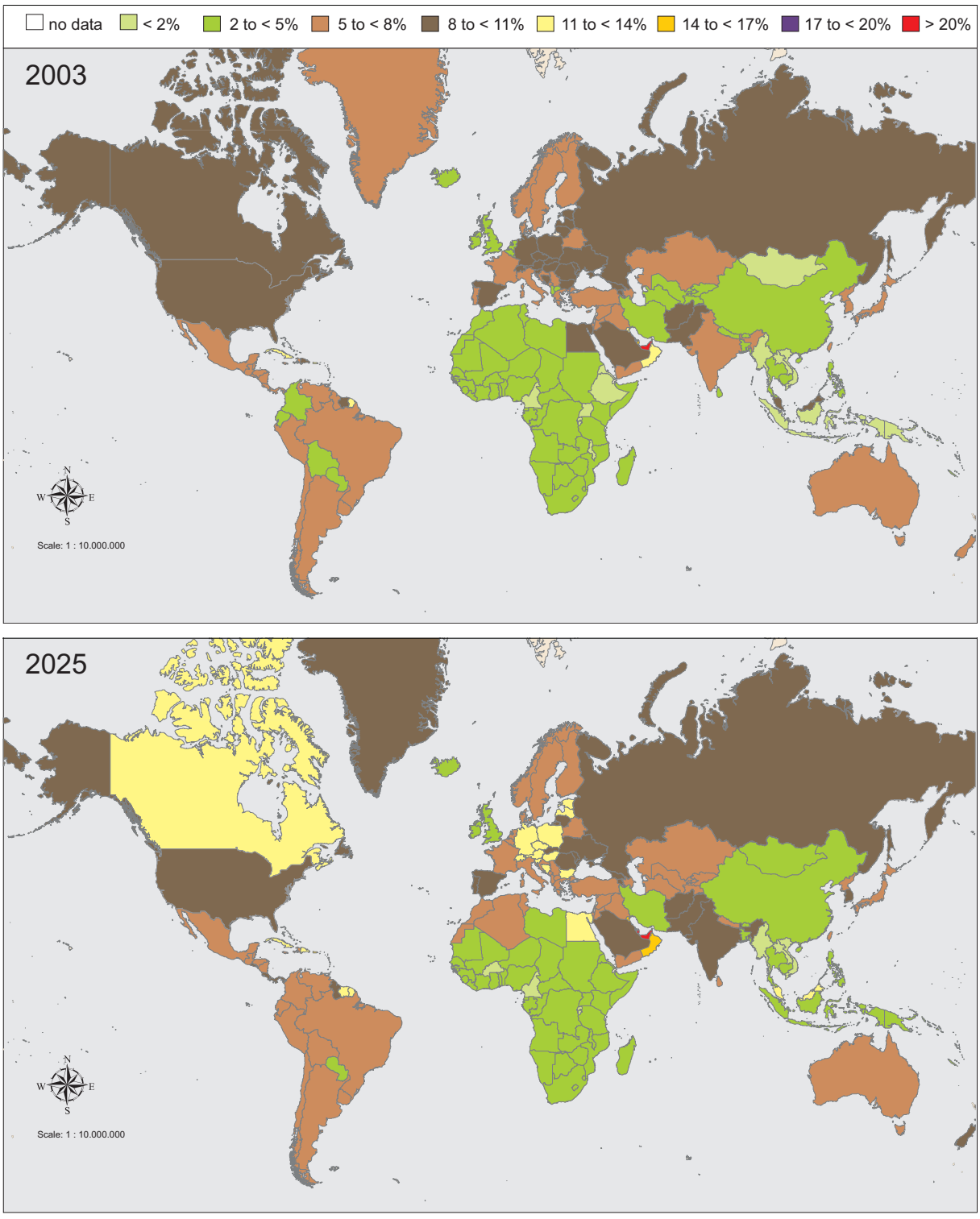

Figure 2: Prevalence estimates of diabetes mellitus 2003 and 2025 adapted from IDF diabetes atlas (IDF 2003).

1.5 Genetic susceptibility in type 2 diabetes mellitus and metabolic syndrome

1.5.1 Approaches for the identification of genetic susceptibilities

1.5.1.1 Linkage

Linkage mapping is a method of genetic localization whereby disease-causing variants are mapped to discrete chromosomal regions by using patterns of inheritance of genetic variants in pedigrees. Linkage analysis is less effective at identifying common variants with modest effects typical for complex traits, and has therefore proved to be largely unsuccessful.

1.5.1.2 Candidate gene approach

In candidate gene approach genes likely to be involved in MetS by their function or a role in an affected metabolic pathway are chosen for investigation of association, in terms of correlation, between genetic variation (single nucleotide polymorphisms) in these genes and a specified disease or quantitative trait. Single nucleotide polymorphisms (SNPs) are substitutions of single base pairs (bp), mostly biallelic, with, per definition, a minor allele frequency (MAF) grater than 1%. They are found approximately every 1,000 bp in the human genome (Kruglyak et al. 2001).

1.5.1.3 Genome wide association analysis

Recently developed genome-wide association studies are also SNP based but investigate a large number of SNPs over the whole genome without prior hypothesis of plausibility for a specific disease.

1.5.2 Genetics of metabolic syndrome

1.5.2.1 Heritability

Environmental factors clearly play a role in development of MetS. However, gender and ethnic group differences in prevalence rates suggest involvement of a genetic component as well. Increased incidence of MetS in individuals with parental history of MetS (Chen et al. 1999, Ford et al. 2002, Liese et al. 1997) and general familial clustering of MetS and its components (Hong et al. 1998, Li et al. 2006, Meigs et al. 1997) supported by twin studies (Carmelli et al. 1994, Edwards et al. 1997, Hong et al. 1997, Mayer et al. 1996) provide evidence for genetic influences. Resulting from a number of factor analysis using either NCEP definition of MetS or weighted components of MetS heritability of NCEP defined MetS has been estimated to be about 30% (Kraja et al. 2005a). In contrast single component heritability varies from 11% to 37% for blood pressure, from 47% to 66% for obesity and insulin, and from 43% to 54% for lipid and insulin traits, as defined by principal component (PC) or principal factor (PF) analysis procedures (Kraja et al. 2005b).

1.5.2.2 Results from linkage studies

Several genome-wide linkage scans for MetS have been reported performed on some of the MetS components or on linear combinations of phenotypes derived from PC or PF explorations (Arya et al. 2002, DeWan et al. 2001, Duggirala et al. 2001, Kissebah et al. 2000, Kraja et al. 2005b, Kraja et al. 2005a, Lehman et al. 2005, Loos et al. 2003, Ng et al. 2004). In total 38 QTLs with a LOD score equal to or higher than 2 were detected for at least one component of MetS (Teran-Garcia et al. 2007). Major findings were the region on chromosome 1q21-q25 linked to NCEP:ATPIII definition detected in the Hong Kong Family Diabetes Study (Ng et al. 2004) and the locus 3q27 strongly linked to six relevant traits in the TOPS cohort (Kissebah et al. 2000). A further key finding was the observation of ethnic-group-specific linkages in US families (Loos et al. 2003).

1.5.2.3 Results from candidate gene studies

Up to now evidence from association studies is quite promising for a number of candidate genes for MetS. The most important candidate genes with known evidence for a role in MetS are summarised below.

11-β-hydroxysteroid dehydrogenase (HSD11B1, HSD11B2) enzymes convert cortisol into inactive cortisone and vice versa. HSD11B1 should explain the MetS-related cluster although impact of increased HSD11B1 on obesity or other MetS related phenotypes is unclear. A mouse model characterized by increased visceral fat mass, glucose intolerance, insulin resistance, dyslipidemia and hypertension due to overexpressing HSD11B1 in adipose tissue became a model for MetS (Seckl et al. 2004) as well as knock-out and transgenic mice (Wake et al. 2006). Associations between genetic variability in the *HSD11B1* gene and enzyme activity with obesity, glucose intolerance, T2DM, or hypertension has been reported by several studies (Draper et al. 2002, Franks et al. 2004, Lindsay et al. 2003, Nair et al. 2004). *HSD11B2* gene may contribute to MetS development due to associations of polymorphism with essential hypertension (Odermatt et al. 2001, Poch et al. 2001, Sugiyama et al. 2001).

Adiponectin (*APM1*) is a significant factor in energy homeostasis, food intake and energy metabolism (Hsueh et al. 2001). Although MetS is associated with lower adiponectin levels, SNPs in *APM1* locus were associated with adiponectin concentrations but not with parameters of the MetS (Heid et al. 2006a). **Adiponectin receptors 1 and 2 (*ADIPOR1/2)*** mediate several adiponectin functions such as increase in AMP-activated protein kinase, PPARA ligand activities, and glucose uptake, and fatty acid oxidation. Adiponectin receptors are expressed in specific tissues where they show different affinity to globular or full length adiponectin (Yamauchi et al. 2003b). Expression was shown to be lower in obese (Kern et al. 2003) and normal-glucose tolerant individuals with a family history of T2DM (Civitarese et al. 2004). Several investigations of genetic variation in both *ADIPOR* genes suggest a role in

MetS development (Broedl et al. 2006, Vaxillaire et al. 2006, Kantartzis et al. 2006, Jang et al. 2006, Damcott et al. 2005, Wang et al. 2004), supported by decreased adiponectin receptor expression together with reduced adiponectin levels prior to onset of hyperglycemia, which related to regulation of mitochondrial number and function in T2DM individuals (Civitarese et al. 2006).

Adrenergic receptor ß-3 (*ADRB3*) is expressed in peripheral brown adipose tissue in infants and several tissues involved in lipid metabolism in adults suggesting a potential minor role in lipid metabolism and TG storage and mobilization in adipose tissues (Lafontan 1994, Krief et al. 1993). Associations between *ADRB3* variants and MetS are population-specific maybe due to ethnic differences, sample sizes or variations in diet, exercise and other behaviors (Teran-Garcia et al. 2007). Thus, SNP Trp64>Arg was found to be associated with insulin-resistance in Japanese-Americans (Kawamura et al. 1999) and with visceral obesity but lower TG levels in Japanese men (Kim-Motoyama et al. 1997), while no association was found with T2DM, hypertension or dyslipidemia in Chinese subjects (Thomas et al. 2000a).

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) inhibits insulin receptor (IR) tyrosine kinase activity (Maddux et al. 2000). As IR kinase is impaired in muscle, fibroblasts, and other tissues of T2DM patients, ENPP1, which identified a cluster of insulin resistance-related alterations when concentration is lower than 19 ng/ml (Frittitta et al. 1998, Frittitta et al. 1999), could be an explanation for IR kinase impairment. One *ENPP1* SNP was associated with insulin resistance, higher fasting glucose and insulin levels and higher systolic blood pressure in diabetic and healthy subjects (Pizzuti et al. 1999). Thus, although mechanism of its effects are unclear ENPP1 was suggested to play a role in etiology of MetS (Duggirala et al. 2001).

Leptin (LEP) participates in regulation of body mass by inhibiting food intake and stimulating energy expenditure (Frederich et al. 1995). It is also involved in regulation of hematopoesis, angiogenesis, wound healing, and immune and inflammatory processes (Fantuzzi et al. 2000). LEP acts through the **leptin receptor (*LEPR*)**, which is found in many tissues in several alternative spliced forms. Although physiological data on LEP suggest a significant role in development of obesity or other components of MetS, there is only few data on genetic variation in *LEP* and *LEPR* genes. Some *LEP* SNPs have been associated with MetS related traits independently of obesity (Shintani et al. 2002), as well as with T2DM (Lakka et al. 2000). Seufert et al. suggest a mild dysfunction in LEP mediated signaling pathway resulting in impairment of peripheral LEP effects induced by genetic variation in *LEP* and *LEPR* (Seufert et al. 1999).

Glucocorticoid receptor (NR3C1) has functional properties given its hormone binding ability only in the NR3C1α isoform possibly functioning with the basal transcriptional machinery to enhance or repress transcription (Rosmond 2002). Several *NR3C1* SNPs have been associated with MetS altered glucocorticoid sensitivity changes in body composition (Rosmond 2002, van Rossum et al. 2004), hyperactivity or abnormal regulation of hypothalamic-pituitary-adrenal axis (Bjorntorp et al. 2000, DeRijk et al. 2002). Thus, genetic variation in *NR3C1* may also play a role in etiology of MetS.

Peroxisome proliferative activated receptor gamma (*PPARG*) a member of the nuclear hormone receptor family of transcription factors produces two isoforms, PPARG1 and PPARG2, whereas the latter is considered more specific to adipose tissue and its differentiation. *PPARG* was considered as strong candidate gene for MetS due to its concomitant effects on adipocyte differentiation, obesity, dyslipidemia, and insulin resistance (Yen et al. 1997). Regulation of a variety of adipose tissue specific genes is the reason why *PPARG* and its agonists are subject of intense investigations as therapeutic agents for obesity, insulin resistance, and MetS (Olefsky et al. 2000). Most investigated SNP Pro12>Ala was associated with increased adiposity and insulin resistance (Gonzalez Sanchez et al. 2002) but decreased risk of MetS (Frederiksen et al. 2002). However, a haplotype including the rare allele of Pro12>Ala was associated with 2.37 fold increased risk for MetS (Meirhaeghe et al. 2005).

The proinflammatory cytokine **tumor necrosis factor alpha (TNFα)** has effects on lipid metabolism, coagulation, insulin resistance, and endothelial function. Diversity of TNFα including mediating expression of growth factors, cytokines, transcription factors, and receptor genes is similar to the cells producing TNFα (Carswell et al. 1975). Expression of TNFα in adipose tissue is proportional to the degree of adiposity. There are positive correlations between TNFα expression, BMI and LEP, and negative correlations between TNFα and LPL activity. Thus, TNFα could affect several metabolic functions but might need insulin resistance for its effects to become evident. Association studies on genetic variants and MetS were inconsistent. While several studies support contribution of two promoter variants to etiology of insulin resistance, obesity, or T2DM (Brand et al. 2001, Dalziel et al. 2002, Fernandez-Real et al. 1997, Herrmann et al. 1998, Hoffstedt et al. 2000, Nicaud et al. 2002, Padovani et al. 2000) a lot of studies reported negative findings (da Sliva et al. 2000, Day et al. 1998, Furuta et al. 2002, Lee et al. 2000, Rasmussen et al. 2000, Romeo et al. 2001, Valenti et al. 2002). Nevertheless, genetic variants in *TNFα* might play a role in development of MetS.

1.5.3 Genetics of Type 2 diabetes

1.5.3.1 Heritability

Similar to MetS evidence for a genetic component of T2DM comes from ethnic group differences in prevalence rates. These differences in prevalence range from 1% in Mapuche Indian tribes or Chinese population living in rural areas in mainland China, to extremely high levels found in Nauru and Pima Indians in Arizona (King et al. 1993). Further support for genetic susceptibilities of T2DM is the fact that prevalence is higher in full-blooded Nauruan and Pima Indians than in those with admixture (Knowler et al. 1988, Serjeantson et al. 1983). Differences between ethnic groups were also observed when sharing the same environment. For example prevalence in the UK is about 2.4% but three to six fold higher in individuals of African-Caribbean and South Asian descent (Chaturvedi et al. 1993, Greenhalgh 1997, Mather et al. 1985, Simmons et al. 1991). Another source of evidence for genetic contribution in T2DM is familial aggregation. Lifetime risk of T2DM development is 40% in offspring of one diabetic parent and increases up to 70% if both parents have T2DM (Groop et al. 1997). In the Framingham Offspring Study T2DM risk increased in offspring with one diabetic parent to an OR of 3.4 to 3.5 and nearly doubled to 6.1 if both parents were affected (Meigs et al. 2000). In studies on monozygotic and dizygotic twins concordance rates of 0.20 to 0.91 and 0.10 to 0.43 were observed, respectively (Barnett et al. 1981, Committee DT 1988, Kaprio et al. 1992, Matsuda et al. 1994, Medici et al. 1999, Newman et al. 1987, Poulsen et al. 1999). Regarding long follow-up ranges 76% of monozygotic twins initially selected as discordant became concordant (Medici et al. 1999). Thus, age-adjusted concordance rate was suggested as high as 70 to 80% for T2DM (Ghosh et al. 1996).

1.5.3.2 Results from linkage studies

Initial linkage studies confined to investigate markers around certain regions prior suggested to be associated with T2DM or including genes with plausible function in T2DM development (Elbein et al. 1995, Lesage et al. 1995, Mitchell et al. 1995, Prochazka et al. 1993, Stern et al. 1996). Up to now about 50 linkage studies, including some covering the whole genome, have been conducted with a number of regions suggesting evidence for linkage with T2DM (Morton et al. 1998). However, few regions have been replicated in multiple studies. The only T2DM gene identified by linkage was calpain 10 (CAPN10) (Horikawa et al. 2000) until recently. In contrast to the initial finding in Mexican-Americans (Serjeantson et al. 1983) attempts to replicate these findings in other populations revealed conflicting results (Baier et al. 2000, Cox 2001, Daimon et al. 2002, Elbein et al. 2002, Evans et al. 2001, Garant et al. 2002, Hegele et al. 2001, Tsai et al. 2001) as well as meta-analyses (Song et al. 2004, Weedon et al. 2003). Regions with consistent linkage in multiple populations included chromosomes 1q25.3 (Elbein et al. 1999, Hanson et al. 1998, Vionnet et al. 2000, Wiltshire et al. 2001), 2q37.3 (Elbein et al.

1999, Hanis et al. 1996, Luo et al. 2001), 3p24.1 (Duggirala et al. 1999, Ehm et al. 2000, Hanis et al. 1996, Lindgren et al. 2002), 3q28 (Busfield et al. 2002, Mori et al. 2002, Vionnet et al. 2000, Wiltshire et al. 2001), 10q26.13 (Duggirala et al. 1999, Wiltshire et al. 2001), 12q24.31 (Bowden et al. 1997, Mahtani et al. 1996, Parker et al. 2001, Shaw et al. 1998), and 18p11.22 (Elbein et al. 1999, Parker et al. 2001). However, the most promising T2DM gene was identified in a region on chromosome 10q that gave only suggestive evidence for linkage (Reynisdottir et al. 2003), but was already observed in Mexican-Americans (Duggirala et al. 1999). This gene - *TCF7L2* - was further analyzed by microsatellite marker analysis indicating high risk alleles of one marker (DG10S478) that were in high LD with a specific SNP (rs12255372) (Grant et al. 2006).

1.5.3.3 Results from candidate studies

Beside *TCF7L2* several other genes have been reproducibly associated with T2DM. The ***PPARG*** Pro12Ala polymorphism showed a small increase in T2DM risk for the common Pro12 allele in a meta-analysis (OR = 1.25) (Altshuler et al. 2000).

KCNJ11 encodes a subunit of an inwardly rectifying ATP-sensitive potassium channel I(KATP). I(KATP) channels are crucial for the regulation of glucose-induced insulin secretion in pancreatic beta cells. More recently, evidence has accumulated for the association of the E23K variant with T2DM (Barroso et al. 2003, Gloyn et al. 2001, Gloyn et al. 2003, Hani et al. 1998, Love-Gregory et al. 2003, Nielsen et al. 2003).

Evidence for a role of the ***HNF4A*** gene in T2DM predisposition is also mounting (Barroso et al. 2003, Love-Gregory et al. 2004, Silander et al. 2004, Zhu et al. 2003). *HNF4A* regulates genes involved in glucose and fatty acid metabolism, as well as insulin secretion, and is therefore critical for maintaining lipid and glucose homeostasis (Wang et al. 2000). Several other genes with less well-established impact on T2DM risk, but where recent evidence has lent support for their involvement, are ***IRS1*** (Jellema et al. 2003) substrate of the insulin receptor and ***SCL2A2***, which encodes glucose transporter 2 (GLUT2) (Barroso et al. 2003). Furthermore, ***ABCC8*** encoding the sulphonylurea receptor SUR1, which is beside KIR6.2 a part of a pancreatic beta-cell potassium channel (Inagaki et al. 1995) was suggested to be involved in T2DM (Barroso et al. 2003, Hansen et al. 2001). Suggested role of the insulin gene (***INS***) in T2DM yet less clearly defined (Bennett et al. 1996, McCarthy 1998, Ong et al. 1999), was recently tightened (Barroso et al. 2003, Huxtable et al. 2000).

1.5.3.4 Results from genome wide association studies

Recently, genome wide association studies revealed new susceptibility loci for T2DM and validated some of the known candidates. Association with T2DM was confirmed for *PPARG* (The Welcome Trust Case Control Consortium 2007), *KCNJ11 (The Welcome Trust Case Control Consortium 2007),* and again with strong signals, *TCF7L2* (The Welcome Trust Case

Control Consortium 2007, Sladek et al. 2007). Strong signals for T2DM were observed also for new genes, whereas one mapped to ***FTO***, which is also highly associated with fat mass and obesity (Dina et al. 2007). The effect of *FTO* variants on T2DM risk has been replicated and seems to be mediated entirely by their marked effect on adiposity (Frayling et al. 2007). Another signal mapped to ***CDKAL1***, a gene of unknown function. However, ***CDKAL1*** shares homology at protein domain level with CDK5 regulatory subunit associated protein 1 (CDK5RAP1), which is known to inhibit the activation of CDK5, a cyclin-dependent kinase which has been implicated in the maintenance of normal beta cell function (Ubeda et al. 2006). This finding was also replicated repeatedly (Saxena et al. 2007, Scott et al. 2007, Steinthorsdottir et al. 2007, Zeggini et al. 2007). Another signal mapped to ***SLC30A8*** (Sladek et al. 2007) encoding a zinc transporter expressed solely in the secretory vesicles of beta cells and thus implicated in the final stages of insulin biosynthesis, which involve co-crystallization with zinc. Overexpression of *SLC30A8* in insulinoma cells increases glucose-stimulated insulin secretion (Chimienti et al. 2006). A locus including the genes ***IDE, KIF11, HHEX*** (The Welcome Trust Case Control Consortium 2007, Sladek et al. 2007) mapped to an interval confirmed in more than one linkage study on chromosome 10 (Duggirala et al. 1999, Ghosh et al. 2000, Meigs et al. 2002, Wiltshire et al. 2001). *HHEX* encoding a homeodomain protein, which is essential for hepatic and pancreatic development (Bort et al. 2004, Bort et al. 2006). *HHEX* is a target of the Wnt signaling pathway (Foley et al. 2005), as is *TCF7L2*. *HHEX* regulates cell proliferation and tissue specification underlying vascular and hepatic differentiation (Bort et al. 2006, Hallaq et al. 2004). Reduction of IDE activity by a pharmacological inhibitor increases islet amyloid polypeptide (amylin) accumulation and amylin-mediated cytotoxicity in cultured β cells (Bennett et al. 2003), whereas IDE ablation causes glucose intolerance in knock-out mice (Farris et al. 2003). IDE showed weak T2DM association (Groves et al. 2003, Karamohamed et al. 2003), which was not confirmed in a third well-powered study (Florez et al. 2006b). Significance of the biological function of the third gene in this region, *KIF11* is yet unknown. Signals for T2DM risk were also obtained in a region including exostosin 2 (***EXT2***) and ***ALX4***, a homeodomain protein with possible involvement in the Wnt pathway (Boras-Granic et al. 2006). EXT 2 is implicated in bone cell proliferation (Stickens et al. 1996) It modulates hedgehog signaling pathway, which is involved in early pancreatic development (Apelqvist et al. 1997) and the regulation of insulin synthesis (Thomas et al. 2000b).

Association of the hypothetical gene ***LOC387761*** (Sladek et al. 2007) and *EXT2* with T2DM could not be confirmed yet (The Welcome Trust Case Control Consortium 2007). For *IGF2BP2* and *CDKN2A/CDKN2B* regions weak, but consistent signals were found (Saxena et al. 2007, Scott et al. 2007, Zeggini et al. 2007). Finally, genome-wide association studies found signals for association with T2DM in several loci that did not map to genes or regions

previously implicated in T2DM pathogenesis (The Welcome Trust Case Control Consortium 2007), which could not be replicated yet (Zeggini et al. 2007).

1.6 Aims

MetS, which is closely connected to DM, is one of the most challenging diseases of the 21st century, with T2DM as most common form. Although definition of MetS is still discussed, both disorders increase enormously mainly driven by the world epidemic of obesity. Both disorders are influenced by overlapping genetic susceptibility besides environmental factors.

This work addresses these genetic influences in a candidate gene approach by investigating genetic variation in selected genes with a putative role in development of MetS or T2DM. In particular SNPs were analyzed for association with MetS, T2DM or related traits.

IL-6 was chosen as candidate gene for its role in inflammatory processes, which are involved in MetS development. *APOA5*, *MCP-1* and *Resistin* were chosen because they all seem to play a role in development of at least one component of MetS.

As replication studies of initially reported associations between genetic variants and complex diseases with polygenic etiology have often revealed false positive findings in the past, associations of *TCF7L2* variants should replicated enhancing analysis to traits related to T2DM and MetS.

Furthermore candidate genes investigated in extern cooprations should be validated in appropriate sub populations, which were assembled within this work.

2. Methods

2.1 SNP selection

There are two main approaches in selecting genetic markers in association studies of complex diseases. The first is a direct or functional approach. SNPs are proposed to influence complex diseases by a variety of mechanisms. They may lead to an amino acid exchange in the corresponding protein, where functions like DNA binding or catalytic activity are altered or abolished. Additionally, SNPs may interrupt the initiation, the termination codon or introduce errors in the reading frame shift, all with consequences for insufficient or prematurely truncated peptides (Wjst 2004). Polymorphisms may also affect transcription, RNA processing, stability, and translation. SNPs in known promoter motifs can lead to changes in mRNA levels. mRNA splicing mutants are most commonly found at the beginning and end of the donor and acceptor consensus splice sequence and cause either exon skipping or utilization of cryptic splice sites resulting in the absence of normally spliced mRNA (Wjst 2004). Finally, RNA cleavage-polyadenylation mutants can occur in the AAUAAA sequence upstream of the polyadenylation sites (Wjst 2004). The 5' untranslated regions (UTRs) are proposed to play a role in controlling mRNA translation while sequence variants in the 3' UTR control RNA cleavage, stability, export, and intracellular localization (Wjst 2004).

The second approach is an indirect or positional one, in which markers in a particular region or the whole genome are systematically screened, on the basis of linkage disequilibrium (LD) structure of the gene region (Sham et al. 2007). For the second approach, efficiency of the selection can be improved by recognizing the redundancy between near-by markers through the presence of LD. A subset of SNPs, called tag SNPs, can be selected for genotyping and analysis with minimal loss of information (Halldorsson et al. 2004, Johnson et al. 2001).

A third possibility is to combine both srategies. This was used in this work. SNPs were chosen on the basis of positional and functional aspects to enhance the chance of detecting associations. Following the functional approach, SNPs were chosen on the basis of occurrence in or near exons and hypothetical promoter regions, hypothetical transcription factor-binding sites (TFBS), human–mouse conserved sequences (HMCS) and 5' and 3'-UTRs. SNPs were also chosen on the basis of density (if possible, distance <5 kb), frequency and level of validation. In addition, DNA variants previously showing an association with MetS, T2DM or related features were selected for analysis dependent on gene locus. For SNP selection, the National Center for Biotechnology (NCBI) SNP database dbSNP, the Innate Immunity Program for Genomic Applications (IIPGA) database and the Seattle SNPs Program for Genomic Applications were used.

2.2 DNA extraction

The method used for DNA extraction within this thesis was a slightly modified one based on Miller (Miller et al. 1988) and involves salting out of the cellular proteins by dehydration and precipitation with a saturated NaCl solution.

Genomic DNA was extracted from 9 ml frozen EDTA anticoagulated blood or 1 ml buffy coats depending on availability. DNA obtained from this technique yielded average quantities from 300-600 µg of genomic DNA for 9 ml EDTA blood and 150-400 µg of genomic DNA for the 1 ml buffy coat samples. DNA samples were stored at temperatures from 4 °C down to -80 °C.

2.2.1 DNA extraction from 9 ml EDTA blood

The 9 ml frozen EDTA samples were resuspended in 50 ml polypropylene tubes with 30 ml RBC lysis buffer to separate the erythrocytes from the rest of the cells with intact nuclei. After centrifugation at 2,500 rpm the supernatant was discarded. The cell lysates were mixed with 25 µl protease K solution, 5 ml SE buffer and 250 µl of 20% SDS and digested overnight at 55 °C, the optimum temperature for the enzyme. After digestion was complete, 3 ml of saturated NaCl together with 5 ml SDS buffer was added to each tube and shaken vigorously, followed by centrifugation at 3,500 rpm for 15 minutes. The precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another tube. 13 ml of absolute isopropanol was added and the tubes were inverted several times until the DNA precipitated. After that the DNA was washed using 10 ml 70% ethyl alcohol and then dried at room temperature. The DNA was dissolved in 1.2 ml TE buffer.

2.2.2 DNA extraction from 1 ml buffy coats

Buffy coats of nucleated cells obtained from anticoagulated blood were resuspended in 15 ml polypropylene tubes with 4 ml RBC lysis buffer for erylysis followed by centrifugation at 2,500 rpm. The cell lysates were mixed with 6.5 µl protease K solution, 1.3 ml SE buffer and 63 µl of 20% SDS and digested overnight at 55 °C. The remaining proteins were removed by salt precipitation, adding 0.75 ml of saturated NaCl together with 1.25 ml SDS buffer and centrifugation at 3,500 rpm. The pellet was solved in the supernatant and precipitated by addition of 3.4 ml 100% isopropanol. The DNA pellet was washed with 2.5 ml 70% ethyl alcohol and the dried pellet was dissolved in 0.6 ml TE buffer.

2.3 DNA quantification

2.3.1 Spectrophotometry

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. DNA absorbs UV light very efficiently. The nitrogenous bases in nucleotides have an absorption maximum at 260 nm. Using a 1 cm light path, the extinction coefficient for nucleotides at this wavelength is 20. Based on this extinction coefficient, the absorbance at 260 nm in a 1 cm quartz cuvette of a 50µg/ml solution of double

stranded DNA is equal to one. A solution of a 50µg/ml double strand DNA has thus an optical density (OD) of one at a wave length of 260 nm. The sample concentrations are automatically calculated as follows:

DNA concentration (µg/ml) = (OD 260) x (dilution factor) x (50 µg DNA/ml)/(1 OD260 unit)

In contrast to nucleic acids, proteins have a UV absorption maximum of 280 nm, due mostly to the tryptophan residues. The absorbance of a DNA sample at 280 nm gives an estimate of the protein contamination of the sample based on the fact, that the OD 260 is twice as high as that at 280 nm, if the solution contains pure DNA. The ratio of absorbance (260 nm/ absorbance at 280 nm) is a measure of the purity of a DNA sample. It should be between 1.70 and 2.00. Concentration and quality of the DNA samples were measured with the photometer GeniOS (Tecan, Crailsheim, Germany) following the manufacturers' protocol and using standard settings. 5 µl DNA were diluted within 95 µl TE buffer. TE buffer was also used as the reference. The OD 260 / OD 280 ratios measured were consistently 1.7 to 2.0, demonstrating good deproteinization during DNA extraction.

2.3.2 Nanodrop

The NanoDrop® ND-1000 is a full-spectrum (220-750 nm) spectrophotometer that measures 1 µl samples with high accuracy and reproducibility. It utilizes a patented sample retention technology that uses surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the ND-1000 has the capability to measure highly concentrated samples without dilution.

2.4 Agarose gel electrophoresis

Agarose gel electrophoresis is used to separate DNA fragments by size. The size of the fragments can be estimated by comparison with commercially available known fragments (DNA ladders). Nucleic acids migrate to the anode in electric fields due to the natural negative charge carried on their phosphodiester backbone. The smaller the DNA fragment, the farther it will move. Depending on the size of the analyzed DNA fragments, gels containing 0.8 to 3% agarose were made, dissolving agarose in TBE buffer by heating in the microwave. Loading buffers are added with the DNA in order to visualize and sediment it in the gel well. After the separation is completed, the fractions of DNA fragments of different length are visualized using ethidium bromide, an intercalating agent that fluoresces at 266 nm.

2.5 Polymerase Chain Reaction (PCR)

Within this work PCR (Saiki et al. 1988), a standard method for amplification of a specific DNA regions, was used to amplify an approximately 200 bp sequence around the SNP of interest. A PCR reaction starts with a denaturing step where samples are heated to 95°C. Prior to the first cycle, an additional denaturation step of several minutes is used to disentangle the complex

structure of the template DNA. For annealing, the temperature is lowered. This allows the excess of primers to anneal to their complementary sequences. The primers are usually only 18 to 25 bp long and designed to bracket the DNA region to be amplified. The annealing temperature of this stage depends on the primers and is usually 5°C below their melting temperature. During the following elongation step, the temperature is raised to 72°C, the optimum temperature of the Taq polymerase. The Taq polymerase attaches at each priming site and uses the 3'OH ends of the primers and the provided deoxynucleotides (dNTPs) to catalyze the synthesis of the new DNA strands. A final elongation step is frequently used after the last cycle to ensure that any remaining single stranded DNA is completely copied. The amplification consists of 30 to 45 cycles denaturation, annealing and elongation.

2.6 SNP detection via MALDI-TOF mass spectrometry

The introduction of the Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) by Karas and Hillenkamp (Karas et al. 1988) has offered a solution for fast and accurate genotyping of SNPs in a high-throughput manner. During MALDI-TOF MS the sample is staggered with a 100 to 1,000 fold excess of matrix, co-crystallized on a sample plate and irradiated with an intensive laser pulse for a few nanoseconds in the high vacuum chamber of the mass spectrometer (Karas et al. 1988, Kirpekar et al. 1998). With the development of this method, the molecular weight of DNA fragments in a mass range of 1,000 to 9,000 Dalton can be determined with an accuracy of 0.1 to 0.01%, making it valuable for SNP analysis (Griffin et al. 2000, Leushner 2001, Pusch et al. 2002, Storm et al. 2003). A widely-used and well developed method for high-throughput genotyping of SNPs is the homogeneous MassExtend™ (hME) Assay (Leushner 2001). In this method, allele-specific extension products are generated by primer extension and can be distinguished by their molecular weight using MS. Advancements of this technology are the iPLEX and iPLEX Gold assays. Due to slight configuration of chemical processes these assays provide possibility to detect up to 28 and 40 SNPs respectively in one approach.

2.6.1 Homogeneous MassExtend™ Assay

The hME assay is based upon the annealing of an oligonucleotide primer (extension primer) adjacent to the polymorphic site of interest. The addition of a DNA polymerase (Thermosequenase), plus a mixture of nucleotides and terminators, allows the extension of the primer through the polymorphic site. The resultant mass of the primer extension product is then analyzed and used to determine the sequence of the nucleotides at the polymorphic site. To analyze a SNP by MALDI-TOF MS, the genomic sequence around the SNP has to be amplified, generating PCR products of approximately 100 bp which are afterwards used as a template for primer extension.

2.6.2 iPLEX and iPLEX Gold Assay

iPLEX and iPLEX Gold are enhanced forms of hME with slight difference in extension reaction resulting in extreme increase of plexing possibilities. In extension of iPLEX and iPLEX Gold assays all four mass-modified nucleotides are present. During iPLEX reaction, the primer is extended by one of the nucleotides, which terminates the extension of the primer. Through mass-modified nucleotides difference in mass of allele specific extension products is much smaller. Thus, number of detectable extension products could be increased and an additional expansion of mass interval further enhances plexing levels.

2.6.3 PCR amplification in scope of hME and iPLEX

The PCR was carried out in a 384-well plate format with 5 µl genomic DNA (1ng/µl) that was dried overnight. The reaction volume of 6 µl (hME) or 5 µl (iPLEX and iPLEX gold) was pipetted by means of the pipette station Genesis RSP 150 workstation (Tecan, Crailsheim, Germany). Depending on plexing level the PCR mix was adapted in hME, iPLEX and iPLEX gold, respectively Table 2.

Table 2: PCR master mix per reaction

Reagent	Volume hME [µl]	Volume iPLEX Gold [µl]
PCR-Buffer with MgCl2 (10x)	0.600 / 0.750[a]	0.625
MgCl2 (25 mM)	0.240 / 0.390[a]	0.325
dNTPs (2 mM)	0.600 / 1.500[a]	0.100
Forward PCR primer (100 mM)	0.010[b]	0.005[c]
Reverse PCR primer (100 mM)	0.010[b]	0.005[c]
HotStar Taq® (5 U/µl)	0.020-0.060[b]	0.100 / 0.200[d]
Nanopure water	Fill up to 6 µl	Fill up to 5 µl

[a])Up to 6 primer pairs / higher than 6 primer pairs, [b]) Primer concentration 100 mM,[c]) Primer concentration 100 µM, [d]) Taq addition ≤ 27-plex 0.1 µl, ≥ 28-plex 0.2 µl, 0.2 for iPLEX

PCR primers and extension primers were designed using Assay Design software Version 2.0 to 3.1 (Sequenom, Hamburg, Germany), minimizing chances for overlapping peaks in spectra. The software also considers potential unwanted intra- and inter-primer interactions in order to avoid non-template extensions. Additionally to the primer sequence, each PCR primer consists of a 10-mer tag (5`-ACGTTGGATG-3`), on its 5`end. The tag increases the masses of unused PCR primers so they fall outside the mass range of analytical peaks. PCR primers with the same masses as the extension products would interfere with the following MALDI-TOF analysis as they are not removed from the reaction. The tags are also useful in balancing amplification. Thermal cycling was performed on PCR DNA Engine Tetrad (MJ Research, South San Francisco, USA) following the conditions shown in Table 3.

Table 3: Temperature conditions for a 384-well plate format PCR

Step	Temperature [°C]	Time	Number of cycles
Denaturation	94	15 '	1x
Denaturation	94	20 "	
Annealing	56	30 "	45x
Elongation	72	60 "	
Final elongation	72	10 '	1x

2.6.4 Shrimp alkaline phosphatase (SAP) reaction

After the PCR reaction remaining dNTPs have to be deactivated with the shrimp alkaline phosphatase (SAP). This step is necessary to avoid the incorporation of the remaining dNTPs during the primer extension reaction, leading to other than the specified extension products and thus problems with the correct detection of the alleles. The enzyme deactivates dNTPs by splitting off the phosphate group. The master mix for the SAP reaction is shown in Table 4.

Table 4: SAP reaction master mix

Reagent	Volume [µl]
SAP buffer (10x)	0.170
Nanopure water	1.530
SAP enzyme (1U/µl)	0.300

The SAP master mix was dispensed using the pipetting robot Multimek 96 (Beckmann/Sagian, Unterschleißheim, Germany). The reaction was performed in two steps on PCR DNA Engine Tetrad. In the first step, the reaction mix was incubated 20 minutes at 37 °C, the optimum temperature for the enzyme. After that, the enzyme has to be denatured at 85 °C for 10 minutes (or 5 minutes for iPLEX) to avoid disturbances in primer extension amplification reaction.

2.6.5 Primer adjustment

2.6.5.1 hME Assay

The extension primers may not have comparable heights in the mass spectrum. Variations in peak height may stem from inconsistent oligonucleotide quality or concentration and different desorption/ionization behavior in MALDI-TOF MS. Thus they have to be adjusted prior to the primer extension reaction to even out peak heights (intensities) in the mass spectrum. An assay with a very low primer peak will systematically fail when applied to samples as part of a multiplex. For primer adjustment a mix of all required primers in one assay is prepared with a final concentration of 9 µM of each primer in the mix. 1 µl of the primer mix is then pipetted into a well of a microplate and 24 µl nanopure water is added to obtain a 360 nM dilution of the primer mix. 3 mg ion exchanger (SpectroCleanTM, Sequenom, Hamburg, Germany) is added to remove ions and the sample was dispensed to a silicon chip with Spectro Point Nanoliter Pipetting Systems™ (Sequenom, Hamburg, Germany). Spectra are analyzed and peaks with

less than 50% of the height of the highest peak are adjusted using MassARRAY Typer software 3.0.1. (Sequenom, Hamburg, Germany). Primer adjustment was performed for all assays with a plexing level above six.

2.6.5.2 iPLEX Assay

Applying high multiplex levels primer adjustment becomes even more important. There is an inverse correlation between primer mass and peak intensity assessed by MALDI-TOF. Thus, as the extension primer with the highest mass has 25% less intensity as the average of the low mass primers, primers were adjusted by concentration dependent on their mass. Three different methods could be used for adjustment, dividing primers into two or four groups or adjusting each primer separately. While lower plexes (up to 19-plex) were processed adjusting each primer separately, higher plexes were adjusted in four groups. However, extension primers were ordered in constant concentrations of 300 µM. Thus, added volumes of the primers were adapted according to the adjustment result from the plate editor tool included in the Spectro Typer software.

2.6.6 Primer extension reaction

To initiate the primer extension reaction, the extension primers, Thermosequenase and the extend mixture of dNTPs and dideoxynucleotide (ddNTPs) and buffer are added to the hME reaction. The extension primer is hybridized directly to the polymorphic site of interest. Nucleotide mixes of one single ddNTP and three dNTPs are selected to maximize mass differences for all potential extension products. Appropriate dNTPs are incorporated through the polymorphic site of interest until a single ddNTP is incorporated and the reaction terminates. Since the termination point and the number of nucleotides is allele specific, the mass difference of the extension products generated can be detected via MALDI-TOF MS. The reaction volume of 2 µl was pipetted by means of the pipetting robot Multimek 96 (Beckmann/Sagian, Unterschleißheim, Germany). Similarly like for the PCR reaction, two types of primer extension mixes were used since the concentrations in the mixes had to be adapted to the multiplex level (Table 5).

Table 5: Primer extension mixes

hME	Reagent	Volume [µl] ≤6 -plex	Volume [µl] >6-plex
	Nanopure H_2O	1.728	0.760
	hME Extend mix (buffer and d/ddNTPs)	0.200	0.200
	Extension primers	0.054	1.000
	Thermosequenase	0.018	0.040
iPLEX	Reagent	Volume [µl]	
	Nanopure H_2O	0.755	
	iPLEX buffer (10x) / iPLEX Gold buffer (10x)	0.200	
	iPLEX termination mix	0.100 / 0.200[a]	
	Primer mix (7 µM: 14 µM)	0.804	
	iPLEX enzyme	0.0205 / 0.0410[a]	

[a]) ≤ 18-plex / ≥ 19-plex

Thermal cycling was performed on PCR DNA Engine Tetrad following the conditions presented in Table 6.

Table 6: Temperature conditions for primer extension in hME and iPLEX

	hME			iPLEX / iPLEX Gold			
Step	Temperature [°C]	Time	Cycles	Temperature [°C]	Time	Cycles	
Denaturation	94	2 '	1x	94	30 "	1x	
Denaturation	94	5 "		94	5 "	1x	
Annealing	52	5 "	75x	52	5 "	5x	40x
Elongation	72	10 "		80	5 "		
				72	3 '	1x	

After extension reaction, samples were purified with ion exchanger (Spectro Clean, Sequenom, Hamburg, Germany) to remove extraneous salts that interfere with MALDI-TOF MS (3 mg hME, 6 mg iPLEX, and iPLEX Gold).

2.6.7 MALDI-TOF MS

Following the primer extension reaction, 1 to 2nl from the ion removed sample are transferred to a silicon chip with Spectro Point Nanoliter Pipetting Systems™ (Sequenom Hamburg, Germany). The chip matrix consists of 3-hydroxypicolinic acid, which is especially well applicable for DNA analysis (Gut 2001, Little et al. 1997a, Little et al. 1997b). Functions of the matrix include absorbing the applied laser energy and supporting, respectively inducing, the ionization of the analyte molecule. Additionally the matrix should prevent a photolytic damage of the analyte and avoid interaction of analyte molecules with each other or with the sample carrier (Hillenkamp et al. 1991).

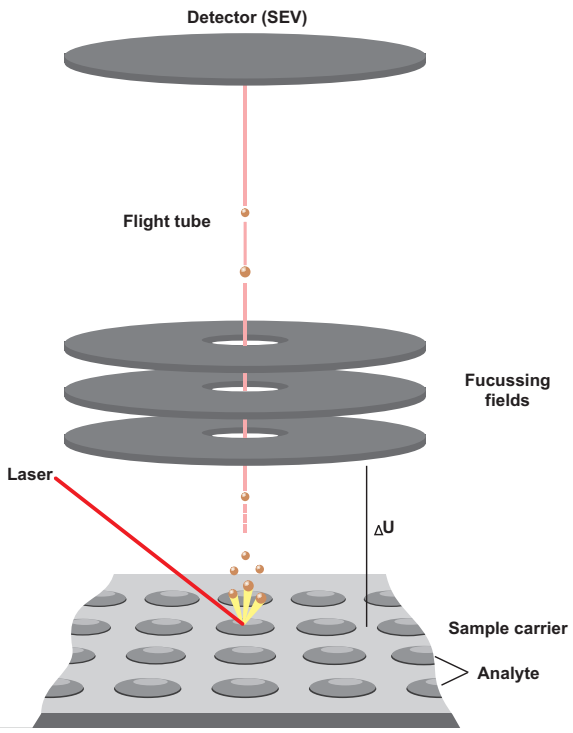

Figure 3: Principle of MALDI-TOF.
ΔU: accelerating voltage; SEV: secondary electron multiplier

The silicon chip contains 384 matrix spots consisting of 3-hydroxypicolinic acid for the samples and ten matrix spots for the calibrant. The calibrant, a mix of three oligonucleotides with known masses is also applied onto the chip for calibrating the analysis system. After loading the chip was transferred to a metallic sample carrier and put in the vacuum lock of the MassARRAY™ mass spectrometer. Measurement of the samples and calibration of the system was done automatically with standard setting.

The transfer of laser energy to sample molecules in the matrix generates mainly single charged molecule ions that trespass into the gas phase (Hillenkamp et al. 1991). Under high vacuum conditions, the matrix crystals were irradiated with nanosecond duration laser pulses, leading to formation of a plume of volatilized matrix and analyte as well as charge transfer from matrix ions to analyte molecules. After electric field-induced acceleration in the mass spectrometer source region, the gas phase ions travel through a field-free region at a velocity inversely proportional to their mass-to-charge ratios (m/z), until they hit the detector (Buetow et al. 2001, Griffin et al. 2000). Ions with low m/z values are faster than ions with higher m/z values and reach the detector earlier. The TOF-analyzer measures exactly the time until the

ions hit the detector (Griffin et al. 2000). The resulting time-resolved spectrum is translated into a mass spectrum upon calibration. These mass spectra were further processed and analyzed by the software Spectrotyper (Sequenom, Hamburg, Germany) for baseline correction and peak identification. Due to multiplexing this method allows genotyping of up to 40 SNPs at once in up to 384 samples at a time.

2.7 Allelic discrimination with TaqMan

Genotyping with TaqMan SNP Assays is based on annealing of to allele specific primers labeled with different fluorescent markers. The SNP Assay contains two primers for amplification of the sequence of interest and the two allele specific primers labeled with the reporter dyes VIC and 6-FAM at the 5' end. Furthermore, a minor groove binder and a non fluorescent quencher are bound to the 3' end of allele specific primers. Minor groove binder increases melting temperature (Tm). Thus, shorter primers are possible resulting in greater differences between Tm of matched and mismatched probes. The quencher allows more accurate measurement of reporter dye contribution by suppressing fluorescents of the reporter dye when the probe is intact. In case of a perfect match the allele specific primers hybridize to target locus. AmpliTaq Gold® DNA polymerase cleaves the bond allele specific primers during amplification and thus releases the reporter dye resulting in detectable fluorescence. This fluorescence identifies corresponding alleles. Figure 4 displays the principle of TaqMan SNP Assays. Equal to MALDI TOF MS 384 samples can be processed at once. However, multiplexing is limited with TaqMan SNP Assays due to limited variaty of fluorescent reporter dyes.

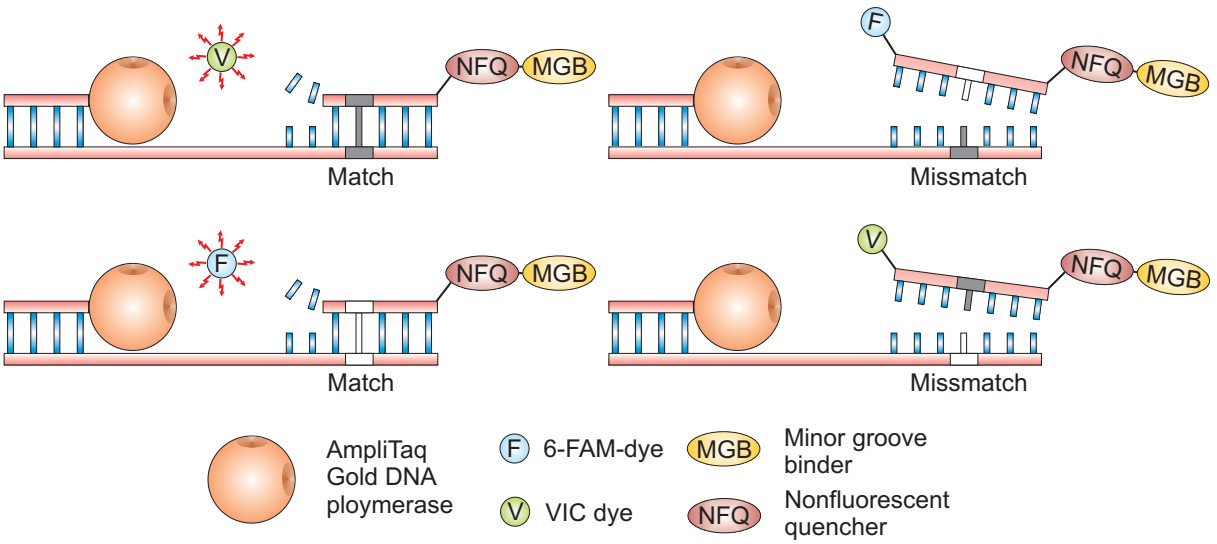

Figure 4: Principle of TaqMan allelic discrimination according to manufacturers guide

2.7.1 PCR Reaction and allelic discrimination

5 µl genomic DNA of working concentration were pipetted into each well of an ABI PRISM™ 384-Well Clear Optical Reaction Plate and dried over night. Subsequently 5 µl per well PCR

reaction mix, which is presented in Table 7, were added. After PCR amplification, an endpoint plate read was performed using SDS software, which calculates the fluorescence measurements made during the plate read and plots R_n values based on the signals from each well. Thus, alleles can be determined.

Table 7: PCR mix and conditions

Reagent	Volume [µl]	Step	Temp. [°C]	Time	Cycles
TaqMan Universal PCR Master Mix	2.50	Denaturation[a]	95	10 '	1x
SNP Assay Mix	0.25	Denaturation	95	15 "	40x
DNase-free water	2.25	Annealing	60	1 "	

[a]) AmpliTaq Gold Enzyme Activation

2.8 Quality assurance during genotyping

To avoid sample mix-up and contamination errors, eight asymmetric negative (0.1 TE) as well as eight positive (defined DNA) controls were included on each 384-well plate. A minimum of 10% of the samples were double genotyped. Furthermore, sex determination was performed with validated genotyping assays. These assays detected DNA variants within AMELX (Amelogenin) and GYG2 (Glycogenin) on chromosome X and their homologous pseudogenes on chromosome Y. Samples with discordant sex determination results were excluded from analysis.

2.9 Statistical methods

All analyses unless otherwise noted were performed using the statistical package SAS Version 9.1 (SAS Institute Inc., Cary, USA).

2.9.1 Hardy-Weinberg-Equilibrium

Hardy and Weinberg independently discovered that frequency of alleles and genotypes in a population will remain constant from generation to generation if the population is stable and in genetic equilibrium (Hardy 2003, Weinberg 1908). Riquired are a sufficient large population, panmixie, no new mutations, no selection and no migration. The Hardy-Weinberg equilibrium (HWE) is calculated by $p^2+2pq+q^2= (p+q)^2 = 1$, whereas p represents the frequency of one allele and q the frequency of the other allele at a single locus. Each SNP was tested for departures from HWE by means of a chi-square test, Fisher's exact test or Pearson's test depending on allele frequency and number of analyzed subjects. SNPs showing deviations from HWE were checked for indication of genotyping errors. Thus, SNPs with $p<0.05$ in Fisher's exact test or Pearson's test were handled with care in statistical analysis. In few cases deviation from HWE could imply evidence for genetic effects arguing against exclusion of such SNPs .

2.9.2 Linkage disequilibrium

The LD analysis (Weir et al. 1986) reveals a possible co-segregation and the non-random association of alleles across two ore more linked polymorphic loci due to lacking recombination events. As measures for pair wise LD between each pair of SNP loci, Lewontin´s disequilibrium coefficient D´ and the squared correlation coefficient r^2 were estimated using the JLIN V 1.0 software (http://www.genepi.com.au/jlin).

Three estimators of LD were computed:

1) D explains the raw difference in frequency between the observed number of AB pairs and the expected number: $D = p(AB) - p(A)*p(B)$

2) D' is a scaled D spanning the range [-1,1]: $D' = D / D_{max}$, where, if D > 0: $D_{max} = min (p(A)p(b), p(a)p(B))$ or if D < 0: $D_{max} = max (-p(A)p(B), -p(a)p(b))$

3) r is the correlation coefficient between the markers: $r = -D / sqrt(p(A) * p(a) * p(B) * p(b))$ where p(A) is defined as the observed probability of allele 'A' for marker 1, $p(a) = 1-p(a)$ is defined as the observed probability of allele 'a' for marker 1, p(B) is defined as the observed probability of allele 'B' for marker 2, and $p(b) = 1- p(b)$ is defined as the observed probability of allele 'b' for marker 2, and p(AB) is defined as the probability of the marker allele pair 'AB'.

2.9.3 Descriptive statistics and association analysis

Quantitative variables selected for association analysis were tested for normal distribution. If median/mean ratio was in range between 0.9 and 1.1, and three fold standard deviation was below mean the trait was regarded as normally distributed. In case of violation of one of these criteria variable was logarithmized and tested under same conditions. Traits that did not accomplish normal distribution in logarithmic scale, were analyzed by Kruskal-Wallis test. In the three genotype groups the values of each parameter were ranked between the groups. For each group a sum of squares was estimated and calculated. The difference between calculated and estimated sum of squares indicates changes in values due to specific genotypes.

Traits accomplishing normal distribution in normal or logarithmic scale were generally analyzed by model free linear regression comparing subjects with heterozygous genotype or subjects homozygous for the minor allele with the major allele homozygous reference group. In analysis of APOA5, MCP-1, and RETN p-value from F-test was used as criteria for further modeling. Thus, SNPs with significant differences between the genotype groups, were further investigated using dominant, recessive or additive models, respectively. Quantitative analysis was mainly restricted to fasting subjects or normoglycemic subjects.

Linear regression was adjusted for potential confounders, generally for age and gender, and BMI if relevant. P-values were considered statistically significant, if p-values were lower than 0.05 divided by number of effective loci, which was calculated by spectral decomposition of

the correlation matrix of all variants analyzed (Nyholt 2004), multiplied with the number of analyzed independent traits. The one exception was IL-6 analysis were significance level was defined as $p<0.01$. Participants taking lipid-lowering drugs were excluded from analysis of lipid traits (TG levels, HDL-, LDL-, and total cholesterol levels). Subjects with antidiabetic medication were excluded from the analysis of fasting glucose, 2-h glucose (from oral glucose tolerance test (OGTT)), and fasting insulin. For analysis of blood pressure, subjects using antihypertensive medication were excluded.

Analysis of association between SNPs and categorical outcomes like T2DM or MetS, was performed by logistic regression. Just like in linear regression models named above were also applied in logistic regression.

2.9.4 Haplotype analysis

Haplotype reconstruction was performed within blocks of high D' using expectation-maximization (EM) algorithm haplo.em (Schaid et al. 2002), which is included in the haplo.glm procedure as it is available within the R software library haplo.stats. This procedure performs an iterative two-step EM, with the posterior probabilities of pairs of haplotypes per subject used as weights to update the regression coefficients, and the regression coefficients used to update the posterior probabilities. To avoid large reconstruction errors resulting from missing data, haplotype estimation is based only on patients from whom all genotypes were available. The number of copies of best-guess haplotypes per subjects (i.e., the haplotype pair with the highest probability) was calculated for each haplotype variable and haplotype frequencies were evaluated. Haplotypes with frequencies <5% were collected into a separate group of rare haplotypes ("haplo rare"). Haplotypes were included in the haplo.glm regression model simultaneously, except for the most common haplotype. Using the expected number of copies of haplotype implies an additive model for each haplotype.

3. Material

3.1 Devices

Centrifuges	Sigma 4K15C (Sigma laboratory centrifuges, Osterode, Germany) Rotanta 46 RS (Hettich, Tuttlingen, Germany) Mikrozentrifuge (NeoLab, Heidelberg, Germany) Centrifuge 5417R (Eppendorf AG, Hamburg, Germany)
Gel electrophoresis documentation system	UVT-40 M Transilluminator (Herolab, Wiesloch, Germany) E.A.S.Y. 429 K Camera (Herolab, Wiesloch, Germany)
Gel electrophoresis gadget	Bio-Rad Power Pac 300/3000 (BIO-RAD Laboratories, Munich, Germany)
Gel tray	Sub-Cell GT Systems (BIO-RAD Laboratories, Munich, Germany)
Ice machine	AF30 (Scotsman, Milano, Italy)
Mass spectrometer	Bruker™ Autoflex (Sequenom, Hamburg, Germany) BIFLEX II-TOF (Bruker Franzen Analytik, Bremen, Germany)
TaqMan	ABI PRISM® 7900HT Sequence Detection System, (Applied Biosystems, Germany)
Microwave	Micromaxx (Medion AG, Essen, Germany)
Nanodispenser	Mass Array™ Nanodispenser (Sequenom, Hamburg, Germany) Spectro Point™ Nanoliter Pipetting Systems (Sequenom, Hamburg, Germany)

PCR cycler	DNA Engine DYAD™
	(MJ Research, South San Francisco, USA)
	DNA Engine Tetrad
	(MJ Research, South San Francisco, USA)
Photometer	Genios® Flourescence Plate Reader
	(Tecan AG, Crailsheim, Germany)
Pipetting robots	Multimek 96 Automated 96-Channel Pipettor
	(Beckman Coulter, Fullerton, USA)
	Temo (Tecan AG, Crailsheim, Germany)
	Genesis RSP 150 Work Station
	(Tecan AG, Crailsheim, Germany)
	Aquarius (Tecan AG, Crailsheim, Germany)
Scale	572 precision balance
	(Schott Duran, Mainz, Germany)
Shaker	Roto-Shake Genie
	(Scientific Industries, NewYork, USA)
Thermal mixer	Thermomixer Comfort
	(Eppendorf AG, Hamburg, Germany)
Ultrapure water purification system	Milli-Q
	(Millipore GmbH, Schwalbach, Germany)
Vortex	MS2 Minishaker
	(IKA-Labortechnik, Staufen, Germany)

3.2 Software

3.2.1 Software for pipetting robots

Gemini 3.2 (Visual Basic Programme) Normalisation WorklistMaker (Visual Basic Programme) Xflour4	Tecan, Crailsheim, Germany

3.2.2 Software for MALDI-TOF mass spectrometry

Genotype Analyser Nanodispenser software Spectro READER Spectro Typer RT	Sequenom, Hamburg, Germany

Software for Allelic discrimination (TaqMan)

SDS	Applied Biosystems, Germany

3.2.3 Software for assay design and sequence analysis

Spectro DESIGNER	Sequenom, Hamburg, Germany
Vector NTI Suite 8/9	www.informaxinc.com

3.2.4 Statistical software

JLIN	www.genepi.com.au/jlin
Haploview	www.broad.mit.edu/mpg/haploview
R 2.2.3	www.r-project.org
SAS 9.1	SAS Institute Inc., Cary, USA

3.2.5 Online databases and programs for SNP selection and sequence analysis

Ensemble	www.ensembl.org
Innate Immunity Program for Genomic Applications (IIPGA)	http://innateimmunity.net
International HapMap Project	www.hapmap.org
MatInspector	www.genomatix.de/products/MatInspector
National Center for Biotechnology Information	www.ncbi.nlm.nih.gov
PromoterInspector	www.genomatix.de/products/PromoterInspector

Seattle SNPs Program for Genomic Applications	http://pga.gs.washington.edu
UCSC Genome Browser	http://genome.ucsc.edu/cgi-bin/hgGateway
UTRScan	www.ba.itb.cnr.it/BIG/UTRScan

3.2.6 Datamanagement

Ingres II	Ingres Corporation, Redwood City, CA, USA
Ingres VDBA	Ingres Corporation, Redwood City, CA, USA
SNP-AT	Genotype data processing tool programmed by Guido Fischer, GSF

3.3 Buffer, solutions and reagents

3.3.1 Solutions for DNA extraction

RBC lysis buffer (pH 7.4)	NH_4Cl (155 mM)
	$KHCO_3$ (20 mM)
	Na_2EDTA (0.1 mM)
SE buffer (pH 8.0)	NaCl (75 mM)
	Na_2EDTA (25 mM)
NaCl solution (saturated)	NaCl (~6 M)
TE buffer (pH 8.0)	Tris/HCl (10 mM)
	EDTA (1 mM)
SDS solution	SDS (20%)

3.3.2 Buffers, solutions and reagents for agarose gel electrophoresis

6x Loading Dye Solution	MBI Fermentas, St. Leon-Rot, Germany
Agarose ultra pure	GibcoBRL, Eggenstein, Germany
DNA agarose	Biozym, Oldendorf, Germany
Ethidium bromide	Biomol, Hamburg, Germany
Gene Ruler 100 bp DNA-Ladder Plus	MBI Fermentas, St. Leon-Rot, Germany
Tris-Borate-EDTA buffer (TBE)	Sigma-Aldrich, Osterode, Germany

3.3.3 Buffers and reagents for PCR

dNTP mix (25 mM)	MBI Fermentas, St. Leon-Rot, Germany
$MgCl_2$ (25 mM)	Quiagen GmbH, Hilden, Germany
PCR-Buffer with $MgCl_2$ (10x)	

3.3.4 Buffers and reagents for SNP detection

3-point calibrant	Sequenom, Hamburg, Germany
hME MassEXTENT Nucleotide Tri-Mix Pack	
hME buffer	
Spectro CLEAN	
iPLEX	
iPLEX gold buffer	

3.4 Enzymes

HotStar Taq DNA polymerase	Quiagen, Hilden, Germany
Proteinase K	Merck, Darmstadt, Germany
Shrimp Alkaline Phosphatatse (SAP)	Amersham, Freiburg, Germany
Thermosequenase	Amersham, Freiburg, Germany

3.5 Primer

All primers were ordered by Metabion (Martinsried, Germany). Extension primers were purified by high performance liquid chromatography. Used primers are listed in Appendix 1.

3.6 Reagents for TaqMan

TaqMan® Universal PCR Master Mix	Applied Biosystems, Germany
SNP Assay	

4. Study populations

The fundament of this work were the populations available in house or from cooperations, which were sufficiently large and well phenotyped for detection of associations with MetS, T2DM or related parameters. All populations used in this work are described in detail below.

4.1 Describtion of study populations

4.1.1 MONICA (KORA S1-S3)

The population based monitoring trends and determinants on cardiovascular diseases (MONICA) Augsburg (Southern Germany) studies conducted between 1984 and 1995 were part of the multinational WHO MONICA project (Bothig 1989, Keil et al. 1998). Three independent cross-sectional surveys were carried out in the city of Augsburg and the counties Augsburg and Aichach–Friedberg (Figure 5) in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3) to estimate the prevalence and distribution of cardiovascular risk factors among men and women. Altogether 13,427 persons (6,725 men, 6,702 women, response 77%) aged 25 to 74 years participated in at least one of the three cross-sectional studies. All persons who took part in more than one survey were included once only with data collected at the first visit. All subjects were prospectively followed within the framework of the Cooperative Health Research in the Region of Augsburg (KORA). Methods of data collection and clinical measurements were discribed in detail elsewhere (Keil et al. 1998).

4.1.2 KORA S4

The KORA S4 is a population-based study of adults performed in Southern Germany (Wichmann et al. 2005). This survey was conducted under the same conditions as the previous three surveys within the WHO MONICA Augsburg project (Holle et al. 2005).

Basis population for the major projects of this work was a sub sample of the KORA S4. This sub sample comprised all participants in the age range from 55 to 74 years, 1,653 subjects in total including 842 men and 811 women. In candidate gene analysis further restricted sub samples were used according to specific questions.

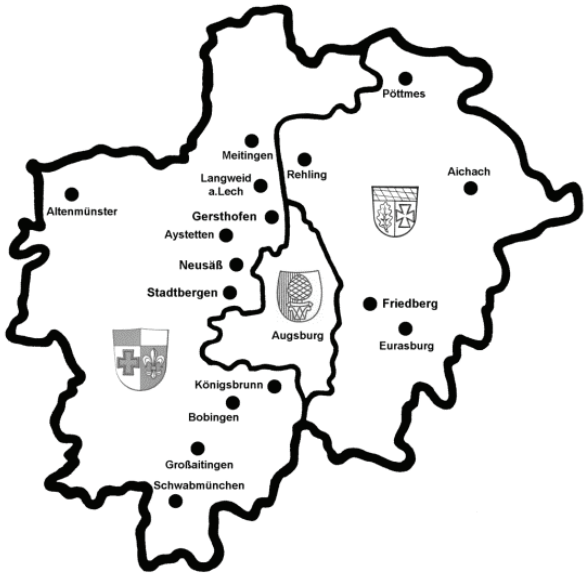

Figure 5: Region of ascertainment within the MONICA/KORA surveys

Standardized personal interviews were performed with all study participants. Known diabetes was found out by self-reported physician diagnosis or the use of antidiabetic agents.

Anthropometric measurements were carried out by trained investigators. Body weight was measured in light clothing to the nearest of 0.1 kg. Height had to be measured to the nearest of 0.5 cm. Waist circumference was measured at the minimum abdominal girth and hip circumference was assessed at the maximum protrusion of the hips at the level of the symphysis pubis to the nearest 0.1 cm. BMI $\geq$ 30 kg/m^2 and waist circumference above 109 cm in men and 100 cm in women were marker for obesity. Body fat mass was measured by biological impedance analysis with BIA 2000-S analyser (Data Input, Frankfurt, Germany). Venous blood samples were drawn and prepared according to the recommendations of the International Committee for Standardization in Hematology (International Committee for Standardization in Hematology 1994). Blood samples were used for determination of biochemical parameters like serum glucose with hexokinase method (Roche Diagnostics, Mannheim, Germany), HBA1c with turbidimetric immunologic method (Roche Diagnostics, Mannheim, Germany) and serum MCP-1 with a sandwich ELISA (R&D Systems, Minneapolis, MN, USA). Serum cholesterol was measured with enzymatic methods (CHOD-PAP, Roche Diagnostics), HDL cholesterol with phosphotungstic acid method (Boehringer Mannheim), LDL cholesterol with precipitation with dextran sulphate (Quantolip LDL, Immuno AG, Vienna, Austria). The Boehringer GPO-PAP was used for measuring triglycerides and serum uric acid was assessed with the uricase method (Roche Diagnostics).

An Oral glucose tolerance test (OGTT) was only carried out in subjects with unknown diabetes history. The protocol was standardized with a fasting period of ten hours overnight, execution of the test before 11:00 am, avoiding heavy physical activity the day before examination and refrain smoking. Fasting glucose was measured before donation of 75 g anhydrous glucose and 2-hour plasma glucose after a period of 120 ± 6 min. Newly diagnosed T2DM was defined as $\geq$ 7.0 mmol/l fasting or $\geq$ 11.1 mmol/l 2-hour plasma glucose. Impaired glucose tolerance (IGT) was defined as 2-h plasma glucose between 7.8 and 11.1 mmol/l and impaired fasting glucose (IFG) was defined as fasting plasma glucose between 6.1 and 7.0 mmol/l. HOMA-IR was calculated as fasting glucose multiplied with fasting insulin divided by 22.5 (Rathmann et al. 2003).

4.1.3 SAPHIR

The Salzburg Atherosclerosis Prevention program in subjects at High Individual Risk (SAPHIR) was an observational study conducted in Austria from 1999 to 2002 involving 1,770 unrelated fasting subjects, including 663 women aged 50 to 70 years and 1,107 men aged 40 to 60 years (Esterbauer et al. 2001, Heid et al. 2006b). The differential age range between the sexes was chosen in order to match cardiovascular risk, which is about the same if women are ten years older then men. Study participants were recruited by health screening programs in large companies in and around the city of Salzburg.

4.2 Characteristics of study populations

This chapter embraces characteristics of all analyzed phenotypes in the appropriate populations and sub samples used in this work.

4.2.1 KORA S4

Table 8 displays characteristics of the basis sub sample of the KORA S4 population including all participants aged 55 to 74 years, which were known diabetic patients or had a status determined by OGTT. Thus, maximum dataset from KORA S4 elderly subjects included 254 diabetic individuals. 23 of these subjects were identified as non-T2DM subjects in validation. MetS was identified in 816 subjects using IDF and in 687 subjects using NCEP definition.

Table 8: Characteristics of KORA S4 subjects (55 to 74 years)

	n (m/f)	total	male	female
Age [years]	1485(770/715)	64.1 ± 5.4	64.2 ± 5.5	64.0 ± 5.3
BMI [kg/m²]	1477(764/713)	28.68 ± 4.38	28.38 ± 3.77	29.01 ± 4.93
Triglycerides [mmol/l]	1445(750/695)	1.36(0.98/1.91)	1.45(1.02/2.10)	1.26 (0.96/1.77)
HDL cholesterol [mmol/l]	1457(757/700)	1.49 ± 0.42	1.35 ± 0.35	1.63 ± 0.44
LDL cholesterol [mmol/l]	1455(756/699)	3.95 ±1.02	3.90 ± 1.03	4.01 ± 1.03
Total cholesterol [mmol/l]	1458 (758/700)	6.27 ± 1.09	6.13 ± 1.09	6.41 ± 1.07
Waist circumference [cm]	1460 (756/704)	96.26 ± 11.62	100.88 ± 9.72	91.30 ± 11.46
Uric acid [mg/dl]	1459 (758/701)	5.68 ± 1.42	6.30 ± 1.36	5.02 ± 1.17
Fasting plasma glucose [mg/dl]	1458 (757/701)	108.35 ± 32.84	111.23 ± 32.37	105.24 ± 33.09
2-h plasma glucose [mg/dl]	1347 (694/653)	125.61 ± 50.00	127.43 ± 52.83	123.68 ± 46.75
Systolic BPR [mmHg]	1457 (755/702)	136.25 ± 20.32	140.44 ± 19.97	131.75 ± 19.73
Diastolic BPR [mmHg]	1457 (755/702)	80.36 ± 10.49	82.41 ± 10.53	78.15 ± 9.98
Fasting insulin [mU/l]	1448 (754/694)	10.5(7.2/15.9)	10.5(7.2/16.1)	10.4 (7.2/15.8)

[a]) sex difference, significantly higher value p<0.05. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, triglycerides).

Restriction to fasting subjects is assembled in Table 9. Despite exclusion of non-fasting subjects with known diabetes there were 123 diabetic patients included, all detected by OGTT. In the fasting sample there were 699 IDF defined and 569 NCEP defined MetS subjects.

Table 9: Characteristics of fasting KORA S4 subjects (55 to 74 years)

	n (m/f)	total	male	female
Age [years]	1354(697/657)	64.0 ± 5.5	64.1 ± 5.6	63.9 ± 5.3
BMI [kg/m²]	1348(693/655)	28.4 ± 4.2	28.2 ± 3.6	28.6 ± 4.7
Triglycerides [mmol/l]	1187(602/585)	1.28(0.94/1.77)	1.39(0.98/1.92)	1.20(0.91/1.62)
HDL cholesterol [mmol/l]	1195(606/589)	1.51 ± 0.43	1.36 ± 0.36	1.67 ± 0.43 [a]
LDL cholesterol [mmol/l]	1194(605/589)	4.00 ± 1.01	3.97 ± 1.00	4.04 ± 1.03
Total cholesterol [mmol/l]	1196(607/589)	6.31 ± 1.07	6.18 ± 1.07	6.44 ± 1.06
Waist circumference [cm]	1353(696/657)	95.6 ± 11.4	100.4 ± 9.5 [a]	90.4 ± 11.0
Uric acid [mg/dl]	1354(697/657)	5.7 ± 1.4	6.3 ± 1.3 [a]	4.9 ± 1.1
Fasting plasma glucose [mg/dl]	1351(695/656)	102.2 ± 16.9	105.0 ± 17.9	99.2 ± 15.2
2-h plasma glucose [mg/dl]	1350(695/655)	125.9 ± 50.0	127.6 ± 52.8	124.0 ± 46.7
Systolic BPR [mmHg]	881(455/426)	133.2 ± 19.4	137.9 ± 18.6[a]	128.0 ± 19.1
Diastolic BPR [mmHg]	881(455/426)	79.3 ± 9.8	81.2 ± 9.8[a]	77.2 ± 9.4
Fasting insulin [mU/l]	1342(692/650)	10.1(7.1/14.6)	10.2(6.9/14.8)	9.9(7.2/14.4)

[a]) sex difference, significantly higher value p<0.05. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, triglycerides).

The most limited sub sample included normoglycemic subjects only (Table 10). This sample included 334 IDF defined MetS subjects.

Table 10: Characteristics of normoglycemic subjects from KORA S4 (55-74 years)

	n (m/f)	male	female
Age [years]	(417/460)	63.6 ± 5.6	63.4 ± 5.4
Body mass index [kg/m^2]	(414/460)	27.4 ± 3.3	27.9 ± 4.6
Height [cm][a]	(416/461)	172.3 ± 6.3	159.3 ± 6.1
Weight [kg] [a]	(414/461)	81.4 ± 10.9	70.8 ± 12.1
Body fat [%][a]	(412/457)	32.18 ± 4.09	39.67 ± 5.06
WHR [a]	(416/460)	0.95 ± 0.05	0.83 ± 0.06
Waist circumference [cm] [a]	(416/460)	98.6 ± 8.9	88.3 ± 10.8
Hip circumference [cm] [a]	(416/460)	103.7 ± 6.2	106.6 ± 9.4
Serum uric acid [mg/dl] [a]	(417/460)	6.13 ± 1.26	4.78 ± 1.07
HDL cholesterol [mmol/l] [a]	(416/459)	1.40 ± 0.34	1.71 ± 0.44
LDL cholesterol [mmol/l]	(415/460)	3.97 ± 0.10	4.02 ± 1.07
Fasting plasma glucose [mg/dl] [a]	(417/460)	97.1 ± 6.8	94.2 ± 7.0
2-h plasma glucose [mg/dl]	(417/460)	100.4 ± 21.3	103.3 ± 20.6
Systolic BPR [mmHg] [a]	(290/337)	134.3 ± 17.6	125.9 ± 18.1
Diastolic BPR [mmHg] [a]	(290/337)	80.1 ± 9.6	76.7 ± 9.4
Leukocytes [x10^{-3}/µl]	(417/460)	6.07 ± 1.56	5.79 ± 1.31
HbA1c [%][a]	(417/460)	5.52 ± 0.33	5.60 ± 0.33
HOMA-IR	(416/460)	2.06(1.49/2.98)	2.05(1.48/3.01)
Fasting insulin [mU/l]	(416/460)	8.70(6.15/12.15)	8.70(6.60/12.90)
Triglycerides [mg/dl] [a]	(412/456)	113.0(79.5/155.5)	101.0(78.0/137.0)
Interleukin-6 [pg/ml]	(413/460)	1.88(0.95/3.24)	1.62(0.94/2.61)

[a]) p<0.01 for sex differences. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, HOMA-IR, triglycerides, IL-6).

Generally in the KORA S4 elderly samples significant sex differences were observed for waist circumference, waist-to-hip ratio (WHR), height, weight, fasting plasma glucose, triglycerides, uric acid and blood pressure, which were all higher in men, and for hip circumference, percent body fat, HDL cholesterol levels and HbA1c, which were higher in women ($p < 0.01$).

4.2.2 KORA case-control study

The KORA case-control study includes all diabetic subjects form the three MONICA surveys and the KORA S4. In total there were 729 subjects with T2DM in the MONICA/KORA surveys. Controls were matched in 2:1 ratio for age and sex to all known patients with T2DM. However, DNA was not available for all T2DM subjects. Hence, the final case-control study included 630 subjects with T2DM, only. Together with the 1,458 control subjects the sample includes 2,088 subjects in total. Characteristics of the KORA case-control sample are presented in Table 11.

Table 11: Characteristics of KORA case-control study

	n(m/f)	total	male	female
Age [years]	2088(1157/931)	60.8 ± 9.2	60.5 ± 9.0	61.2 ± 9.3
BMI [kg/m²]	2075(1148/927)	28.6 ± 4.6	28.5 ± 4.0	28.8 ± 5.1
Triglycerides [mmol/l]	1010 (564/446)	1.30(0.95/1.79)	1.38(0.97/1.95)[a]	1.21(0.94/1.66)
HDL cholesterol [mmol/l]	2079(1153/926)	1.42 ± 0.44	1.29 ± 0.37	1.58 ± 0.46
LDL cholesterol [mmol/l]	2015(1116/899)	3.91 ± 1.08	3.88 ± 1.04	3.94 ± 1.11
Total cholesterol [mmol/l]	2063(1143/920)	6.24 ± 1.17	6.14 ± 1.14	6.38 ± 1.18
Waist circumference [cm]	2018(1117/901)	96.1 ± 12.4	100.7 ± 10.4 [a]	90.4 ± 12.2
Hip circumference [cm]	2017(1116/901)	106.3 ± 9.2	105.3 ± 7.5	107.6 ± 10.8 [a]
Uric acid	2063(1142/921)	5.50 ± 1.46	6.04 ± 1.40	4.84 ± 1.25
Systolic BPR [mmHg]	2062(1142/920)	137.3 ± 20.3	140.1 ± 19.4 [a]	133.8 ± 20.8
Diastolic BPR [mmHg]	2062(1142/920)	81.0 ± 11.1	82.7 ± 11.1 [a]	78.9 ± 10.7
Leukocytes [$x10^{-3}$/µl]	1805(997/808)	6.5 ± 1.8	6.5 ± 1.8	6.5 ± 1.8
HbA1c [%][a]	1804(996/808)	5.9 ± 1.1	5.9 ± 1.2	6.0 ± 1.1

[a]) sex difference, significantly higher value p<0.05. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, triglycerides).

4.2.3 SAPHIR

Characteristics of the SAPHIR study are presented in Table 12. The SAPHIR study was used as replication sample for KORA S4 in the analysis of *APOA5*. In comparison to the elderly KORA S4 sample SAPHIR participants were younger. BMI was higher in KORA S4 in all groups.

Table 12: Characteristics of SAPHIR subjects

	n(m/f)	total	male	female
Age [years]	1770(1107/663)	51.8 ± 6.1	49.2 ± 5.5	56.2 ± 4.3
BMI [kg/m²]	1770(1107/663)	26.8 ± 4.1	26.9 ± 3.7	26.6 ± 4.7
Triglycerides [mmol/l]	1681(1073/608)	1.15(0.82/1.69)	1.21(0.85/1.92) [a]	1.06(0.79/1.45)
HDL cholesterol [mmol/l]	1681(1073/608)	1.54 ± 0.41	1.43 ± 0.35	1.75 ± 0.42 [a]
LDL cholesterol [mmol/l]	1681(1073/608)	3.78 ± 0.95	3.79 ± 0.93	3.77 ± 0.98
Total cholesterol [mmol/l]	1681(1073/608)	5.93 ± 1.04	5.85 ± 1.03	6.07 ± 1.05
Waist circumference [cm]	1728(1081/647)	94.6 ± 12.6	98.0 ± 10.5 [a]	88.9 ± 13.6
Uric acid [mg/dl]	1764(1105/659)	5.9 ± 1.4	6.4 ± 1.3 [a]	4.9 ± 1.1
Fasting plasma glucose [mg/dl]	1736(1036/650)	92.5 ± 14.1	93.4 ± 15.2 [a]	90.9 ± 11.8
2-hour plasma glucose [mg/dl]	654(495/159)	102.2 ± 36.8	99.5 ± 37.9	110.7 ± 31.7
Systolic BPR [mmHg]	1388(919/469)	132.8 ± 12.8	134.6 ± 12.4	129.3 ± 12.9
Diastolic BPR [mmHg]	1388(919/469)	81.4 ± 7.7	82.1 ± 7.8	80.1 ± 7.4
Fasting insulin [mU/l]	1726(1081/645)	6.0(4.2/8.9)	6.0(4.0/8.9)	6.0(4.3/8.8)

[a]) sex difference, significantly higher value p<0.05. Data are presented as means ± standard deviations or medians (25th/75th percentiles) for traits that were not normally distributed (insulin, triglycerides).

5. Results

5.1 Candidate gene selection and strategies

The genes, which were investigated in the central analysis of this work were chosen from different pathways involved in MetS or T2DM development. Depending on the specific candidate gene, the strategy of the genetic analysis differs in study design, gene coverage due to prior association information and statistical analysis.

IL-6 was chosen as elevated circulating levels of IL-6 contribute to development of insulin resistant states and T2DM. Thus, for this analysis two SNPs were selected that were proposed to influence IL-6 expression. As the *IL-6* SNPs -174 G>C and -573 G>C have previously been investigated in several studies, the study population was limited to normoglycemic subjects to investigate influences on MetS and T2DM related parameters, before outbreak of T2DM.

The analysis strategie for ***APOA5***, which was chosen for its promising role in lipid metabolism, was different. A recent finding in Japanese, where the minor allele of *APOA5* SNP -1131T>C was associated with higher risk of MetS, supported the choice for *APOA5*. To get all genetic information the *APOA5* locus was systematically analysed by a selection of SNPs covering the whole gene and potential promoter region, enhancing selection of previous studies but also trying to replicate the Japanese finding in a Caucasian population. Statistical analysis was performed in fasting subjects only, especially concerning variability of lipid measurements depending on food intake.

MCP-1 was chosen as second inflammatory candidate, as higher MCP-1 levels were associated to insulin resistance and T2DM. Another aspect was the interaction between MCP-1 and IL-6 concerning expression. Similar to *APOA5*, SNPs were selected to cover most of the genetic information of the *MCP-1* locus. SNPs in the *MCP-1* locus were investigated for association with outcome MetS and T2DM in a case control design as well as parameters related to both diseases in fasting subjects.

The analysis strategie for ***RETN*** was similar to that of *MCP-1*. This locus was chosen as there were a lot of association studies of single SNPs linking *RETN* to insulin resistance, obesity and T2DM. Therfore, SNPs were selected by prior information from the literature and additionally by tagging information from the HapMap catalouge. In contrast to previous studies, statistical analysis was enhanced to parameters of MetS and T2DM but investigated also association with both diseases themselves.

5.2 Genotyping

Genotyping success rates were >93% for all SNPs. One of the SNPs in *RETN* locus violated HWE ($p<0.05$) and was not further investigated. Table 13 displays genotype frequencies, success rates, and further genotyping information for all statistically analyzed SNPs.

Table 13: Genotype Information for all SNPs included in statistical analysis

	Variant	Alias[a]	Position on Chr[b]	Gene locus	n	GSR [%]	Base change	AA change	MAF	HWE[c]
Elderly KORA S4	rs1800795	-174G>C	7:22733170	IL-6	1598	98.0	G→C		0.43	0.08
	rs1800796	-573G>C	7:22732771	IL-6	1603	98.3	G→C		0.05	0.57
	rs2542061	-9655A>G	11:116177441	APOA5	1581	97.0	A→G		0.50	0.82
	rs633867	-4904C>T	11:116172690	APOA5	1559	95.6	C→T		0.02	0.74
	rs662799	-1131T>C, SNP3	11:116168917	APOA5	1607	98.6	T→C		0.08	0.18
	rs1729411	-1099G>A	11:116168885	APOA5	1589	97.5	G→A		0.13	0.18
	rs651821	-3A>G	11:116167789	APOA5	1596	97.9	T→C		0.07	0.17
	rs3135506	S19W, c.56C>G	11:116167617	APOA5	1533	94.1	G→C	S→W	0.06	0.22
	rs2072560	476G>A, SNP2	11:116167036	APOA5	1603	98.3	G→A		0.07	0.25
	rs3135507	c.457G>A, Val 153Met	11:116166698	APOA5	1595	97.9	C→T	V→M	0.03	0.70
	rs619054	1764C>T	11:116166023	APOA5	1608	98.7	G→A		0.26	0.80
	rs2266788	1259T>C, SNP1	11:116165896	APOA5	1597	98.0	T→C		0.08	0.05
SAPHIR	rs662799	-1131T>C, SNP3	11:116168917	APOA5	1702	96.2	T→C		0.07	0.57
	rs3135506	S19W, c56C>G	11:116167617	APOA5	1722	97.3	G→C	S→W	0.07	0.06
	rs2266788	1259T>C, SNP1	11:116165896	APOA5	1694	95.7	T→C		0.07	0.42
Elderly KORA S4	rs1860188	c.-3813C>T	17:29602669	MCP-1	1569	95.8	C→T		0.15	0.56
	rs1024611	c.-2138A>T	17:29603901	MCP-1	1620	98.9	A→T		0.20	0.73
	rs1024610	c.-928G>C	17:29604344	MCP-1	1617	98.7	G→C		0.22	0.36
	rs3760396	c.77-109C>G	17:29605554	MCP-1	1616	98.7	C→G		0.20	0.74
	rs2857657	c.105T>C	17:29607245	MCP-1	1559	95.2	T→C		0.38	0.68
	rs4586	c.*65C>T	17:29607382	MCP-1	1604	97.9	C→T		0.28	0.68
	rs13900	c.*3879C>T	17:29608024	MCP-1	1620	98.9	C→T		0.28	0.86
	rs4804762		19:7635195	RETN	1517	93.9	C→T		0.17	0.06
	rs3760678	A-1093G, A-852G	19:7639120	RETN	1593	98.6	A→G		0.14	0.94
	rs1862513	C-420G, C-394G, C-180G, C-179G	19:7639793	RETN	1587	98.2	C→G		0.29	0.28
	rs3219177	C156T, C157T, C39T, IVS2+39C>T	19:7640369	RETN	1586	98.1	C→T		0.21	1.00
	rs3745367	G299A, G298A, G181A, IVS2+181G>A	19:7640511	RETN	1574	97.4	A→G		0.23	0.76
	rs3219178	C167G, IVS3+167C>G	19:7640951	RETN	1576	97.6	C→G		0.46	0.14
	rs3745369		19:7641475	RETN	1593	98.7	G→C		0.48	0.76
KORA CC S1-S4	rs12255372		10:114798892	TCF7L2	2325	98.3	G→T		0.29	0.16
	rs7903146		10:114748339	TCF7L2	2292	97.3	C→T		0.30	0.40
KORA S4	rs2084202		2:119841499	ACBP	1583	97.1	A→G		0.16	0.11
	rs8192506		2:119846311	ACBP	1585	97.2	A→G		0.04	0.54
	rs13283456	Arg298His	9:129924574	PTGES2	1617	99.2	C→T	R→H	0.17	0.79

[a] Synonymous names used in the literature [b] Positions according to Ensemble database, [c] p-values of Pearson's Test for HWE; AA= amino acid; CC= Case Control; GSR = Genotyping success rate; MAF = Minor allele frequency

5.3 Candidate gene analysis

5.3.1 Interleukin-6

5.3.1.1 IL-6 KORA S4 (Grallert et al. 2006)

Two promoter polymorphisms potentially affecting IL-6 expression were investigated in the *IL-6* locus (Figure 6) in KORA S4 normoglycemic subjects aged 55-74 years. The used sample was limited to the specified subjects to investigate influence of the selected genetic variants before outbreak of T2DM. Comparing the 332 subjects with IDF-defined MetS (37.8 % of the sample) with healthy normoglycemic subjects no genetic association with MetS could be found for the *IL-6* -174 G>C or -573 G>C polymorphism. Association results with MetS related parameters are presented for the -174 G>C polymorphism in Table 14 and for the -573 G>C polymorphism in Table 15.

In men lower waist ($\beta_{CCvs.GG}$= -1.91 cm, p= 0.002) and hip ($\beta_{CCvs.GG}$= -1.39 cm, p= 0.01) circumferences as well as higher BMI values ($\beta_{CCvs.GG}$= -0.65 cm, p= 0.004) were associated with the -174 C allele. These associations were only observable with additional adjustment for BMI or waist and hip circumference, respectively. Analyzing the single components of BMI a negative association of the -174 C allele with height in men was observed ($\beta_{CCvs.GG}$= -2.23 cm, p= 0.01), while weight was not significantly different in C allele carriers. For the -573 G>C polymorphism there was a tendency for the C allele to be associated with lower age ($\beta_{CC/GCvs.GG}$= -1.37 years, p= 0.03). Another tendency for an association of the -573 C allele was found with higher diastolic blood pressure ($\beta_{CC/GCvs.GG}$= 3.00 mmHg, p= 0.03). No genetic association with weight, percent body fat, WHR ratio, uric acid, HDL and LDL cholesterol levels, fasting plasma glucose, 2h glucose, leukocyte count, $HbA1_C$, HOMA-IR, fasting insulin, triglycerides, or IL-6 levels could be found in this sub sample for any of the two polymorphisms.

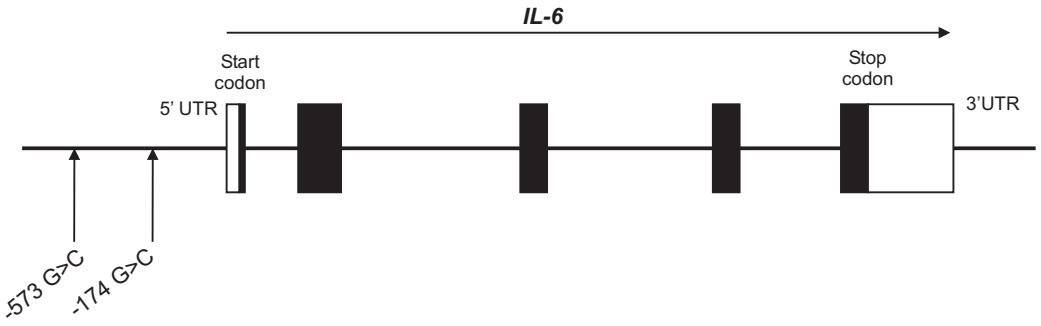

Figure 6: Genetic structure of the *IL-6* locus (chr: 7p21; length: 4796; aa: 212)

5.3.1.2 *IL-6* joint analysis of 21 studies (Huth et al. 2006, Huth et al. 2007)

Among 21 studies from eight different countries the results of the *IL-6* joint analyses included genotyping data from KORA S4 produced within this work. Thus, results are briefly

summarized below. Including 18 studies (including KORA S4 data) fulfilling analysis criteria pooled OR for 4,746 T2DM cases and 16,230 control subjects for an association between

Table 14: Estimated associations in the nominal linear regression model and scores of the Kruskal-Wallis test for the *IL-6* -174 G>C polymorphism on quantitative traits related to the metabolic syndrome.

1) Linear regression	**GC versus GG**			**CC versus GG**		
	total	**men**	**women**	**total**	**men**	**women**
Age [years] n=857(408/449)	0.03 (0.94)	0.08 (0.90)	-0.16 (0.98)	0.09 (0.88)	0.50 (0.55)	-0.30 (0.70)
BMI [kg/m^2] n=857(406/451)	0.39 (0.22)	0.64 (0.08)	0.16 (0.74)	-0.06 (0.89)	-0.03 (0.95)	-0.10 (0.88)
BMI [kg/m^2][a] n=857(406/451)	**0.33 (0.01)**	0.41 (0.02)	0.26 (0.17)	0.26 (0.13)	**0.65 (0.004)**	-0.14 (0.56)
Waist circumference [cm][b] n=857(406/451)	0.41 (0.59)	0.76 (0.44)	0.10 (0.93)	-0.77 (0.44)	-2.02 (0.11)	0.33 (0.83)
Waist circumference [cm] n=857(406/451)	-0.40 (0.30)	-0.74 (0.14)	-0.22 (0.70)	-0.61 (0.22)	**-1.91 (0.002)**	0.56 (0.45)
Hip circumference [cm][b] n=857(406/450)	-0.23 (0.73)	0.28 (0.68)	-0.70 (0.53)	-0.87 (0.31)	-1.46 (0.10)	-0.38 (0.79)
Hip circumference [cm] n=857(406/450)	-0.70 (0.02)	-0.68 (0.11)	-1.03 (0.04)	-0.55 (0.16)	**-1.39 (0.01)**	-0.19 (0.77)
Height [cm] n=859(408/451)	-0.74 (0.11)	-1.33 (0.05)	-0.19 (0.76)	-0.75 (0.21)	**-2.23 (0.01)**	0.58 (0.48)
Weight [kg] n=857(406/451)	0.48 (0.59)	0.71 (0.56)	0.27 (0.84)	-0.75 (0.52)	-2.10 (0.19)	0.42 (0.84)
Percent body fat [%] n=851(404/447)	0.07 (0.83)	0.41 (0.31)	-0.25 (0.65)	-0.33 (0.47)	-0.11 (0.83)	-0.59 (0.40)
WHR n=857(406/451)	0.0034 (0.36)	-0.001 (0.83)	0.006 (0.30)	0.0006 (0.89)	-0.006 (0.30)	0.007 (0.34)
Serum uric acid [mg/dl] n=857(406/451)	-0.09 (0.29)	-0.11 (0.43)	-0.08 (0.45)	-0.13 (0.26)	-0.18 (0.33)	-0.07 (0.63)
HDL cholesterol [mmol/l] n=855(405/450)	0.019 (0.51)	0.035 (0.36)	0.002 (0.97)	-0.012 (0.76)	-0.005 (0.92)	-0.020 (0.72)
LDL cholesterol [mmol/l] n=854(404/450)	-0.10 (0.22)	-0.19 (0.09)	-0.01 (0.93)	-0.04 (0.73)	-0.18 (0.22)	0.11 (0.46)
Triglycerides (log) n=849(401/448)	-0.016 (0.65)	-0.016 (0.78)	-0.018 (0.68)	0.002 (0.96)	-0.034 (0.64)	0.048 (0.40)
Fasting glucose [mg/dl] n=857(406/451)	0.82 (0.12)	0.88 (0.25)	0.74 (0.32)	1.11 (0.11)	1.07 (0.28)	1.20 (0.22)
2 h glucose [mg/dl] n=857(406/451)	0.83 (0.60)	2.60 (0.28)	-0.83 (0.70)	0.92 (0.66)	2.80 (0.37)	-0.71 (0.80)
Systolic BPR [mmHg] n=611(283/328)	3.04 (0.06)	1.15 (0.62)	4.25 (0.06)	3.22 (0.13)	3.95 (0.19)	2.28 (0.44)
Diastolic BPR [mmHg] n=611(283/328)	-0.08 (0.92)	-1.27 (0.41)	0.91 (0.45)	-0.29 (0.80)	-0.46 (0.79)	-0.13 (0.75)
Leukocytes [x10^{-3}/µl] n=857(406/451)	-0.02 (0.88)	-0.25 (0.17)	0.19 (0.18)	-0.12 (0.40)	-0.11 (0.63)	-0.14 (0.44)
HbA1c [%] n=857(406/451)	0.005 (0.85)	0.05 (0.16)	-0.04 (0.28)	-0.003 (0.93)	0.03 (0.56)	-0.03 (0.50)

2) Kruskal-Wallis test		**total**		**men**		**women**	
n=858(408/450)		median	p-value	median	p-value	median	p-value
IL6 levels	GG	1.67 (0.91/2.52)		1.73 (0.89/2.55)		1.66 (0.91/2.49)	
	GC	1.74 (1.02/3.07)	0.20	2.02 (1.20/3.55)	0.07	1.60 (0.95/2.72)	0.95
	CC	1.68 (0.89/3.02)		1.78 (0.81/3.42)		1.64 (0.89/2.72)	
HOMA-IR	GG	2.04 (1.47/2.92)		2.02 (1.44/2.90)		2.09 (1.48/2.93)	
	GC	2.05 (1.50/3.04)	0.81	2.05 (1.51/3.08)	0.76	2.04 (1.47/3.02)	0.95
	CC	2.13 (1.47/3.00)		2.22 (1.47/2.97)		2.08 (1.47/3.02)	
Fasting insulin	GG	8.70 (6.30/12.60)		8.70 (6.00/12.00)		8.70 (6.53/12.90)	
	GC	8.70 (6.60/12.45)	0.90	8.70 (6.30/12.30)	0.87	8.70 (6.60/12.53)	0.96
	CC	9.15 (6.30/12.60)		9.30 (6.15/11.70)		9.08 (6.60/13.50)	

1) Linear regression: ß-values (p-values) are given after adjustment for BMI and age. The traits BMI, height, weight and percentbody fat were only adjusted for age. The trait age was only adjusted for gender in the whole group; [a]) additionally adjusted for waist and hip circumference. [b]) Adjustment without BMI. All analyses for the total group were additionally adjusted for sex. p-value < 0.01 was regarded as significant.
2) Kruskal-Wallis test: medians (25th/75th percentiles).

-174 C allele T2DM was 0.91 ([95%CI]= [0.83-0.99], p= 0.037). For the -573 G>C polymorphism pooled OR for 2,392 T2DM case and 9,265 control subjects from 8 studies (including KORA S4 data) was 1.05 ([95%CI]= [0.86-1.27], p= 0.65).

Analysis of quantitative traits and *IL-6* polymorphisms revealed a significant decrease of fasting glucose (β= -0.09 mmol/L [95%CI]= [0.16,0.02]) for the -174 C allele carriers when summarizing estimate, while the seven individual studies did not reach significance. Stratification by T2DM status revealed a substantial and statistically significant fasting glucose reduction of -0.32 mmol/L [95%CI]= [-0.58,0.05] among diabetic subjects comparing the CC- or GC-genotypes with the GG-genotype. For 2 h glucose analyzed in six studies no statistically significant association was found, although estimates pointed at the same direction (β= -0.08 mmol/L, [95%CI]= [0.20,0.05]).

Table 15: Estimated effects of the IL-6 -573 G>C polymorphism on quantitative traits related to the metabolic syndrome - comparing genotype effects of the minor allele in a dominant model (CC/GC versus GG)

	N (m/w)	all	men	women
Age [years]	858 (411/447)	-1.37 (0.03)	-1.27 (0.15)	-1.47 (0.12)
BMI [kg/m^2]	858 (411/447)	-0.21 (0.65)	-0.08 (0.87)	-0.37 (0.65)
Height [cm]	860 (413/447)	1.43 (0.04)	1.75 (0.07)	1.05 (0.30)
Weight [kg]	858 (411/447)	0.78 (0.57)	1.35 (0.43)	0.09 (0.96)
Body Fat [%]	853 (409/444)	0.30 (0.56)	0.27 (0.64)	0.34 (0.69)
WHR	858 (411/447)	0.004 (0.45)	0.004 (0.54)	0.004 (0.62)
Waist circumference [cm]	858 (411/447)	0.70 (0.23)	0.92 (0.18)	0.41 (0.66)
Hip circumference [cm]	857 (411/446)	0.25 (0.58)	0.53 (0.36)	-0.09 (0.89)
Serum uric acid [mg/dl]	858 (411/447)	0.18 (0.16)	0.22 (0.26)	0.14 (0.41)
HDL cholesterol [mmol/l]	856 (410/446)	-0.017 (0.70)	-0.053 (0.31)	0.026 (0.71)
LDL cholesterol [mmol/l]	855 (409/446)	0.01 (0.90)	-0.06 (0.70)	0.10 (0.57)
Triglycerides (log)	848 (406/442)	0.014 (0.79)	0.028 (0.72)	-0.005 (0.94)
Fasting glucose [mg/dl]	858 (411/447)	-0.183 (0.82)	-0.210 (0.84)	-0.160 (0.89)
2h glucose [mg/dl]	858 (411/447)	-0.74 (0.76)	-1.48 (0.66)	0.13 (0.97)
Systolic blood pressure [mmHg]	609 (285/324)	1.91 (0.45)	5.46 (0.09)	-2.21 (0.57)
Diastolic blood pressure [mmHg]	609 (285/324)	3.00 (0.026)	4.19 (0.022)	1.49 (0.46)
Leukocytes [x10^{-3}/µl]	858 (411/447)	-0.12 (0.49)	-0.04 (0.86)	-0.20 (0.38)
HbA1c [%]	858 (411/447)	-0.011 (0.77)	0,047 (0.37)	-0,082 (0.16)

ß-values (p-values) are given after adjustment for BMI and age. The traits BMI, height, weight and body fat were only adjusted for age. The trait age was only adjusted for gender in the whole group; [a]) additionally adjusted for waist and hip circumference. [b]) Adjustment without BMI. All analyses for the total group were additionally adjusted for sex. p-value < 0.01 was regarded as significant.

Analysis of BMI was performed in 15 studies including 19,007 non-diabetic and 5,026 subjects with T2DM. Although most studies showed increased BMI none except one showed significantly association between the -174 C allele and BMI. Stratified analysis for T2DM status did not reveal a significant p-value either. However, non-diabetic men (n= 11,457) with

a -174 C allele were significantly associated with higher BMI (C dominant: β= 0.185, p= 0.01). There was no evidence for an association between -174 C allele and IL-6 levels analyzing 5,659 subjects of seven studies. Only among 966 subjects with T2DM from four studies -174 CC-genotype was associated with higher IL-6 levels (β_{CCvsGG}= 0.16, [95%CI]= [0.004, 0.31], adjusted for age, sex, and BMI), whereas there was no evidence for an association of heterozygous individuals with T2DM (β_{GCvsGG}= 0.004, [95%CI]= [0.10,0.11]). Sex-specific association estimates were similar, although they were non significant due to reduced sample size.

5.3.2 Apolipoprotein A5 (APOA5) (Grallert et al. 2007)

Covering the *APOA5* locus, ten polymorphisms were investigated for association with lipid parameters and quantitative traits related to NCEP-defined MetS in KORA S4 fasting subjects aged 55-74 years. An overview of the polymorphism locations within *APOA5* locus and localization within the *APOA1/C3/A4* cluster is given in Figure 7 A. An LD plot of the ten polymorphisms analyzed in KORA S4 is presented in Figure 7 B.

Primary analysis of lipids

Triglyceride levels were significantly different between the three genotype groups of variants 1259T>C, -3A>G, -1131T>C, and 476G>A (p≤ 0.001), and borderline significantly different for c.56C>G. Table 16 presents the results for -1131T>C and c.56C>G. Variant -1131T>C is reported as representative as it was highly correlated with -3A>G (r^2= 0.98), 476G>A (r^2= 0.95) and 1259T>C (r^2= 0.84) (Figure 7 B). In the additive model, variant -1131T>C showed a statistically significant association with 13.30% (p= 5.0×10^{-4}) higher TG levels per copy of the minor allele for -1131T>C. Variant c.56C>G showed a borderline significant difference for TG levels between genotype groups (p= 0.009, f-test). β-estimates were in the same range as for the variants described above, but were insignificant after correction for multiple testing (additive model: % change of geometric mean (PCGM)= 12.3%, p= 0.005). No associations were observed with total, LDL and HDL cholesterol.

Variants -1131T>C, 1259T>C (data not shown) and c.56C>G were selected for replication. The observed associations of these variants with lipid levels were clearly replicated in the SAPHIR sample (Table 16B). The minor allele of variant c.56C>G was significantly associated with higher TG levels in the dominant model (PCGM= 22.08%, p= 6.7×10^{-7}). Minor alleles of both variants were significantly associated with further lipid parameters (Table 16B).

Haplotype analysis

Haplotype analysis performed in the KORA study revealed six haplotypes with frequencies of >5% (Table 17). The major haplotype was used as reference. Haplotype APOA5_3, containing the minor alleles of variants -3A>G, -1131T>C, 476G>A, and 1259T>C, was significantly associated with higher TG levels (p= 0.002). Haplotype APOA5_5, tagged by variant c.56C>G,

was also significantly associated with higher TG levels (PCGM= 15.2%, p= 0.003). This association was stronger in women (PCGM= 22.5%, p= 0.002) than in men.

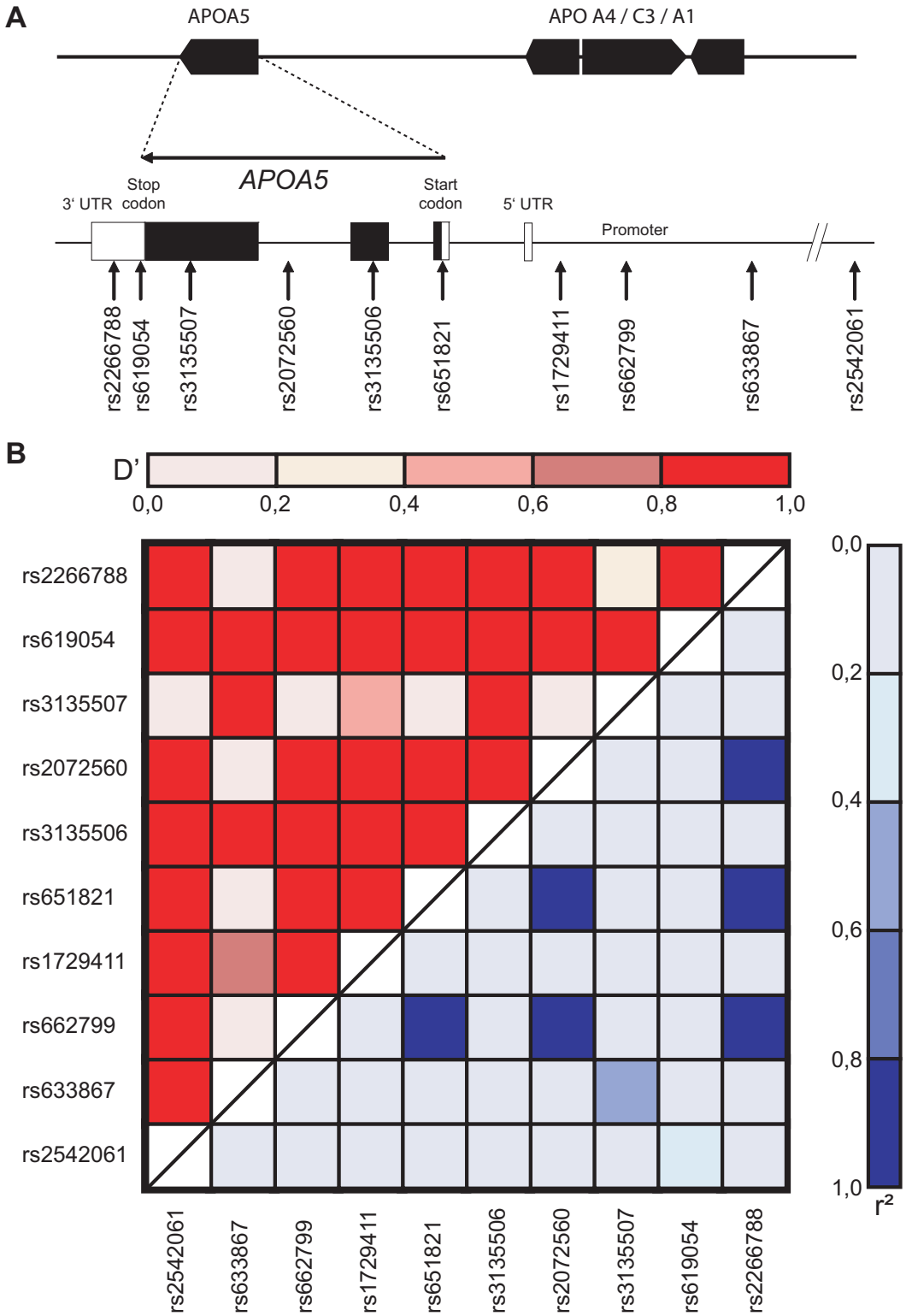

Figure 7: (A) Genomic structure and variant location within the *APOA5* gene. (B) LD plot of the analyzed variants in the KORA S4 sample. The upper triangle depicts the recombination (D') and the lower triangle the correlation coefficient (r^2) between the variants. (chr: 11q23; length: 2,499 bp; aa: 366)

Table 16: Primary analysis in KORA S4 (A) and SAPHIR (B). Estimated associations in linear regression models between the *APOA5* polymorphisms and lipid parameters.

				aa vs. AA[c]			Aa vs. AA[c]			additive			dominant		
A) KORA S4		n	p[b]	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p
-1131T>C	Triglycerides [%] [a]	1163	**8.3×10^{-4}**	54.23	[16.02, 105.04]	0,003	10.29	[1.9, 19.36]	0.015	13.30	[5.62, 21.54]	**5.0×10^{-4}**	12.66	[4.31, 21.67]	0.002
	HDL cholesterol [mmol/l]	1171	0.486	-0.02	[-0.26, 0.21]	0.847	-0.04	[-0.11, 0.03]	0.233	-0.03	[-0.09, 0.02]	0.259	-0.04	[-0.1, 0.02]	0.232
	LDL cholesterol [mmol/l]	1170	0.051	0.60	[-0.07, 1.26]	0.079	0.16	[-0.02, 0.33]	0.081	0.18	[0.03, 0.34]	0.021	0.18	[0.01, 0.35]	0.038
	Total cholesterol [mmol/l]	1172	0.008	0.85	[0.19, 1.51]	0.012	0.18	[0, 0.36]	0.054	0.23	[0.07, 0.4]	0.005	0.22	[0.04, 0.4]	0.015
c.56C>G	Triglycerides [%] [a]	1108	0.009	-0.71	[-33.69, 48.69]	0.973	14.72	[5.15, 25.15]	0.002	12.31	[3.61, 21.75]	0.005	14.07	[4.72, 24.25]	0.003
	HDL cholesterol [mmol/l]	1116	0.424	0.03	[-0.3, 0.37]	0.844	-0.05	[-0.12, 0.02]	0.197	-0.04	[-0.1, 0.03]	0.268	-0.04	[-0.11, 0.03]	0.221
	LDL cholesterol [mmol/l]	1115	0.230	0.05	[-0.84, 0.94]	0.916	0.17	[-0.02, 0.36]	0.087	0.15	[-0.03, 0.32]	0.107	0.16	[-0.03, 0.35]	0.090
	Total cholesterol [mmol/l]	1116	0.048	0.09	[-0.85, 1.02]	0.856	0.25	[0.05, 0.45]	0.014	0.22	[0.03, 0.41]	0.020	0.25	[0.05, 0.44]	0.015
B) SAPHIR															
-1131T>C	Triglycerides [%] [a]	1596	**1.0×10^{-6}**	4.17	[-33.61, 63.46]	0.859	22.68	[13.7, 32.37]	**1.5×10^{-7}**	20.51	[12.09, 29.57]	**5.1×10^{-7}**	22.19	[13.34, 31.73]	**2.0×10^{-7}**
	HDL cholesterol [mmol/l]	1596	**0.001**	-0.31	[-0.62, -0.01]	0.046	-0.08	[-0.13, -0.03]	**0.002**	-0.09	[-0.14, -0.04]	**3.8×10^{-4}**	-0.09	[-0.14, -0.04]	**7.6×10^{-4}**
	LDL cholesterol [mmol/l]	1596	0.040	0.01	[-0.82, 0.84]	0.984	0.18	[0.04, 0.32]	**0.011**	0.16	[0.03, 0.3]	0.016	0.18	[0.04, 0.32]	**0.012**
	Total cholesterol [mmol/l]	1596	**0.011**	-0.25	[-1.16, 0.65]	0.581	0.23	[0.08, 0.38]	**0.003**	0.20	[0.05, 0.34]	**0.009**	0.22	[0.07, 0.37]	**0.005**
c.56C>G	Triglycerides [%] [a]	1630	**3.0×10^{-6}**	3.94	[-42.05, 86.41]	0.897	21.25	[12.53, 30.64]	**4.6×10^{-7}**	20.05	[11.64, 29.08]	**8.9×10^{-7}**	22.08	[12.88, 32.03]	**6.7×10^{-7}**
	HDL cholesterol [mmol/l]	1630	0.057	0.06	[-0.33, 0.46]	0.756	-0.06	[-0.11, -0.01]	0.018	-0.06	[-0.11, -0.01]	0.026	-0.06	[-0.11, -0.01]	0.021
	LDL cholesterol [mmol/l]	1630	0.042	1.11	[0.04, 2.18]	0.043	0.11	[-0.03, 0.24]	0.124	0.13	[0, 0.27]	0.050	0.12	[-0.01, 0.26]	0.080
	Total cholesterol [mmol/l]	1630	0.010	1.26	[0.1, 2.42]	0.034	0.17	[0.02, 0.32]	0.027	0.19	[0.05, 0.34]	**0.009**	0.18	[0.03, 0.33]	0.016

[a] β-values of logarithmized variables (TG levels and fasting insulin) are given as geometric mean change estimated by (EXP(log(variable))-1)*100; [b] p-value from f-test [c] -1131T>C: AA=AA, Aa=GA, aa=GG, number of minor allele homozygote in KORA n=13, in SAPHIR n=6; c.56C>G AA=GG, Aa=GC, aa=CC, number of minor allele homozygote in KORA n=16, in SAPHIR n=3; significant p-values are printed in bold; corrected significance level: KORA ($p< 0.0017$), SAPHIR ($p< 0.0125$)

Table 17: Haplotype analysis for triglyceride levels in KORA S4.

Haplotypes	-9655A>G	-4904C>T	-1131T>C	-1099G>A	-3A>G	c.56C>G	476G>A	c.457G>A	1764C>T	1259T>C	total				men				women			
											freq	PCGM [%]	95 % CI [%]	p	freq	PCGM [%]	95 % CI [%]	p	freq	PCGM [%]	95 % CI [%]	p
APOA5_01	2	1	1	1	1	1	1	1	2	1	0.26	1.91	[-3.68, 7.83]	0.511	0.26	-2.69	[-10.79, 6.14]	0.538	0.26	6.11	[-1.17, 13.93]	0.103
APOA5_02	2	1	1	1	1	1	1	1	1	1	0.15	-2.66	[-8.93, 4.05]	0.429	0.15	-7.37	[-16.33, 2.54]	0.141	0.14	0.98	[-7.24, 9.93]	0.822
APOA5_03	2	1	**2**	1	**2**	1	**2**	1	1	**2**	0.06	14.96	[5.4, 25.38]	**0.002**	0.06	15.89	[1.68, 32.09]	0.028	0.06	15.00	[2.78, 28.68]	0.015
APOA5_04	1	1	1	2	1	1	1	1	1	1	0.13	6.60	[-0.6, 14.32]	0.074	0.13	9.94	[-1.27, 22.43]	0.085	0.13	1.98	[-6.65, 11.41]	0.665
APOA5_05	1	1	1	1	1	**2**	1	1	1	1	0.06	15.24	[4.91, 26.59]	**0.003**	0.06	10.49	[-3.47, 26.46]	0.148	0.05	22.54	[7.85, 39.24]	**0.002**
Rare	*	*	*	*	*	*	*	*	*	*	0.05	9.62	[-2.08, 22.73]	0.111	0.05	6.74	[-10.23, 26.92]	0.461	0.04	12.34	[-1.92, 28.67]	0.094
Reference	1	1	1	1	1	1	1	1	1	1	0.30				0.28				0.31			

Triglyceride levels are given as geometric mean change, estimated by (EXP(log(variable))-1)*100. Highly correlated variants are highlighted in grey. Significance level for haplotype analysis corrected for multiple testing was p=0.0125. Haplotype tagging variants and statistically significant values are shown in bold. freq= frequency; PCGM = percent change of geometric mean

Secondary analysis – MetS and its features

Results of the secondary analysis are presented in Table 18. In both samples, no significant associations of gene variants with waist circumference, fasting plasma glucose, 2 h plasma glucose, systolic or diastolic blood pressure, and fasting insulin were found (p> 0.05). In the SAPHIR sample uric acid was different between the genotype groups of variant -1131T>C (p= 0.015). The CC genotype was associated with lower uric acid levels (β= -1.31 mg/dl, p= 0.006). After correction for multiple testing, this observation was no longer significant (correction for 10 of traits and 2 effective loci in SAPHIR p= 0.05/10*2= 0.0025).

348 KORA S4 subjects (26%) and 502 SAPHIR subjects (28%) fulfilled the NCEP criteria for MetS. Comparing these individuals with MetS-free subjects, variant c.56C>G showed increased OR estimated for MetS for the minor allele in the additive model (KORA S4 OR [95% CI]= 1.43 [1.04, 1.99] p=0.03; SAPHIR OR [95% CI]= 1.48 [1.10, 1.99] p=0.009). According to corrected significance levels association with MetS loses significance in KORA but remains significant in SAPHIR. For minor alleles of variants -4904C>T, and c.457G>A, which were not in LD with any other variant, ORs indicated a tendency for increased MetS risk (-4904C>T OR [95% CI]= 1.48 [0.87, 2.54] p=0.12; c.457G>A 1.33 [0.85, 2.08] p=0.21). Variant -1131T>C indicated a tendency for higher MetS risk in SAPHIR only (KORA S4 OR [95% CI]= 1.00 [0.75, 1.34] p= 0.99; SAPHIR OR [95% CI]= 1.24 [0.92, 1.68] p= 0.16). No tendency was observable for any other variant. Combining samples variant -1131T>C had an OR [95% CI] of 1.03 [0.84, 1.27] (p= 0.77) and variant c.56C>G an OR [95% CI] of 1.28 [1.03, 1.58] (p= 0.026). Subjects heterozygous in both variants showed insignificant OR of 2.11 [1.00,4.49] p=0.05.

Table 18: Secondary analysis in KORA (A) and SAPHIR (B). Estimated associations in linear regression models between the *APOA5* polymorphisms and quantitative traits related to the MetS.

				aa vs. AA[c]			Aa vs. AA[c]			additive			dominant		
A) KORA S4		n	p[b]	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p
-1131T>C	Waist circumference [cm]	1328	0.818	-0.21	[-2.99, 2.58]	0.884	-0.25	[-1.03, 0.53]	0.534	-0.21	[-0.91, 0.48]	0.544	-0.25	[-1.00, 0.51]	0.527
	Uric acid [mg/dl]	1328	0.197	0.62	[-0.05, 1.29]	0.072	0.00	[-0.18, 0.19]	0.962	0.07	[-0.09, 0.24]	0.380	0.04	[-0.14, 0.23]	0.638
	Fasting plasma glucose [mg/dl]	1325	0.366	-4.76	[-13.9, 4.39]	0.308	1.24	[-1.33, 3.81]	0.344	0.40	[-1.88, 2.68]	0.734	0.84	[-1.66, 3.35]	0.508
	2 h plasma glucose [mg/dl]	1324	0.624	-12.50	[-39.62, 14.63]	0.366	1.24	[-6.38, 8.87]	0.749	-0.56	[-7.31, 6.20]	0.871	0.28	[-7.13, 7.69]	0.941
	Systolic BPR [mmHg]	859	0.570	0.45	[-12.24, 13.15]	0.944	2.06	[-1.76, 5.88]	0.290	1.59	[-1.73, 4.91]	0.348	1.94	[-1.75, 5.63]	0.302
	Diastolic BPR [mmHg]	859	0.555	-2.70	[-9.25, 3.86]	0.420	0.70	[-1.27, 2.67]	0.488	0.17	[-1.55, 1.89]	0.846	0.45	[-1.46, 2.35]	0.647
	Fasting insulin [%] [a]	1319	0.177	-19.68	[-41.6, 10.48]	0.178	5.82	[-3.27, 15.78]	0.217	1.79	[-6.01, 10.23]	0.664	3.90	[-4.80, 13.41]	0.391
c.56C>G	Waist circumference [cm]	1273	0.893	0.35	[-7.11, 7.82]	0.926	-0.42	[-2.18, 1.34]	0.643	-0.17	[-0.95, 0.60]	0.661	-0.38	[-2.10, 1.34]	0.665
	Uric acid [mg/dl]	1268	0.283	-0.59	[-1.47, 0.30]	0.193	0.09	[-0.12, 0.30]	0.382	0.03	[-0.17, 0.22]	0.794	0.06	[-0.14, 0.26]	0.558
	Fasting plasma glucose [mg/dl]	1266	0.530	4.87	[-7.21, 16.95]	0.429	-1.13	[-3.98, 1.71]	0.435	-0.51	[-3.11, 2.09]	0.701	-0.85	[-3.64, 1.94]	0.549
	2 h plasma glucose [mg/dl]	1265	0.907	-6.59	[-42.15, 28.97]	0.716	-1.12	[-9.50, 7.26]	0.793	-1.50	[-9.16, 6.16]	0.701	-1.38	[-9.58, 6.82]	0.742
	Systolic BPR [mmHg]	821	0.853	-4.64	[-22.46, 13.17]	0.609	0.44	[-3.42, 4.30]	0.823	0.02	[-3.55, 3.60]	0.990	0.24	[-3.55, 4.03]	0.902
	Diastolic BPR [mmHg]	821	0.643	-0.34	[-9.59, 8.91]	0.943	-0.96	[-2.96, 1.05]	0.348	-0.84	[-2.7, 1.02]	0.375	-0.93	[-2.90, 1.04]	0.352
	Fasting insulin [%] [a]	1260	0.402	-1.15	[-34.95, 50.21]	0.957	7.02	[-3.04, 18.11]	0.178	5.64	[-3.46, 15.61]	0.232	6.62	[-3.19, 17.43]	0.194
B) SAPHIR															
-1131T>C	Waist circumference [cm]	1637	0.566	0.49	[-4.6, 5.58]	0.850	-0.50	[-1.43, 0.44]	0.296	-0.41	[-1.30, 0.47]	0.359	-0.47	[-1.39, 0.45]	0.318
	Uric acid [mg/dl]	1673	0.015	-1.31	[-2.24, -0.37]	0.006	0.08	[-0.09, 0.25]	0.378	0.00	[-0.16, 0.16]	0.956	0.04	[-0.13, 0.20]	0.663
	Fasting plasma glucose [mg/dl]	1646	0.876	-1.95	[-12.43, 8.53]	0.715	0.35	[-1.57, 2.26]	0.723	0.20	[-1.61, 2.01]	0.830	0.28	[-1.61, 2.17]	0.771
	2 h plasma glucose [mg/dl]	621	0.236	-33.91	[-73.23, 5.41]	0.091	0.42	[-7.54, 8.38]	0.917	-1.90	[-9.36, 5.56]	0.617	-0.75	[-8.60, 7.10]	0.851
	Systolic BPR [mmHg]	1318	0.511	3.66	[-5.73, 13.06]	0.445	-0.82	[-2.72, 1.07]	0.394	-0.47	[-2.25, 1.31]	0.605	-0.67	[-2.54, 1.20]	0.483
	Diastolic BPR [mmHg]	1318	0.461	2.34	[-3.61, 8.30]	0.440	-0.59	[-1.79, 0.62]	0.340	-0.35	[-1.48, 0.77]	0.542	-0.48	[-1.67, 0.70]	0.423
	Fasting insulin [%] [a]	1642	0.433	-5.71	[-34.97, 36.72]	0.757	4.42	[-2.43, 11.76]	0.212	3.57	[-2.87, 10.44]	0.284	4.12	[-2.63,11.34]	0.238
c.56C>G	Waist circumference [cm]	1674	0.397	7.25	[-5.90, 20.39]	0.280	0.70	[-0.93, 2.34]	0.399	0.86	[-0.73, 2.46]	0.288	0.79	[-0.84, 2.42]	0.341
	Uric acid [mg/dl]	1710	0.250	1.01	[-0.31, 2.33]	0.134	0.06	[-0.10, 0.23]	0.452	0.09	[-0.07, 0.25]	0.286	0.08	[-0.09, 0.24]	0.365
	Fasting plasma glucose [mg/dl]	1683	0.499	3.04	[-11.08, 17.17]	0.673	-0.99	[-2.75, 0.78]	0.274	-0.85	[-2.57, 0.88]	0.335	-0.93	[-2.69, 0.83]	0.299
	2 h plasma glucose [mg/dl]	646	0.916	-12.91	[-81.99, 56.16]	0.714	0.80	[-7.19, 8.80]	0.844	0.46	[-7.36, 8.27]	0.908	0.65	[-7.31, 8.60]	0.873
	Systolic BPR [mmHg]	1346	0.809	5.23	[-17.66, 28.11]	0.654	-0.44	[-2.29, 1.41]	0.640	-0.37	[-2.20, 1.46]	0.693	-0.41	[-2.26, 1.44]	0.664
	Diastolic BPR [mmHg]	1346	0.875	0.55	[-14.01, 15.11]	0.941	-0.31	[-1.48, 0.87]	0.609	-0.29	[-1.46, 0.87]	0.621	-0.30	[-1.48, 0.87]	0.614
	Fasting insulin [%] [a]	1675	0.527	31.15	[-22.88, 123.06]	0.317	1.88	[-4.68, 8.88]	0.585	2.53	[-3.90, 9.39]	0.448	2.21	[-4.33, 9.20]	0.515

Description Table 18: [a] β-values of logarithmized variables (TG levels and fasting insulin) are given as geometric mean change estimated by (EXP(log(variable))-1)*100; [b] p-value from f-test [c] -1131T>C: AA=AA, Aa=GA, aa=GG, number of minor allele homocygotes in KORA n=13, in SAPHIR n=6; c.56C>G AA=CC, Aa=CG, aa=GG, number of minor allele homocygotes in KORA n=16, in SAPHIR n=11; significant p-values are presented in bold; significance level if corrected for multiple testing: KORA (p<0.0007), SAPHIR (p<0.005).

5.3.3 MCP-1 (Sedlmeier, Grallert et al. 2007)

Eight polymorphic SNPs covering the *MCP-1* locus (Figure 8) were investigated for association with IDF-defined MetS, T2DM in all or with related quantitative traits in fasting KORA S4 subjects aged 55-74 years. Several trends were observed for different SNPs (Table 19). SNP c.-3813C>T showed difference in the genotype groups for TG levels (p= 0.0084), 2-h glucose (p= 0.014), and uric acid (p= 0.027). Differences between genotype groups were further observed for c.-928G>C and fasting glucose (p= 0.033), c.2138A>T and IL-6 levels (p= 0.044), c.105T>C and leukocyte count (p= 0.047), c.*65C>T, c.*3879C>T and MCP-1 levels (p= 0.012 each). Analyzing single components of BMI distribution of height was different between genotype groups of c.-3813C>T and c.-928G>C. Stratification for sex revealed a significant association between c.-928 C allele and increased height (p= 0.0004). Haplotype analysis estimated five major haplotypes whereas MCP1*1 containing the c.-928 C allele was significantly associated with increased height in men (β= 1.30 cm, p= 0.0002).

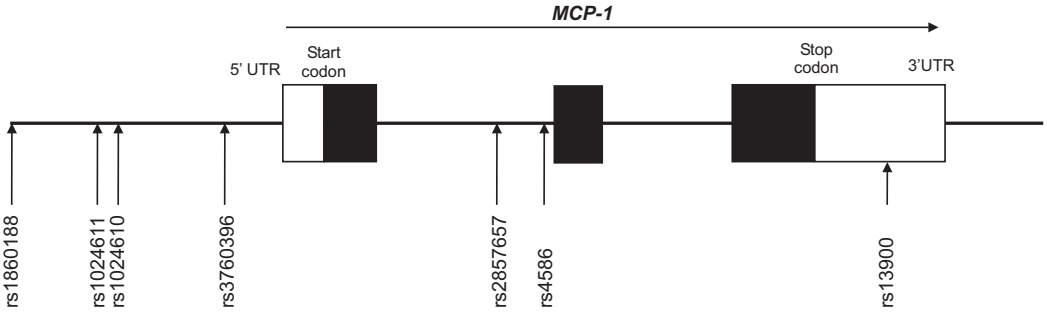

Figure 8: Genetic structure of MCP-1 locus (chr: 17q11.2; length: 1927 bp; aa: 99)

Table 19: Global P values for all single nucleotide polymorphisms and parameters from nominal analysis

	c.-3813C>T		c.2138A>T		c.-928G>C		c.77-109C>G		c.105T>C		c.*65C>T		c.*3879C>T	
Parameter	n	p	n	p	n	p	n	p	n	p	n	p	n	p
MetS†	841/665	0.12	874/683	0.59	872/682	0.97	872/683	0.46	833/667	0.44	866/677	0.71	873/687	0.64
Waist circumference	1167	0.59	1211	0.79	1210	0.97	1209	0.76	1166	0.35	1198	0.7	1214	0.75
Triglycerides[a]	1153	0.0084	1196	0.41	1195	0.34	1194	0.43	1151	0.052	1183	0.34	1199	0.41
HDL cholesterol[a]	1163	0.44	1206	0.2	1205	0.79	1204	0.19	1161	0.069	1193	0.59	1209	0.71
Sys. BPR[a]	770	0.27	791	0.34	790	0.2	790	0.34	764	0.8	782	0.079	794	0.068
Dia. BPR[a]	770	0.27	791	0.7	790	0.64	790	0.62	764	0.29	782	0.11	794	0.1
T2DM†	222/1163	0.23	227/1207	0.46	225/1206	0.089	227/1205	0.46	214/1163	0.83	226/1194	0.93	227/1210	0.67
Fasting glucose[a]	1165	0.088	1208	0.27	1207	0.033	1206	0.19	1163	0.3	1195	0.94	1211	0.91
Parameters related to the metabolic syndrome														
BMI	1165	0.68	1208	0.51	1207	0.88	1206	0.45	1163	0.26	1195	0.83	1211	0.86
Weight	1165	0.96	1208	0.59	1207	0.57	1206	0.53	1163	0.35	1195	0.39	1211	0.37
Height	1167	0.14	1211	0.075	1210	0.0024	1209	0.087	1166	0.0095	1198	0.098	1214	0.063
WHR	1167	0.087	1211	0.65	1210	0.37	1209	0.68	1166	0.63	1198	0.28	1214	0.34
Hip circumference	1166	0.75	1210	0.73	1209	0.67	1208	0.67	1165	0.065	1197	0.53	1213	0.52
Percent body fat	1157	0.49	1200	0.87	1199	0.63	1198	0.75	1156	0.064	1187	0.87	1203	0.8
Adiponectin[a]	1154	0.14	1197	0.78	1196	0.14	1195	0.75	1153	0.1	1184	0.73	1200	0.84
LDL cholesterol[a]	1162	0.96	1205	0.82	1204	0.25	1203	0.81	1160	0.77	1192	0.83	1208	0.69
Total cholesterol[a]	1164	0.92	1207	0.61	1206	0.63	1205	0.6	1162	0.84	1194	0.58	1210	0.56
2 h glucose[a]	1165	0.014	1208	0.56	1207	0.46	1206	0.56	1163	0.68	1195	0.47	1211	0.47
HbA1c[a]	1164	0.38	1207	0.97	1206	0.71	1205	0.96	1162	0.88	1194	0.67	1210	0.82
Insulin[a]	1161	0.93	1204	0.74	1203	0.26	1202	0.79	1159	0.82	1191	0.73	1207	0.81
HOMA-IR*	1164	0.99	1208	0.98	1207	0.44	1206	0.98	1163	0.78	1195	0.91	1211	0.88
Leucocyte count[a]	1165	0.71	1208	0.37	1207	0.97	1206	0.36	1163	0.047	1195	0.47	1211	0.53
MCP-1[a]	454	0.78	479	0.38	478	0.32	477	0.39	453	0.93	476	0.012	480	0.012
IL-6*	1161	0.19	1205	0.044	1204	0.47	1203	0.08	1160	0.77	1192	0.82	1208	0.84
Uric acid[a]	1165	0.027	1208	0.43	1207	0.086	1206	0.44	1163	0.34	1195	0.18	1211	0.29

P = global p value calculated in the model free model; sys, systolic; dia, diastolic; all variables are adjusted for sex and age, [a]BMI; *p values from Kruskal Wallis test; †numbers are given for cases/controls, p values originate from logistic regression; p values below 0.05 are grey shaded

5.3.4 RETN (Sedlmeier, Grallert et al. submitted)

Covering the *RETN* locus nine SNPs were investigated (Figure 9) comparing MetS or T2DM affected subjects with healthy subjects in the whole sample or parameters related to MetS or T2DM in fasting KORA S4 subjects aged 55-74 years. SNP rs3219176, which was monomorphic, and rs1423096 which violated HWE were excluded from statistical analysis. TG were statistically significantly different between the three genotype groups of rs3760678 (p=0.0003) in the entire group (Table 20). The rs3760678 genotypes or alleles in any genetic model did not differ statistically significantly according to a significance level of 0.00055. TG levels differed up to 19% with p values ranging from 0.0009 to 0.1 for nominal, dominant, recessive, and additive models. No statistically significant results were observed for TG levels or any other parameters analyzed in men and women separately. None of the other analyzed parameters exhibited significant differences between genotype groups in model-free analysis of the entire population (p>0.010).

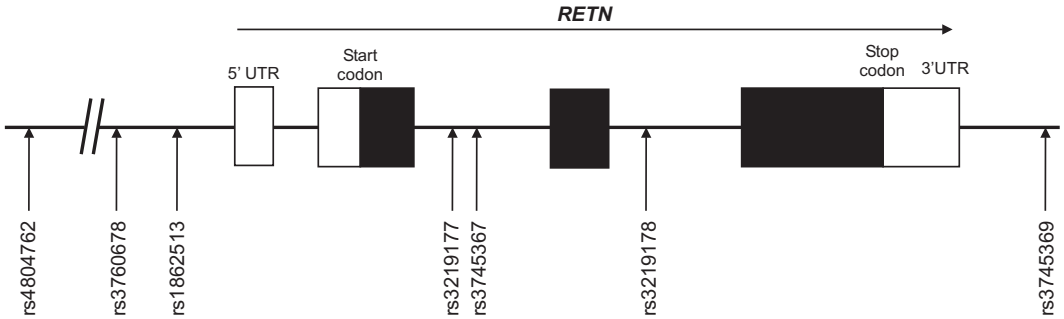

Figure 9: Genetic structure of the *RETN* locus (chr: 19p13.3; length: 1369; aa: 108)

Table 20: Global p values for all RETN SNPs and parameters from model free analysis

	rs4804762		rs3760678		rs1862513		rs3219177		rs3745367		rs3219178		rs3745369	
	n	p	n	p	n	p	n	p	n	p	n	p	n	p
MetS‡	731/611	0.63	779/638	0.36	777/635	0.49	772/637	0.62	766/634	0.34	768/635	0.26	782/632	0.076
T2DM‡	204/1145	0.011	226/1198	0.77	225/1194	0.052	225/1191	0.064	219/1188	0.017	224/1186	0.33	226/1195	0.63
Waist circumference*	1247	0.78	1315	0.21	1309	0.62	1307	0.41	1301	0.65	1302	0.78	1313	0.13
Triglycerides[a]†	1090	0.6	1147	**0.0003**	1143	0.078	1143	0.01	1136	0.077	1139	0.41	1146	0.62
HDL cholesterol[a]	1098	0.28	1155	0.073	1151	0.53	1151	0.38	1144	0.77	1147	0.91	1153	0.57
Pulse pressure[c]	811	0.66	848	0.75	844	0.77	847	0.42	841	0.12	841	0.7	844	0.6
Fasting glucose[b]	1240	0.45	1307	0.79	1301	0.31	1299	0.84	1294	0.53	1294	0.14	1305	0.2
BMI*	1243	0.64	1310	0.18	1304	0.78	1302	0.58	1297	0.95	1297	0.92	1308	0.12
WHR*	1247	0.82	1315	0.53	1309	0.43	1307	0.1	1301	0.15	1302	0.24	1313	0.33
Percent Body fat*	1233	0.93	1299	0.037	1293	0.56	1291	0.12	1286	0.39	1286	0.82	1297	0.033
LDL cholesterol	1097	0.94	1154	0.84	1150	0.47	1150	0.019	1143	0.062	1146	0.4	1152	0.53
Total cholesterol	1099	0.7	1156	0.68	1152	0.43	1152	0.012	1145	0.016	1148	0.55	1154	0.6
2-hour glucose	1240	0.41	1307	0.66	1301	0.55	1299	0.68	1294	0.44	1294	0.78	1305	0.2
HbA1c	1239	0.43	1306	0.3	1300	0.19	1298	0.22	1293	0.36	1293	0.21	1304	0.48
Fasting insulin†	1235	0.17	1302	0.95	1296	0.49	1294	0.36	1290	0.12	1289	0.62	1300	0.65
Leukocytes	1243	0.6	1310	0.61	1304	0.66	1302	0.49	1297	0.38	1297	0.16	1308	0.18
MCP-1†	530	0.92	589	0.059	583	0.055	579	0.13	579	0.053	578	0.89	588	0.97
Uric acid	1243	0.62	1310	0.39	1304	0.84	1302	0.31	1297	0.64	1297	0.71	1308	0.99

p = global p values calculated in a model free analysis; all variables are adjusted for sex, age and BMI, *) only adjusted for sex and age; †) p values calculated by Kruskal Wallis test, ‡) numbers are given for case/controls, p values calculated by logistic regression; subjects with [a]) lipid lowering drugs, [b]) antidiabetic drugs and [c]) antihypertensive drugs are excluded; p values below 0.00055 are grey shaded

5.3.5 TCF7L2 (Marzi, Huth, Kolz, Grallert et al. 2007)

In the *TCF7L2* locus two SNPs in high LD (D' = 0.88, r^2 = 0.86) were analyzed in one case-control sample including 2,369 participants from KORA S1-S4, and one population-based sample of 1,404 elderly KORA S4 participants. The analysis was designed to replicate the priviously reported association with T2DM in the German population and to enhance analysis to related parameters of T2DM and MetS. Frequencies of the minor alleles were similar to those previously reported with 0.27 for rs12255372 and 0.28 for rs7903146, with difference in T2DM cases in the case-control sample (0.33 for rs12255372 and 0.34 for rs7903146). Multiple analyses in the case-control sample identified the T alleles at both SNPs as risk variants for T2DM, with significant associations for model-free estimates and the multiplicative genotype model. The results demonstrated a copy-number effect and provided good fit for the multiplicative genotype model, the OR for T2DM being higher for homozygous than for heterozygous T allele carriers at rs7903146 (model-free estimates: $OR_{TTvs.CC}$ [95 % CI]= 1.92 [1.38, 2.67], p= 0.0001; $OR_{CTvs.CC}$ [95 % CI]= 1.33 [1.09, 1.62], p= 0.006; multiplicative model: $OR_{Tvs.C}$ [95 % CI]= 1.36 [1.18, 1.58], p= 0.00003). Similar results were observed for rs12255372 (model-free estimates: $OR_{TTvs.GG}$ [95 % CI]= 2.00 [1.43, 2.80], p= 0.00005; $OR_{GTvs.GG}$ [95 % CI]= 1.16 [0.95, 1.41], p = 0.14; multiplicative model: OR_{TvsG} [95 % CI]= 1.31 [1.13,1.51], p= 0.0003). Analyses were adjusted for sex, age, and BMI. No major differences between adjusted and unadjusted results as well as no associations for interactions between these covariates and the analyzed genotypes were detected.

Regarding association of *TCF7L2* variants and MetS analyses were performed within the population-based sample. Neither of the two SNPs was statistically significantly associated with MetS according to the multiplicative genotype model. Analyses on associations between the SNPs and quantitative parameters related to MetS revealed an inverse association rs7903146 T allele with log-transformed HOMA- % B (β= -0.07, p= 0.005) as a measure of basal insulin secretion, and log-transformed fasting insulin (β= -0.06, p= 0.02).

5.3.6 Genes from extern cooperation

Following genes were selected in the group of Frank Döring, University of Kiel, Germany. All genes were selected from fat assimilation pathways and were resequenced to detect new SNPs. SNPs that were significantly associated with T2DM or related parameters in the EPIC-Potsdam study were replicated in the elderly KORA S4 participants within this work. Thus, results of analyses of these genes shall be briefly summarized below (5.2.6.1 and 5.2.6.2).

5.3.6.1 ACBP (Fischer et al. 2007)

Resequencing of the *ACBP* locus in 47 unrelated subjects confirmed eight known common SNPs, but did not detect any new SNPs. Four of these SNPs and additionally four validated SNPs from NCBI dbSNP database were considered for genotyping in 192 incident T2DM

patients and 384 controls of the EPIC-Potsdam cohort. SNP rs2084202 was significantly associated with decreased risk of T2DM OR [95%CI]= 0.63 [0.41, 0.96] in the dominant inheritance model calculated. For rs8192506, the heterozygote genotype was significantly associated with increased risk of T2DM in an unadjusted model OR [95%CI]= 1.89 [1.03, 3.47]. No association was found with BMI, WHR, waist circumference, percent body fat, and HbA1c for rs2084202 and rs8192506. Both SNPs were replicated in the elderly KORA S4 comparing 226 T2DM cases with 863 normoglycemic subjects. SNP rs2084202 showed a borderline association with decreased risk of T2DM (OR [95%CI]= 0.72 [0.51, 1.01]).

5.3.6.2 PTGES2 (Nitz et al. 2007)

The *PTGES2* locus was sequenced in 94 unrelated subjects. One coding SNP (rs13283456, C>T) and a previously unknown SNP g.-417G>T in the promoter region were identified. SNP rs13283456 results in an arginine (Arg) to histidine (His) change at position 298 (NP_079348) of PTGES2. These SNPs and additionally three SNPs from CEPH HapMap database were genotyped in 192 incident T2DM patients and 384 controls of the EPIC-Potsdam study. The His allele at position 298 showed significantly lower risk of T2DM (OR [95%CI]= 0.63 [0.41, 0.97], p= 0.04) in the adjusted model. Polymorphisms rs4837240, g,-417G>T, and rs10987883 were not associated with the disease (data not shown).

SNP rs13283456 was investigated for verification in KORA S4 comparing 239 subjects with IGT and 226 subjects with T2DM with 863 normoglycemic controls. Individuals with the minor allele, had decreased risk for developing IGT and T2DM (OR [95%CI]= 0.68 [0.50, 0.93], p= 0.007 and OR [95%CI]= 0.61 [0.43, 0.86], p= 0.004). Investigation of phenotypes related to T2DM revealed lower HOMA-%B (p= 0.036) as a measure of basal insulin secretion in minor allele carriers of rs13283456, but for control subjects only. These subjects showed insignificant lower HOMA-IR (0.086), too.

6. Discussion

MetS and T2DM are closely related complex diseases. Despite environmental influences genetic components are involved in both. Due to the close coherences genetic susceptibilities are overlapping. However, genetic influences can be hardly detected in complex diseases and candidate gene approach provides possibilities to cover only a small part of the manifold genetic susceptibilities. Thus, analysis was focused on several genes from different pathways involved in development of MetS or T2DM.

6.1 Association Analysis

6.1.1 Genotyping

For genotyping of SNPs several methods are available. GSF developed an enormous device pool within the Genome Analysis Center (GAC) providing possibility of high throughput genotyping analyzing 300,000 genotypes per day with MALDI-TOF MS by Sequenom. During this work Sequenom advanced cost/performance ratio by increased plexing possibilities. While initial genotyping was limited to 13 SNPs per probe later trails could access assays with up to 28 and 40 SNPs per probe. In the analysis of *IL-6* genotypes were additionally quality checked by the TaqMan method. This test revealed no relevant discordant rate between the two genotyping methods. Thus, further genotyping analysis was based on MALDI-TOF MS only. With a discordance rate of <0.5% within routine duplicates MALDI-TOF MS personates a reliable genotyping method.

6.1.2 IL-6

IL-6 is a multifunctional cytokine which is produced not only in monocytes and macrophages but in several cell types including most cells of the immune system, chondrocytes, osteoblasts, skeletal and smooth muscle cells, hepatocytes, islet beta cells and adipocytes (Kamimura et al. 2003). This variety of cell types secreting IL-6 indicates the complexity of IL-6 function and regulation. IL-6 plays a central role in host defense as it functions as a main regulator of the acute phase response (Fey et al. 1990). Besides further functions in the immune, nervous and endocrine systems, IL-6 is involved in bone metabolism, hematopoiesis and cancer (Kamimura et al. 2003). As the receptor subunit gp130 is widely expressed, deregulated high-level production of IL-6 combined with its agonistic soluble receptor sIL-6R may induce an undesired inflammatory state in many organs and could thus cause various diseases.

Two promoter polymorphisms (-174 G>C and -573 G>C) in the *IL-6* locus were investigated for association with quantitative traits related to the IDF-defined metabolic syndrome in normoglycemic subjects and with T2DM and related traits in joint analysis of 21 study samples. There was no association found between any *IL-6* polymorphism and IDF-defined MetS. Data suggest a negative association of the -174 C allele with height in men. Moreover,

for men lower waist and hip circumferences as well as higher BMI were associated with the -174 C allele. Association of the -174 C allele with higher BMI was confirmed in joint analysis for non-diabetic men. Joint analysis data suggest reduced risk for T2DM for the -174 C allele, implicated with decreased fasting glucose. For the -573 C allele there was only a tendency to be associated with higher diastolic blood pressure in normoglycemic subjects. There was no evidence for an association between IL-6 -573 G>C and T2DM in joint analysis.

Low-grade inflammation has been postulated as the link between insulin resistance, obesity and diabetes (Dandona et al. 2004). As IL-6 is one of the major cytokines in low-grade inflammation, the physiological and pathophysiological effects of IL-6 have been examined extensively. Elevated circulating levels of IL-6 have been suggested to cause insulin resistance and T2DM (Herder et al. 2005, Hu et al. 2004, Pickup et al. 2000, Pradhan et al. 2001, Spranger et al. 2003). In vitro there is evidence for a role of IL-6 in causing impaired insulin signaling in adipocytes (Kristiansen et al. 2005). High IL-6 levels have been one of the most powerful predictors of morbidity and mortality in the elderly (Ferrucci et al. 1999, Harris et al. 1999). However, IL-6 has also been proposed to have an enhancing effect on glucose and lipid metabolism (Steensberg et al. 2000, van Hall et al. 2003, Wallenius et al. 2003). In skeletal muscle IL-6 increased glucose transport, incorporation into glycogen as well as oxidation (Glund et al. 2007).

Although an impact of *IL-6* promoter variants on IL-6 levels has been proposed (Fernandez-Real et al. 2000) especially in inflammatory situations (Brull et al. 2001, Jones et al. 2001), there was no evidence of an association with IL-6 levels for any variant in non-diabetic subjects. This is consistent with the five largest hitherto published epidemiological studies on healthy or population-based subjects, including 641 to 1,526 Caucasian subjects each, which did not show statistically significant associations of *IL-6* -174 G>C polymorphism with IL-6 levels (Herbert et al. 2006, Qi et al. 2006, van Oijen et al. 2006, Sie et al. 2006, Bennet et al. 2003). However, experimental data (Fishman et al. 1998, Rivera-Chavez et al. 2003, Terry et al. 2000) demonstrate unequivocally that the *IL-6* -174 G>C allele affects promoter activity. *IL-6* transcription and the effect of promoter polymorphism -174 G>C might depend on metabolic state and thus differ between non-diabetic and subjects with T2DM. This hypothesis is supported by the observation that direction of statistically significant associations between the *IL-6* -174 C allele and IL-6 levels also differs between studies conducted in patients with different diseases (Jones et al. 2001). Furthermore, Terry et al. showed that regulation of IL-6 expression is cell-type specific (Terry et al. 2000), which is of relevance as different cell types play a role in different disease processes. Prediabetic subjects and subjects with T2DM are characterized by a subclinical inflammatory state, and inflammatory stimuli were reported to influence the association between *IL-6* -174 G>C and IL-6 levels in vivo (Brull et al. 2001,

Bennermo et al. 2004, Gaudino et al. 2003). Thus, joint analyses detected an association of -174 CC genotype with higher IL-6 levels in subjects with T2DM only. Two other studies showed no (Testa et al. 2005) or an opposite (Libra et al. 2006) association, respectively. The IL-6 level regulation is extremely complex (Terry et al. 2000) and non-diabetic subjects who generally have lower IL-6 levels might be better capable to counterregulate the higher IL-6 expression induced by the *IL6* -174 C allele and to maintain physiologically normal IL-6 levels. In contrast to small but chronically elevated IL-6 levels in T2DM patients, which were proposed to contribute to the pathogenesis of T2DM (Dovio et al. 2001), -174 C allele was associated with 9% lower risk for T2DM accompanied by 0.09 mmol/L lower fasting glucose levels. Thus, the common -174 G allele seems to be the risk allele for T2DM as reported before (Stephens et al. 2004, Vozarova et al. 2003).

In normoglycemic subjects, the -174 C allele was associated with reduced waist circumference in men. The fact that this association only occurred when BMI was included in adjustment is a consequence of the opposite estimated BMI effect. The BMI adjusted association of the -174 C allele with hip circumference and with waist circumference was in the same direction in men. Estimated effects of waist and hip circumferences, independent of BMI, in the same direction are unexpected, because larger waist circumference indicates increased cardiovascular risk whereas a larger hip circumference has a protective influence. However, risk estimates of waist and hip circumferences have been proposed to be independent of each other and of BMI (Lissner et al. 2001). The associations with waist and hip circumference and BMI seem to be due to lower height, which is associated with the C allele in men, and suggest an association with a particular stature (Figure 10). Thus, men carrying a -174 C allele may be smaller but not lighter than men carrying no C allele, as weight and body fat mass do not differ significantly between these genotype groups. Hence, the distribution of fat mass over the body might differ. IL-6 and related cytokines have profound effects on bone metabolism by regulating osteoclast and osteoblast development and function (Manolagas 1998). Men who carry a C allele might have impaired regulation of bone metabolism, inhibiting growth. An explanation of the gender-specific association might be hormonal protection from bone mineral loss by estrogens in women. As estrogens could inhibit IL-6 expression (Bruunsgaard et al. 1999) men could also be more affected by deregulated IL-6 expression. In this context, several other variants showed gender-specific associations with height. Polymorphisms in the parathyroid hormone type 1 receptor (PTHR1) (Scillitani et al. 2006), the vitamin D receptor (d'Alesio et al. 2005), and the estrogen receptor alpha (ESR1) gene were associated with height in women (Schuit et al. 2004) as well as variants in the renin–angiotensin system (Chaves et al. 2004). For men a polymorphic CA repeat in the insulin-like growth factor I (IGF-I) (Rietveld et al. 2004) was associated with height. Furthermore, variants in the aromatase gene (CYP19) were associated

with height, which is even sustained considering interactions with variants in a locus on the Y chromosome (Ellis et al. 2001). If height was reduced more considerably than weight, BMI would increase. This phenomenon would explain the higher BMI in normoglycemic subjects, which is significantly associated with the C allele when adjustment is made for the controversially estimated effects of waist and hip circumference. Several lines of evidence suggest that the cytokine IL-6 plays a role in regulation of body composition, probably by acting catabolic (Lyngso et al. 2002, van Hall et al. 2003, Wallenius et al. 2002). Association between *IL-6* -174 G>C and obesity has been investigated by several, mainly small studies, with inconsistent results (Barbieri et al. 2005, Georges et al. 2001, Klipstein-Grobusch et al. 2006, Berthier et al. 2003). In a previous study, the -174 CC genotype was associated with higher BMI in subjects with T2DM but not in healthy subjects, indicating a higher risk of developing T2DM (Stephens et al. 2004). Controversially, Hamid et al. have reported an association of the G allele carriers with higher BMI in a large study of glucose-tolerant subjects (Hamid et al. 2005). In contrast to these two studies but confirm with normoglycemic subjects -174 C allele was associated with higher BMI in non-diabetic men in joint analysis including all three samples among others. However, -174 C allele was associated with higher IL-6 levels in T2DM subjects only. Thus, observed findings in non-diabetic men could not be explained by higher IL-6 levels provoked by -174 G>C polymorphism.

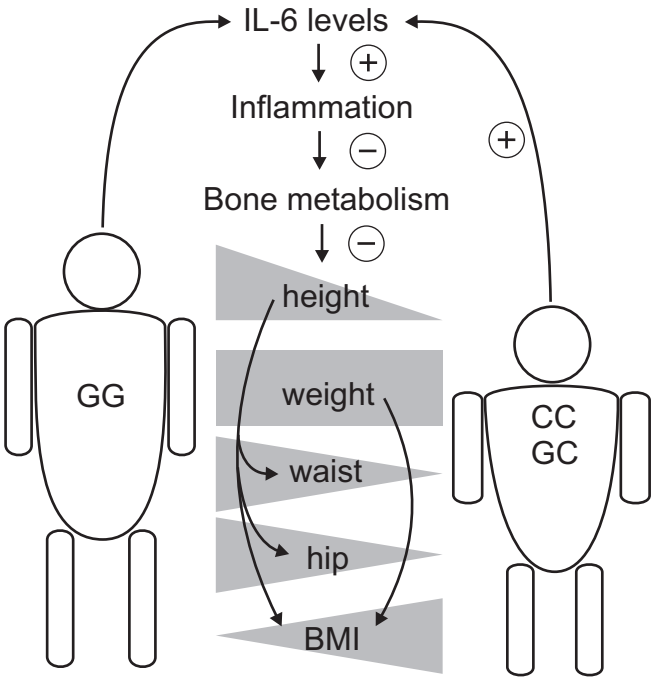

Figure 10: Hypothetical model for the association of the -174 C allele with height in men. C allele carriers might develop enhanced IL-6 expression levels. These would induce inflammatory processes which may inhibit bone metabolism. Thus, men with a -174 C allele would show impaired growth. Lower height may negatively influence waist and hip circumference. As weight of C allele carriers did not differ from the GG homozygotes, the C allele carriers have increased BMI.

There is increasing evidence for an age-related effect of the -174 G>C polymorphism (Bonafe et al. 2001, Fishman et al. 1998, Olivieri et al. 2003). Men with -174 GG genotype were proposed to be disadvantaged for longevity (Bonafe et al. 2001, Ross et al. 2003). This was proposed to be due to higher IL-6 expression (Olivieri et al. 2003) but another study observed lower IL-6 expression in this context (Rea et al. 2003). According to its influence on diseases of aging, IL-6 was proposed to be ‘‘a cytokine for gerontologists’’ (Ershler 1993). In contrast,

some studies propose no influence of the *IL-6* variant in aging (Wang et al. 2001). Likewise, the analysis of normoglycemic subjects reveals no significant impact of the -174 *IL-6* promoter polymorphism on age. However, the scope of this analysis for investigating associations on longevity was limited as the highest investigated age of 74 years was rather moderate. The data for the -573 G>C variant which was not investigated for longevity in previous studies point to the direction that the C allele carriers might be disadvantaged in this context. This effect might correspond to the previously reported association with higher IL-6 levels in elderly people for the -573 C allele (Brull et al. 2001).

In 2003 Sesso et al. found hypertension to be associated with low-grade inflammation (Sesso et al. 2003). In normoglycemic subjects, a trend was observed towards higher diastolic blood pressure for the -573 C allele carriers, especially in men. Blood pressure might be influenced by IL-6 expression but no other study has yet observed an association of this polymorphism with hypertension. Thus, the observed trend could represent a chance finding. Many of the controversies that were found for the -174 G>C polymorphism might be due to insufficient statistical power or lack of correction for multiple testing. Ethnic and gender differences and genetic heterogeneity might bias association studies as well. For analysis of the elderly normoglycemic KORA S4 subjects, the significance level was lowered to 0.01. As traits and SNPs are correlated no consistent Bonferroni correction was performed. Furthermore, Barbieri et al. suggested additive effects of *IL-6* and *PPARγ* variants. Thus, interactions with the effects of other variants should be investigated in further studies (Barbieri et al. 2005).

In conclusion, SNPs in the promoter region of IL-6 were associated with components of MetS in German Caucasians. The data from joint analysis supported association of -174 G>C polymorphism with T2DM. Higher IL-6 level were found for patients with T2DM with -174 CC genotype only. In normoglycemic men, the -174 CC genotype seems to be associated with lower height, waist and hip circumference and higher BMI. As height seems to be the central factor causing these associations, a role of the -174 G>C polymorphism in bone metabolism is indicated. Association of -174 C allele with higher BMI in non-diabetic men indicates assignment of the scenario, observed in normoglycemic subjects, to non-diabetic subjects.

6.1.3 APOA5

The *APOA5* gene was discovered independently by two groups in 2001 (Pennacchio et al. 2001, van der Vliet et al. 2001). Despite sequence homology (20 to 28%) to other apolipoproteins, a family of surface-active lipid binding proteins, of this cluster (van der Vliet et al. 2001), APOA5 differs significantly from other apolipoproteins. The APOA5 is exclusively expressed in the liver and is secreted into the plasma, where it is mainly present in HDL particles (van Dijk et al. 2004). For the function of APOA5 which is still not clarified in detail two mechanisms were proposed. One is that APOA5 may inhibit hepatic VLDL production, the

other that APOA5 is an activator of intravascular TG hydrolysis by LPL (Merkel et al. 2005). APOA5 seems to be the counterpart of APOC3 which acts in an opposite mode of action (van Dijk et al. 2004). With plasma concentrations of 0.15 µg/ml APOA5 circulates at rather low levels compared to other apolipoproteins (O'Brien et al. 2005). *APOA5* is considered as an important modifying gene for familial combined hyperlipidemia (FCHL), a disorder characterized by higher plasma TG levels and lower HDL levels (Naukkarinen et al. 2006, Shoulders et al. 2004). As these parameters are two components of MetS (Eckel et al. 2005b), APOA5 may also have a role in this complex disorder.

Ten polymorphisms, covering the *APOA5* locus, were investigated for association with lipid parameters to NCEP-defined MetS and its features in KORA S4. Minor alleles of highly correlated variants -3A>G, -1131T>C, 476G>A, 1259T>C, and variant c.56C>G were associated with higher TG levels as reported before (Chaaba et al. 2005, Pennacchio et al. 2001, van der Vliet et al. 2001, Jakel et al. 2006). The associations of the minor alleles of variant -1131T>C and variant c.56C>G, which was associated with TG levels borderline significantly in KORA S4, were replicated in a second population (SAPHIR). Furthermore, associations with lower HDL levels and higher LDL and total cholesterol levels were found for -1131T>C and c.56C>G in the SAPHIR population, but not in KORA S4. Variant c.56C>G was associated with higher risk of MetS in the SAPHIR study with similar ORs in the KORA study or in combined analysis. Especially findings with MetS deserve some attention due to the recent description of an association of -1131T>C, the one *APOA5* polymorphism analyzed in this study, with MetS in a Japanese population investigating a large number of polymorphisms in 133 candidate genes (24). However, this association could not be confirmed.
While association of *APOA5* variants with TG levels could be replicated in different ethnicities, complexity of polygenic susceptibilities of MetS might be an explanation for the lack of association of -1131T>C with MetS in Caucasians. Comparability of the Japanese case-control study with the population-based study samples could be biased by different study designs. Furthermore, definition of MetS is controversially discussed, especially concerning different ethnicities (Kahn et al. 2005).

Although no association was reported with features of MetS besides TG and HDL levels (Martin et al. 2003) or T2DM (Dorfmeister et al. 2007), an association of the functionally relevant polymorphism (c.56C>G) with MetS could be found. This could be due to the fact that MetS is a strongly lipid-driven or lipid-influenced syndrome involving three of five components. Two of these components are clear lipid-components not only correlated with each other but also with further components of MetS. Therefore it is possible that an association found between a genetic variant and TG and HDL levels extends to an association with MetS only marginally triggered by the other components. This interpretation is also supported by an

association of c.56C>G and FCHL which might share aetiological overlap with MetS (Ayyobi et al. 2003, Lewis 2002).

In most (Eichenbaum-Voline et al. 2004, Ribalta et al. 2002, Mar et al. 2004) but not all studies (van der Vleuten et al. 2007) investigating Caucasian FCHL subjects variant 1131T>C was associated with FCHL. More consistent results were reported for c.56C>G so far, showing increased transmission of the G allele in FCHL subjects (Eichenbaum-Voline et al. 2004, Mar et al. 2004). However, major impact on serum triglyceride levels by homozygote c.56C>G found by several studies (Eichenbaum-Voline et al. 2004, Pennacchio et al. 2002, Talmud et al. 2002, Vrablik et al. 2003) could only be confirmed in SAPHIR. It is interesting to note that *APOC3* variants that were reported to be in LD with *APOA5* variants (Olivier et al. 2004, Talmud et al. 2002) were also found to be associated with MetS (Miller et al. 2007).
Haplotype analysis supported the findings of the single variant analysis. Estimated haplotypes were in line with the haplotypes reported by Pennachio et al. (Pennacchio et al. 2002) and the additional variants included did not appear to provide further information.

In conclusion investigations on the association between *APOA5* variants and features of MetS in one of the largest Caucasian study samples to date provide strong support for an association with TG and HDL levels. The association of variant c.56C>G with MetS might mainly be driven by association with TG and HDL levels.

6.1.4 MCP-1

MCP-1 is a member of the CC chemokine family (Baggiolini 2001). The corresponding gene (MCP-1 or CCL2) is mainly expressed by adipocytes, endothelial cells, macrophages and osteocytes (Gerhardt et al. 2001, Rahimi et al. 1995, Nelken et al. 1991). MCP-1 expression is stimulated among others by TNFα, IL-6 and IL-1ß and is suppressed by IL-10 (Bruun et al. 2005, Gerhardt et al. 2001). MCP-1 itself influences the expression of IL-6, β1-integrins and lipoprotein lipase (Ashida et al. 2001, Sartipy et al. 2003, Viedt et al. 2002). MCP-1 actions are mediated by chemokine (C–C motif) receptor 2 (CCR2) (Ashida et al. 2001). MCP-1 seems to play an important role in several of the clustering risk factors of MetS as well as in the pathogenesis of MetS itself (Kanda et al. 2006). MCP-1 has typical proinflammatory properties, like promoting the arrest and transmigration of monocytes (Gerszten et al. 1999, Randolph et al. 1995). Additionally, MCP-1 is involved in adipocyte metabolism (Gerhardt et al. 2001, Sartipy et al. 2003). The association of high MCP-1 levels with obesity is clear in mice, but uncertain in humans (Christiansen et al. 2005, Herder et al. 2006a, Sartipy et al. 2003, Zietz et al. 2005). It was further shown that increased MCP-1 levels are related to insulin resistance and T2DM (Herder et al. 2006a, Kanda et al. 2006). In addition, MCP-1 is involved in foam cell differentiation and progression of atherosclerosis (Gosling et al. 1999, McDermott et al. 2005).

Among seven SNPs analyzed in the *MCP-1* locus, which were investigated for association with IDF-defined MetS and its features in KORA S4, trends for association were observed for TG levels, fasting, and 2 h glucose, and uric acid. There were also trends for leukocyte count and height. SNP c.-928G>C was significantly associated with height in men only.

Until now, studies assessing MetS and MCP-1 levels have been rare, yielded controversial results and have only been conducted on rather small populations (<350 individuals each) (Lee et al. 2006, Liu et al. 2005a, Marso et al. 2005). The association between *MCP-1* SNPs and MetS has not yet been investigated, but Kanda et al. recently suggested that MCP-1 may play an important role in the pathogenesis of MetS (Kanda et al. 2006). However, there was no association between any of the analyzed SNPs and MetS suggesting an indirect influence in MetS development. Data indicated an influence on TG levels for MCP-1. In healthy populations higher MCP-1 levels were associated with higher TG (Deo et al. 2004, Herder et al. 2006a, Joven et al. 2006, McDermott et al. 2005). In small studies with <160 participants suffering from peripheral arterial disease and systemic lupus erythematosus, this association was also observed, although elevated MCP-1 levels may have been correlated with lipid abnormalities (Asanuma et al. 2006, Kowalski et al. 2001, Petrkova et al. 2004). Herder et al. found a significant positive association between systemic MCP-1 and elevated TG levels in 722 subjects of KORA S4, a subgroup of the basis population in this work (Herder et al. 2006b). Other cytokines, like TNFa and IL-1, have been previously shown to be involved in the regulation of serum TG levels (Argiles et al. 1989, Grunfeld et al. 1990).

Inconsistency prevails epidemiologic studies on association between MCP-1 levels with T2DM, too (Cermakova et al. 2005, Herder et al. 2006a, McDermott et al. 2005, Simeoni et al. 2004, Zietz et al. 2005). Confounding effect of cardiovascular and cerebrovascular conditions could be an explanation. Considering this fact, Herder et al. reported that MCP-1 levels were associated with risk for incident T2DM (Herder et al. 2006a). Zietz et al. showed that subjects with T2DM had significantly higher MCP-1 levels, but association with the SNP –2578A>G could not be detected. This might be due to co-medication with angiotensin converting enzyme inhibitors and lipid-lowering drugs, which can influence MCP-1 levels (Zietz et al. 2005). In this work, no association was observed for any analyzed SNP in the *MCP-1* gene with T2DM, even when subjects taking lipid-lowering drugs were excluded from the analysis. This lack of association might be a power problem, as the analysed sample included 254 T2DM cases only. Larger association studies or a meta-analysis may be needed to exclude an influence of *MCP-1* SNPs on T2DM.

Association of MCP-1 SNPs with fasting glucose has also not been previously reported. Epidemiological studies revealed controversial results for a correlation of fasting glucose and MCP-1 levels in diabetic patients. This controversy might be due to the influence of blood

glucose on MCP-1 production in several cell types and differential glycaemic control in diabetic patients (Kim et al. 2005, Mine et al. 2006). The trend for differences in fasting glucose found between genotype groups of c.-928G>C, is supported by the observation that MCP-1 interferes with insulin signaling, which leads to a reduction in glucose uptake by adipocytes (Sartipy et al. 2003).

Association of MCP-1 levels with 2 h glucose was not found whether in a population-based approach nor in patients with massive weight loss by bariatric surgery (Herder et al. 2006b, Schernthaner et al. 2006). Similar to fasting glucose, no study has investigated the influence of *MCP-1* SNPs on 2 h glucose. For c.-3813C>T, a trend for higher 2 h glucose was observed between the genotype groups. This trend is also supported by the observation that MCP-1 reduces insulin-stimulated glucose uptake in adipocytes (Sartipy et al. 2003).

As analysis of IL-6 revealed an association with height suggesting that IL-6 and other related cytokines exert an influence on osteoclast and osteoblast development and function, height was included in this analysis as a component of the BMI obesity parameter, too. This hypothesis was supported by Rahimi et al. who reported that the MCP-1 protein is involved in osteoclast recruitment and development in mice (Rahimi et al. 1995). Until now, no study has investigated an association of MCP-1 levels or *MCP-1* SNPs with height. Due to trends observed for c.-928G>C, c.105T>C and MCP-1*4 with height and significant gender differences in height, it was further analyzed separately for men and women. A significant association was observed in men, which implicated that the trend in the entire group was caused by men. Furthermore, haplotype analysis showed a statistically significant increase in height for carriers of MCP-1*1, which includes minor alleles of the two SNPs showing trends, and c*3879C>T.

Accumulating evidence suggests that the receptor activator NFkB ligand (RANKL), among others, induces the MCP-1 protein production, leading to differentiation and higher activity of osteoclasts, which could lead to increased bone resorption (Kim et al. 2005, Rahimi et al. 1995). Furthermore, Evans et al. showed that osteoclast activity exerts an influence on long bone length (Evans et al. 2003).

SNP -2578A>G, which was analyzed for associations with leukocytes in two studies of 550 or 150 participants respectively, was not associated with leukocyte counts (Franco-Lopez et al. 2005, Kozma et al. 2002). This is in line with the KORA S4 results for two SNPs in strong LD with -2578A>G, which did not show a trend for differences in leukocytes between the genotype groups. However, c.105T>C genotypes exhibited differences in leukocytes. As there is no literature concerning an association of this SNP with serum leukocytes, this finding needs to be replicated.

The SNPs c.*65C>T and c.*3879C>T which were in strong LD with –2578A>G exhibited trends for different MCP-1 levels between the genotype groups. Several studies investigated association between SNP –2578A>G and MCP-1 serum levels (Cermakova et al. 2005, McDermott et al. 2005, Simeoni et al. 2004, Tabara et al. 2003, Zietz et al. 2005). Although functionality of this SNP has been previously demonstrated, the results of the association studies remain contradictory (Rovin et al. 1999). Thus, c.*65C>T and c.*3879C>T may indicate association of –2578A>G, although MCP-1 levels were only measured in subjects with IGT and matched controls within each group comprising about 240 subjects. Glucose tolerance status did not correlate with MCP-1 levels (Herder et al. 2006b).

SNP -2578A>G was also investigated for association with IL-6 levels. Zietz et al. detected a negative correlation ($p< 0.025$) (Herder et al. 2006b). This was not observed for c.*65C>T and c.*3879C>T. The SNP c.-2138A>T exhibited a trend for differences between the genotype groups, although the strongly correlated c.77-109C>G did not. Since MCP-1 is involved in IL-6 expression, an association seems conceivable (Viedt et al. 2002), but further replication is necessary.

Nakagawa et al. suggested uric acid to be related to MetS by inhibiting endothelial dysfunction (Nakagawa et al. 2006) in KORA S4. SNP c.-3813C>T showed a trend for differences in serum uric acid between the genotype groups. There were no previous studies on MCP-1 or *MCP-1* SNPs and uric acid. Thus, replication is recommended.

In conclusion, analyzed SNPs of the *MCP-1* locus revealed trends for differences between the genotype groups for several parameters of MetS. Potential association of *MCP-1* SNPs with triglyceride levels and fasting glucose, two components of the IDF MetS definition are suggested. Although these new findings have to be replicated first, MCP-1 may be an interesting gene for further investigation in MetS research.

6.1.5 Resistin

Resistin is suggested to play a role in lipid metabolism (Rae et al. 2006, Palanivel et al. 2005, Ort et al. 2005). Futhermore, proinflammatory and atherogenic properties were reported (Nagaev et al. 2006, Reilly et al. 2005, Verma et al. 2003). However, circulating resistin levels do not appear to represent the expected link between adipose tissue and insulin resistance (Arner 2005), but may still play a role locally (e.g. within macrophage-infiltrated adipose tissue) (Utzschneider et al. 2005). Obesity and related conditions such as insulin resistance, T2DM, hyperlipidaemia, and hypertension are possibly influenced by resistin expression and secretion (Koebnick et al. 2006, Kunnari et al. 2005, Kusminski et al. 2005, Menzaghi et al. 2006). As MetS represents a cluster of these parameters and is highly heritable (Eckel et al. 2005a, Liu et al. 2005b, Menzaghi et al. 2006), *RETN* is still a potential candidate gene for MetS. However, a clear role of resistin in MetS is not supported within this work.

For the *RETN* locus tagging SNPs from Hap Map and SNPs from literature were chosen as *RETN* is located in a recombination hotspot, according to Hap Map. Recombination hotspots are small regions where crossover occurs more frequently than in surrounding sequences (Lichten et al. 1995). Tagging SNPs available from Hap Map should contain the complete information of the gene. None of the *RETN* SNPs was associated with MetS, its components, or related parameters. However, the applied significance level was conservatively corrected for multiple testing by the Bonferroni correction (p=0.00055). For *RETN* gene variant rs3760678 significant difference between the genotype groups were found for triglyceride levels. As this SNP was not among the tagging SNPs systematic screen was very valuable in this case. However, no statistically significant associations in the analysis of further models for triglyceride levels and rs3760678 were detected.

Several studies suggested *RETN* as a candidate gene for MetS or T2DM (Osawa et al. 2004, Smith et al. 2003, Verma et al. 2003). Correlation studies of the resistin protein with MetS and T2DM are contradictory (Haluzik et al. 2006, Kusminski et al. 2005, Menzaghi et al. 2006, Utzschneider et al. 2005). Until now, there was no association analysis for *RETN* SNPs and MetS. In a meta-analysis, a significant increase in the risk for T2DM was observed for the GG genotype of rs862513 (Osawa et al. 2004). None of the analyzed SNPs were associated with T2DM, which might be a power problem.

Promoter SNP rs3760678, which does not seem to have influence on serum resistin levels (Azuma et al. 2004), was analyzed for an association with TG for the first time. However, association between rs3760678 and TG levels was not present in the nominal model, which showed insignificant opposite directions for TG estimates in AG and GG carriers compared to the AA reference group. As GG carrier group was very small (33 subjects), indicating a chance finding, dominant model was considered as best fitting model showing 8.5% increased TG levels. This finding is biological plausible, as murine, rat, and human models showed an involvement of resistin in lipid metabolism. From these studies it might be proposed that resistin stimulates hormone sensitive lipase in adipocytes via the extracellular signal-regulated kinase (ERK) pathway, which leads to increased lipolysis. This in turn increases non-esterified fatty acids in the blood. Therefore, more fatty acids are incorporated into TG in the liver and released in more VLDL, which increases serum TG levels (Greenberg et al. 2001, Julius 2003, Ort et al. 2005, Pravenec et al. 2006, Sato et al. 2005, Venkatesan et al. 1993). Although Ort *et al.* showed that human and mouse resistin do not have the same effects on the same cell population, TG lipolysis was also observed in human adipocytes stimulated with human resistin protein (Ort et al. 2005). Thus, resistin might play a role in human lipid metabolism, which is supported by another *in vitro* human study and correlation studies (Koebnick et al. 2006, Lu et al. 2006, Rae et al. 2006). Rae *et al.* reported that human resistin caused glucose-

dependent triglyceride accumulation and an increase in total cholesterol mass in macrophages (Rae et al. 2006). Lu *et al.* reported a correlation between resistin protein and triglyceride levels, but not with non-esterified fatty acids in 60 Chinese patients with T2DM and 28 healthy control subjects (Lu et al. 2006). Koebnick *et al.* additionally found a correlation of resistin with plasma non-esterified fatty acids, but not with triglycerides in 20 healthy, lean and 43 overweight Germans (Koebnick et al. 2006). Three studies, all analyzing the same 41 healthy Caucasian men with a wide range in BMI, did not detect a correlation between resistin protein and TG levels (Chan et al. 2005, Farvid et al. 2005, Ng et al. 2005).

In conclusion systematic analysis of the *RETN* gene locus did not reveal any association between the *RETN* SNPs and MetS or T2DM. Association of rs3760678 with TG supports, if replicable, the hypothesis that resistin is involved in lipid metabolism. Thus, *RETN* could still be involved in MetS development if direct association is not detectable due to complexity.

6.1.6 TCF7L2

Only a few genetic variants have been identified as susceptibility genes for T2DM so far (O'Rahilly et al. 2006), altering the T2DM risk by approximately 15 to 20 % (Polonsky 2006). However, risk modifications of newly identified genetic variants in the *TCF7L2* gene seem to be substantially greater (Florez et al. 2006a, Grant et al. 2006). As most early association studies overestimate genetic effects (Ioannidis et al. 2001), validation not only of reported genetic associations, but also of their effect sizes is essential.

Reported associations of the *TCF7L2* SNPs rs12255372 and rs7903146 with T2DM could be replicated in KORA samples reflecting the Southern German population (Holle et al. 2005). Replication results strongly confirmed the T alleles at both SNPs as risk variants for T2DM. However, allele frequencies were slightly lower in diabetic cases as previously reported (Grant et al. 2006). Good fit for the multiplicative genotype models, especially for SNP rs7903146 was also confirmed. In combination with eight other studies conducted independently at the same time providing similar results on *TCF7L2* gene variants and T2DM (Cauchi et al. 2006, Damcott et al. 2006, Groves et al. 2006, Humphries et al. 2006, Saxena et al. 2006, Scott et al. 2006, Vliet-Ostaptchouk et al. 2007, Zhang et al. 2006) data provide strong evidence that *TCF7L2* is a true diabetes susceptibility gene (Table 21). As individuals with MetS are at significantly increased risk of T2DM (Hanley et al. 2005) and pathophysiological mechanism of *TCF7L2* variants is still unclear, analyses were extended to MetS and related components. Among quantitative traits analyzed BMI, fasting insulin, HOMA-IR and HOMA-% B, are highly relevant for the development of T2DM. Although the elderly KORA population had a high prevalence of MetS, neither of the two SNPs was statistically significantly associated with MetS. One explanation might be that not all subjects with MetS will eventually develop T2DM. In the Insulin Resistance Atherosclerosis Study (IRAS), a prospective multi-ethnic

epidemiologic study in the U.S., the population attributable-risk of incident diabetes was 49% for the IDF and 46% for the NCEP definition of MetS, respectively (Hanley et al. 2005). Thus, less than 50% of the risk of T2DM were attributable to the presence of MetS. This might be due to substantial heterogeneity of MetS allowing for many combinations of metabolic disturbances with different implications for diabetes risk, but resulting in the same diagnosis. Second, it is possible that there are distinct polygenic determinants of the MetS with small effect sizes, which could not be observed in this analysis and would require an even larger sample. Thus, it is conceivable that a significant association of *TCF7L2* variants could be found with T2DM, but not with MetS. Among analyzed traits strongest association was found for decreased basal insulin secretion, assessed by HOMA-% B, and the T2DM risk-conferring T allele at rs7903146. This finding is plausible and in line with a previous report based on the T2DM high-risk population of the Diabetes Prevention Program (DPP), demonstrating that persons who are homozygous for the risk allele at rs7903146 have lower first phase insulin secretion in response to an OGTT than homozygous non-carriers of the risk allele (Florez et al. 2006a). In non-diabetic Amish subjects (n= 698), no association of OGTT-derived insulin resistance (HOMA-IR) and insulin secretion measurements with the *TCF7L2* gene variants was observed (Damcott et al. 2006). In addition, intravenous glucose tolerance tests were carried out in a small sample of 48 non-diabetic non-Amish subjects, which indicated that rs7901695 and rs79003146 were significantly associated with both a reduction in insulin sensitivity and a defect in insulin secretion (Damcott et al. 2006). Results of this study were confirm with KORA S4 indicating no association between *TCF7L2* polymorphisms and insulin sensitivity, assessed by HOMA-IR. Thus, higher risk of diabetes in carriers of the T allele might primarily be conferred by an impaired insulin secretion. *TCF7L2* gene product is a high-mobility-group box containing transcription factor that suppresses rather than activates gene expression (Hurlstone et al. 2002, Kikuchi et al. 2006, Nelson et al. 2004). It has been suggested, that the association with T2DM may result from impaired regulation of gene expression of the insulinotropic proglucagon in enteroendocrine cells via the Wnt signaling pathway (Grant et al. 2006, Yi et al. 2005), but the precise mechanisms underlying the reduced insulin secretion require further investigation.

Taken together, a significant association of *TCF7L2* gene variants and T2DM in a large sample from the Southern German KORA study was found, thus confirming previous findings. While no significant association of these SNPs with insulin resistance (HOMA-IR), MetS or any of its components was found, there was a significant trend towards lower levels of fasting insulin and reduced basal insulin secretion (HOMA- % B) in the individuals who were homozygous for the T2DM risk allele of rs7903146. This finding supports the hypothesis that *TCF7L2* variants may primarily affect pancreatic beta cell function, leading to impaired insulin secretion and eventually to T2DM.

Table 21: Recent studies on *TCF7L2* variants rs7903146 and rs12255372 in combined or single analysis.

Reference	Study information	Ethnicity	Patients	Controls	Variation	CT vs. CC[a]	TT vs.CC[a]	Additive model[a]	p[b]
(Grant et al. 2006)	CC	Caucasians (Iceland, Denmark, USA)	1185/228/361	931/539/530	rs7903146			1.49 [1.35, 1.65]	3.9×10^{-15}
					rs12255372			1.52 [1.38, 1.68]	2.5×10^{-16}
					combined	1.45 [1.26, 1.67]	2.41 [1.94, 3.00]	1.56 [1.41, 1.73]	4.7×10^{-18}
(Humphries et al. 2006)	NPHSII	Caucasians (Europe)	158	2518	combined	1.65 [1.13, 2.41]	1.87 [0.99, 3.53]		<0.01
			1459	2518	combined	1.43 [1.24, 1.65]	2.11 [1.69, 2.63]		<0.0001
		Asians (India)	919	919	combined	1.50 [1.14, 1.99]	1.64 [1.03, 2.63]		0.003
		Afro-Caribbean	385	385	combined	1.25 [0.90, 1.75]	1.32 [0.74, 2.33]		0.17
(Vliet-Ostaptchouk et al. 2007)	CC	Caucasians (Netherlands)	502	920	combined		1.38		
(Cauchi et al. 2006)	CC	Caucasians (France)	2367	2499	rs7903146		1.69 [1.55, 1.83]		6.0×10^{-35}
					rs12255372		1.60 [1.47, 1.74]		7.6×10^{-28}
(Saxena et al. 2006)	CC	Caucasians (Scaninavia, Sweden, Poland, US, Botnia, Finnland)	3563	3563	rs7903146	1.40 [1.27, 1.55]	1.86 [1.55, 2.23]	1.39 [1.29, 1.50]	1.55×10^{-17}
	different family samples	Caucasians (Scandinavia, Finnland, Botnia)	569	615	rs7903146			1.48 [1.17, 1.87]	4.69×10^{-4}
(Damcott et al. 2006)	CC	Caucasians (Amish)	137(+139 IGT)	342	rs7903146		1.57		0.008
					rs12255372		1.40		0.04
(Scott et al. 2006)	CC	Caucasians (Finnland)	1151	953	rs7903146			1.33 [1.14, 1.56]	0.00042
					rs12255372			1.36 [1.15, 1.61]	0.00026
(Zhang et al. 2006)	Nurses health study (CC) only women	Caucasians (USA)	687	1051	rs12255372	1.25 [1.01, 1.55]	1.86 [1.30, 2.67]	1.32 [1.13, 1.54]	0.0002
	Health professional Follow-up Study (CC) only men		886	896	rs12255372	1.63 [1.32, 2.02]	2.15 [1.48, 3.13]	1.53 [1.31, 1.80]	<0.0001
	nested CC		1573	1947	rs12255372	1.43 [1.23, 1.66]	1.99 [1.54, 2.59]	1.42 [1.27, 1.59]	<0.0001
	meta analysis		3347	3947	rs12255372			1.48 [1.37, 1.60]	1×10^{-16}
(Groves et al. 2006)	parent offspring trios (388)	Caucasians (UK)	2158	2574	rs7903146	1.35 [1.19, 1.53]	1.90 [1.54, 2.33]	1.36 [1.24, 1.48]	1.3×10^{-11}
					rs12255372	1.30 [1.15, 1.47]	1.66 [1.35, 2.05]	1.29 [1.18, 1.41]	2.2×10^{-8}
(Cauchi et al. 2007)	Meta analysis		17,202	29,195	rs7903146			1.46 [1.42-1.51]	5.4×10^{-140}

[a]) estimated OR are given with 95% convidence interval [b]) p-values for additive models or combined analysis; CC=Case/Control

6.1.7 Fat assimilation

6.1.7.1 ACBP

The human acyl-CoA-binding protein (ACBP) is a highly conserved polypeptide with multiple physiological functions (Kragelund et al. 1999). It has been described as a regulator of insulin release from pancreatic cells (Borboni et al. 1991, Chen et al. 1988). ACBP is a prominent candidate for T2DM because of its central role in determining free intracellular acyl-CoA concentrations that have been reported to inversely correlate with insulin sensitivity in muscle (Ellis et al. 2000, Oakes et al. 1997). According to the assumption that the alteration of acyl-CoA-binding properties, or expression levels of ACBP might be involved in etiology of T2DM, association of common and putative functional variants of the gene with T2DM risk was examined. A moderate but consistent association between one *ACBP* SNP (rs2084202) and T2DM was found in two independent German study populations (EPIC-Potsdam and KORA). In both studies, carriers of the minor allele (A) showed lower risk of T2DM compared to homozygote carriers of the major allele (GG). The obtained OR in the range of 0.5 to 0.7 and borderline significances are explained by limited statistical power on the one hand, but on the other hand, might demonstrate the additional involvement of other (genetic or environmental) factors influencing gene-disease association. Since results were replicated in an independent study, a false-positive finding seems to be unlikely. However, replication studies in larger populations are desirable in the future. Associations between rs2084202 and related traits of the disease (i. e., BMI, percent body fat) were not found. Thus, effect of rs2084202 on T2DM might not be related to body weight or fat mass. In fact, a direct function of the polymorphism on insulin resistance could be expected. SNP rs2084202 is located in the promoter region of splice variant ACBP-1c (Nitz et al. 2005), 466 bp upstream from the translation start. Thus, A>G substitution of this promoter SNP may increase the transcriptional activity of this PPARc dependent variant which is highly expressed in adipocytes. This could lead to decreased free acyl-CoA concentrations in insulin sensitive cells such as hepatocytes or adipocytes and thereby reduce the risk to develop insulin resistance. Of course, this hypothesis has to be tested in future studies.

In conclusion, first evidence that the minor allele of *ACBP* rs2084202 might be associated with reduced risk of T2DM is obtained from two Caucasian study populations. The functional significance of this finding has to be unraveled in future studies.

6.1.7.2 PTGES2

PTGES2 is important for synthesis of the antilipolytic-hypertrophic (Vassaux et al. 1992) metabolite prostaglandin E2 (PGE2). The synthesis of PGE2 from arachidonic acid is mediated by phospholipase A2, cyclooxygenase (PTGS2) and prostaglandin E synthase (PTGES). Terminal PTGESs, which catalyze the conversion of PGH2 to PGE2, exist in three

forms (Murakami et al. 2004): microsomal PTGES (PTGES1), cytosolic PTGES (cPTGES) and the membrane-bound PTGES2. Whereas the physiological role of cPTGES is uncertain (Murakami et al. 2004), PTGES1 seems to be mainly involved in inflammation (Gudis et al. 2005, Jakobsson et al. 1999, Murakami et al. 2000). In contrast, PTGES2 is not induced by inflammatory stimuli and is expressed constitutively in various cells and tissues in which PTGES1 expression is relatively low (Murakami et al. 2003, Tanikawa et al. 2002). These findings argue for a general role of PTGES2 in the production of PGE2 crucial for tissue homeostasis. *As PTGES2* gene maps to a chromosomal locus linked to obesity (Wilson et al. 1991), *PTGES2* polymorphisms were tested for association with T2DM and related traits in two German study populations with KORA S4 as replication sample.

Sequencing (Fisher et al. 2007) of all seven exons of *PTGES2* revealed only one non-synonymous SNP in codon 298 (Arg→His), which had not been genotyped in a large population before. The minor allele (His298) frequencies observed were 12.0% in the screening group comprising 47 individuals, 16.5% in EPIC and 18.6% in KORA. T2DM was only identified by self-report and confirmed by the patient primary care physician. The key finding in the present study was the consistent association between the minor allele of the *PTGES2* Arg298His SNP (rs13283456) and decreased risk of T2DM in both study populations. This finding was confirmed in a subgroup of KORA consisting of 239 subjects with impaired glucose tolerance. In addition, a pooled analysis of data from both study populations revealed a p-value of 0.0005 for association between *PTGES2* Arg298His SNP and T2DM. Among htSNPs selected from a 20kb gene region of *PTGES2* and a novel promoter SNP (g.-417G>T) detected in sequencing approach genotyped in EPIC none was significantly associated with T2DM. A common haplotype including minor allele of Arg298His SNP showed evidence for association with T2DM similar to single SNP Arg298His. Thus, evidence that the Arg298His SNP within the *PTGES2* gene might be important for the association with T2DM is substantiated. Interestingly, a significant association between the *PTGES2* Arg298His and HOMA-%B as an indicator of basal insulin secretion was found in KORA. HOMA-IR values were also insignificantly lower in His-carriers. As both observations were made in controls only putative functionality of the polymorphism could be apparent under physiological conditions. A functional link between PGE2 and beta cell dysfunction or impaired insulin secretion via the Akt (protein kinase B) pathway has been provided by cell culture experiments *in-vitro* and *ex-vivo* as well in animal studies and transgenic approaches (Meng et al. 2006, Oshima et al. 2006, Tran et al. 1999). As shown in cell culture experiments (Sandra et al. 1986) and in human studies, increasing concentrations of PGE2 caused peripheral insulin resistance. A molecular mechanism for this effect has not been elucidated so far, but an alteration of insulin-stimulated phosphoinositide turnover by PGE2 had been discussed

earlier by Sandra *et al.* (Sandra et al. 1986). Beside the investigated *PTGES2* transcript, three other transcripts are described in public databases (NCBI). NM_025072 encodes the 377 amino acids comprising membrane-associated PTGES2 (NP_079348) which was the focus of this investigation.

Amongst others in this isoform rs13283456 causes the described amino acid exchange Arg→His which is located in the Glutathion-S-transferase domain. The change of a basic amino acid residue (Arg) with a pKa-value of 12.5 to a basic residue with a value of 6.0 (His) may cause a partial functional perturbation of the *PTGES2* His298 variant. This would result in lower PGE2 production, which could influence beta cell function and/or insulin sensitivity.
In summary, first evidence from two independent German study populations that the His-variant of the *PTGES2* Arg298His polymorphism is associated with reduced risk of T2DM is provided including explanation hypothesis.

6.2 MetS definitions

Within this work two definitions were used for analysis of genetic associations with MetS. NCEP definition (NCEP 2002), which is the most widely used criteria to identify MetS individuals, and the IDF definition for MetS (Alberti et al. 2005). All independent study populations used in this work showed higher prevalence of IDF defined MetS. Frequencies of the NCEP and IDF definition were in line through all samples. For KORA S4 Rathmann *et al.* compared MetS definitions additionally regarding the WHO definition (Rathmann et al. 2006). Possibly the IDF definition overestimates number of MetS cases due to lower cutoffs and accentuated importance of abdominal obesity. Thus, IDF defined MetS analysis must be validated with care. However, effect sizes of genetic variants with MetS did not significantly differ between the definitions.

6.3 Strength and limitations

Study design
The MONICA/KORA surveys and KORA S4, being representative for the general population of Augsburg, Germany, benefit from the high-quality phenotyping of the internationally approved MONICA/KORA surveys. In particular, the analyzed subgroup included fasting values of parameters relevant to MetS and OGTT data. However, OGTT was not performed in subjects with known T2DM to avoid any threat. Thus, these subjects were unfortunately mostly non-fasting.
The SAPHIR study is representative of the working population of Salzburg, Austria, providing fasting measurements as well. However, OGTT data were available for a fraction of the subjects.

One limitation for investigation of associations between gene variants and MetS are the study designs. The KORA S4 sub sample is a population-based sample with limited age range. Same holds true for the SAPHIR study with further limitation to healthy working population. The KORA sample including subjects with T2DM from S1-S4 and controls of S4 was construed to investigate associations with T2DM. Thus, controls were matched for included subjects with T2DM. Thus, none of the available study samples provides a definite case-control design for MetS.

Multiple testing

Multiple testing is a major problem in association studies, bearing mischief of false positive associations by chance when performing a large amount of tests. While significance level in analysis of *IL-6* was only slightly corrected ($p<0.01$), significance levels were strictly corrected applying Bonferroni correction in analysis of *APOA5*, *MCP-1* and *RETN*. However, strictly corrected significance levels could also lead to false negative associations. Significance levels were notably low in *RETN* analyis. Thus, observed trends should not be ignored.

SNP coverage for *APOA5*, *MCP-1*, and *RETN* was suitable, in contrast to *IL-6* analysis, which investigated two promoter polymorphisms only. However, functional relevance was uprated for these *IL-6* promoter polymorphisms.

6.4 Coherences of analyzed genes

Within this work a small part of yet unforeseen multifarious genetic susceptibilities for MetS and T2DM could be illuminated. Equally multifarious are environmental influences that were marginally incorporated in the present study design. An illustration of these complex coherences is provided in Figure 11, instancing MetS as complex disease. Another inconvenience is mostly small effect of the single gene variants emerging from interaction with other gene variants or environmental influences. Thus, association with a complex disease is hardly detectable. There are only few variants showing consistent associations like TCF7L2 with T2DM. Moreover, this association is one of few with such a strong effect in a complex

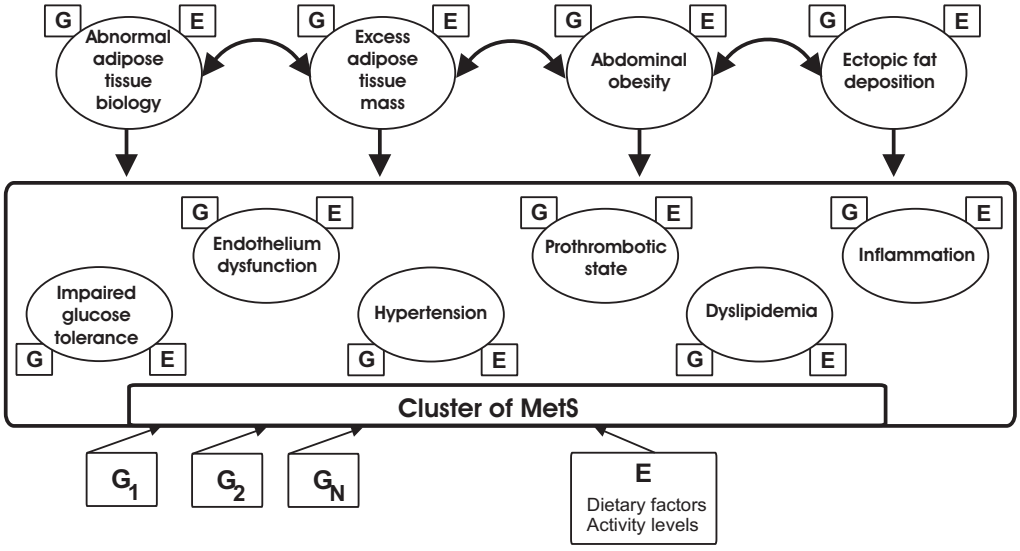

Figure 11: Role of genetic (G) and environmental (E) factors on putative causes of MetS and related traits. Genetic and environmental effects have impact on lipid and adipose tissue related traits that in turn influence individual risk factors and the cluster of MetS. In addition, all individual components of MetS are regulated by both genetic and environmental factors (Teran-Garcia et al. 2007).

disease. In addition, several products of susceptibility genes have complex actions by themselves. A case in point is IL-6, which is not only a major player in inflammatory processes, but is suggested to be involved in several processes of T2DM or MetS development (Kristiansen et al. 2005). However, despite its complex actions, which are obvious due to the manifold tissues secreting IL-6, association with T2DM and BMI seem to be consistent albeit effects are weak. Relevance of the hypothesized explanation for an influence on body composition in men or observed association trends with MetS related parameters have to be further validated regarding MetS development. Figure 12 presents a simple scheme summarizing coherences of the genes analyzed within this work regarding MetS and T2DM. The only analyzed gene showing a direct association with MetS was APOA5. However, as already mentioned, this association might mainly be driven by strong association with two lipid parameters (TG and HDL cholesterol levels). A role in lipid metabolism was suggested for RETN as well, which is supported by association of *RETN* SNPs with higher TG levels. Thus,

RETN could contribute to lipid abnormalities in MetS. Involvement of resistin in insulin resistance (Steppan et al. 2002, Kim et al. 2001) was suggested to be mediated by oxidative stress (Smith et al. 2003), which was shown to impair insulin action in adipocytes *in vivo* (Tirosh et al. 2001, Rudich et al. 1999, Rudich et al. 1997). MCP-1, which interacts with IL-6, has typical proinflammatory properties. It seems to play an important role in several features of MetS. Only potential association of *MCP-1* variation with two features of MetS, TG levels and fasting glucose, was observed. Thus, suggested manifold influences of MCP-1 in features of MetS could not fully be supported by effects of genetic variation. Similar to IL-6, MCP-1 seems to be involved in growth regulation in men. However, relevance for MetS or T2DM development is not distinguishable.

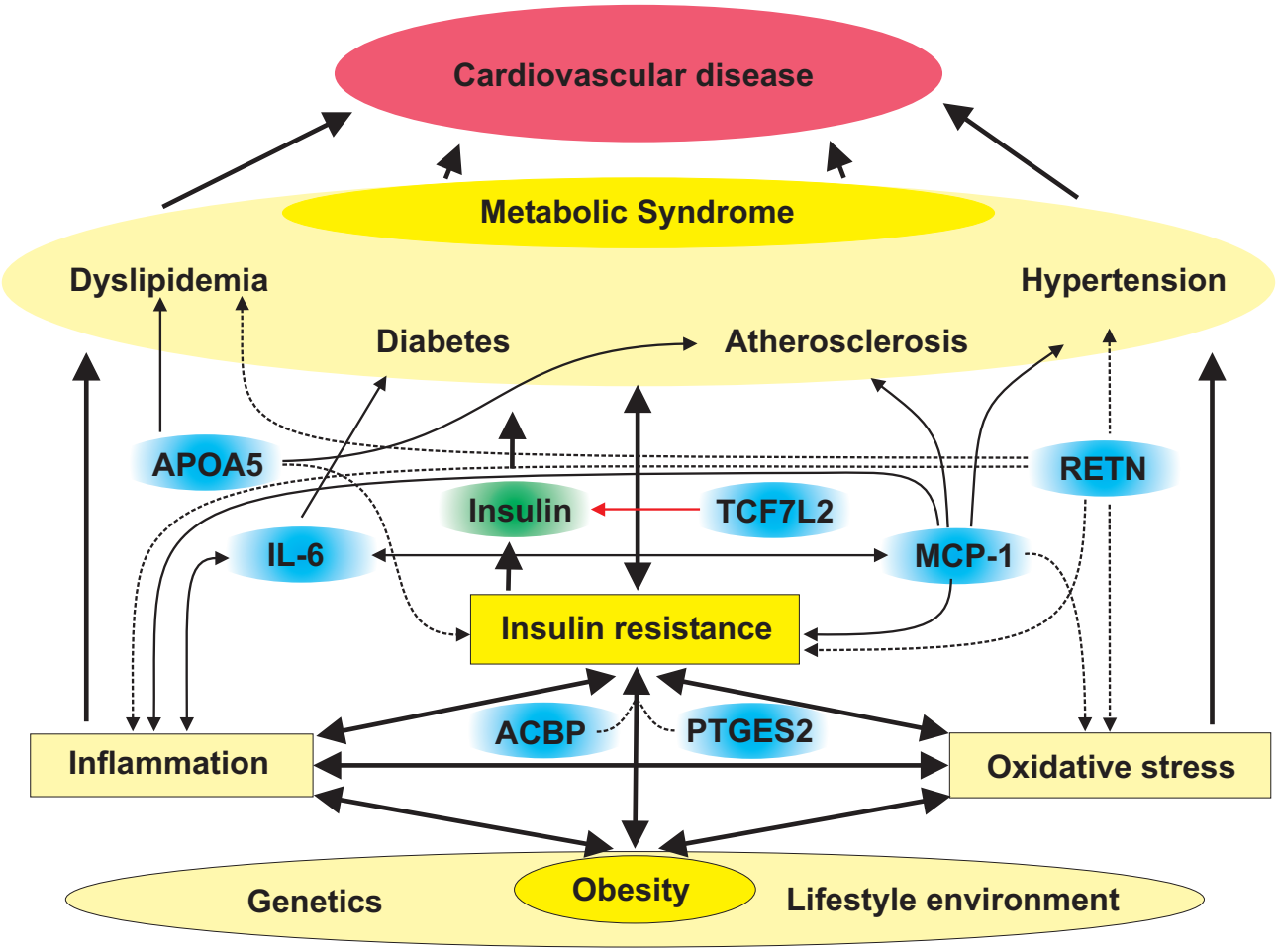

Figure 12: Hypothetical model of coherences in MetS development regarding all genes analyzed within this work (blue) or in related projects.

TCF7L2 is the most promising T2DM gene yet discovered. While replication of the association with T2DM was successful none of the variants was associated with MetS or related features. Defective function of *TCF7L2* was proposed to lead to decreased transactivation of the proglucagon gene, reduced GLP-1 levels and decreased insulin secretion in response to

enteral glucose challenge (Florez 2007). This is in line with the observed significant trend towards lower levels of fasting insulin and reduced basal insulin secretion. Resulting abnormalities in nutrient absorption and metabolic regulation also suggest a role of *TCF7L2* in MetS.

The genes *ACBP* and *PTGES2* are both involved in fat assimilation processes. Both gene products influence insulin regulation. ACBP a regulator of insulin release from pancreatic cells has T2DM risk reducing effects when expression is increased amongst others due to the analyzed promoter variant. PTGES2 acts inderectly through regulation of PGE2 production. Thus, PTGES2 impairement by an amino acid exchange results in lower PGE2 influencing beta cell function and/or insulin sensitivity, finally resulting in reduced risk for T2DM.

In conclusion all analyzed genes have a role in MetS or T2DM development due to their function. Partly genetic variation could be shown to relate to ones genes contribution to features of MetS or T2DM. Association with these complex diseases was replicated for *TCF7L2* variants with T2DM and for the first time for an *APOA5* variant with MetS. However, underlying mechanisms are still speculative and must be further assessed by functional studies. In case of APOA5 association might be detected due to strong association with two MetS components.

6.5 Future perspectives

There is a major problem in investigation of genetic susceptibilities for diseases like MetS and T2DM – complexity. Candidate genes are manifold. Thus, association studies on genetic variations within a certain gene locus deliver insight in a small part of genetic susceptibilities only. New technologies, which provide possibility to investigate most of the genetic variation information over the whole genome will enhance insight. The leading companies in this field Affymetrix and Illumina will soon provide possibilities to genotype up to one million SNPs per subject at once. SNP selection strategies are slightly different for these approaches. While Affymetrix chips include SNPs in 5kb distance over the whole genome, Illumina arrays focus on known coding regions. Hence, both technologies may currently miss some important genetic information. However, this effect might become unessential the higher the provided SNP coverage will be. Sooner or later technologies sequencing the whole genome will be developed closing these gaps completely. In addition larger study samples are needed to detect small effects of gene variation in complex diseases. A approach to account for this need are meta-analyses combining several samples increasing power for association analyis. As association studies can only be the first step on a long way to understand mechanisms, which are behind detected associations between gene variants and disease related parameters, association studies provide mainly hints were functional studies have to head for. Furthermore, analysis should not only be focused on single gene loci. Gene-gene interactions may play a major role and additive or modulating effects of genetic variants in different genes has already been shown. A yet mostly unregarded topic is the information that is hidden in DNA methylations. Thus, there will be a lot of action in the field of epi genetics in the near future. In combination with conventional genotyping new insights will be gained.

7. Publications

1. Grallert,H, Huth,C, Kolz,M, Meisinger,C, Herder,C, Strassburger,K, Giani,G, Wichmann,HE, Adamski,J, Illig,T, Rathmann,W: IL-6 promoter polymorphisms and quantitative traits related to the metabolic syndrome in KORA S4. *Exp.Gerontol.* 41:737-745, 2006

2. Grallert,H, Sedlmeier,EM, Huth,C, Kolz,M, Heid,IM, Meisinger,C, Herder,C, Strassburger,K, Gehringer,A, Haak,M, Giani,G, Kronenberg,F, Wichmann,H-E, Adamski,J, Paulweber,B, Illig,T, Rathmann,W: APOA5 variants and metabolic syndrome in Caucasians. *J.Lipid Res.* 2007. Sep 3 Epub ahead of print.

3. Sedlmeier,EM, Grallert,H, Huth,C, Lowel,H, Herder,C, Strassburger,K, Giani,G, Wichmann,HE, Hauner,H, Illig,T, Rathmann,W: Gene variants of monocyte chemoattractant protein 1 and components of metabolic syndrome in KORA S4, Augsburg. *Eur.J.Endocrinol.* 156:377-385, 2007

4. Sedlmeier,EM, Grallert,H, Huth,C, Meisinger,C, Herder,C, Strassburger,K, Giani,G, Wichmann,HE, Hauner,H, Illig,T, Rathmann,W: The *Resistin* SNP rs3760678 is strongly associated with triglycerides in KORA S4. *Eur J Clin Invest* 2007. submitted

5. Holzapfel,C, Klopp,N, Grallert,H, Huth,C, Gieger,C, Meisinger,C, Strassburger,K, Giani,G, Wichmann,H-E, Laumen,H, Hauner,H, Herder,C, Rathmann,W, Illig,T: Genetic variants in the *LARG* (*ARHGEF12*) gene are not associated with T2DM and related parameters in Caucasians (KORA study). *Eur J Endocrinol* 2007.

6. Nitz,I, Fisher,E, Grallert,H, Li,Y, Gieger,C, Rubin,D, Boeing,H, Spranger,J, Lindner,I, Schreiber,S, Rathmann,W, Gohlke,H, Doring,A, Wichmann,HE, Schrezenmeir,J, Doring,F, Illig,T: Association of Prostaglandin E Synthase 2 (PTGES2) Arg298His Polymorphism with Type 2 Diabetes in two German Study Populations. *J.Clin.Endocrinol.Metab* 2007 Jun 12 Epub ahead of print

7. Marzi,C, Huth,C, Kolz,M, Grallert,H, Meisinger,C, Wichmann,HE, Rathmann,W, Herder,C, Illig,T: Variants of the transcription factor 7-like 2 gene (TCF7L2) are strongly associated with type 2 diabetes but not with the metabolic syndrome in the MONICA/KORA surveys. *Horm.Metab Res.* 39:46-52, 2007

8. Fisher,E, Nitz,I, Gieger,C, Grallert,H, Gohlke,H, Lindner,I, Dahm,S, Boeing,H, Burwinkel,B, Rathmann,W, Wichmann,HE, Schrezenmeir,J, Illig,T, Doring,F: Association of acyl-CoA-binding protein (ACBP) single nucleotide polymorphisms and type 2 diabetes in two German study populations. *Mol.Nutr.Food Res.* 51:178-184, 2007

9. Huth,C, Heid,IM, Vollmert,C, Gieger,C, Grallert,H, Wolford,JK, Langer,B, Thorand,B, Klopp,N, Hamid,YH, Pedersen,O, Hansen,T, Lyssenko,V, Groop,L, Meisinger,C, Doring,A, Lowel,H, Lieb,W, Hengstenberg,C, Rathmann,W, Martin,S, Stephens,JW, Ireland,H, Mather,H, Miller,GJ, Stringham,HM, Boehnke,M, Tuomilehto,J, Boeing,H, Mohlig,M, Spranger,J, Pfeiffer,A, Wernstedt,I, Niklason,A, Lopez-Bermejo,A, Fernandez-Real,JM, Hanson,RL, Gallart,L, Vendrell,J, Tsiavou,A, Hatziagelaki,E, Humphries,SE, Wichmann,HE, Herder,C, Illig,T: IL6 gene promoter polymorphisms and type 2 diabetes: joint analysis of individual participants' data from 21 studies. *Diabetes* 55:2915-2921, 2006

10. Huth,C, Illig,T, Herder,C, Gieger,C, Grallert,H, Vollmert,C, Rathmann,W, Hamid,YH, Pedersen,O, Hansen,T, Thorand,B, Meisinger,C, Doring,A, Klopp,N, Gohlke,H, Lieb,W, Hengstenberg,C, Lyssenko,V, Groop,L, Ireland,H, Stephens,JW, Miller,GJ, Wernstedt,I, Jansson,J-O, Boeing,H, Mohlig,M, Stringham,HM, Boehnke,M, Tuomilehto,J, Fernandez-Real,JM, Lopez-Bermejo,A, Gallart,L, Vendrell,J, Humphries,SE, Kronenberg,F, Wichmann,H-E, Heid,IM: Joint Analysis of Individual Participants' Data from 17 Studies on the Association of the IL6 Variant -174G>C with Circulating Glucose and Interleukin-6 Levels, and Body-Mass Index. Circulation 2007. submitted

8. References

Aizawa, T., Y. Funase, M. Katakura, N. Asanuma, K. Yamauchi, K. Yoshizawa, and K. Hashizume. 1997. Ketosis-onset diabetes in young adults with subsequent non-insulin-dependency, a link between IDDM and NIDDM? Diabet.Med. **14**: 989-991

Alberti, K. G., P. Zimmet, and J. Shaw. 2005. The metabolic syndrome--a new worldwide definition. Lancet **366**: 1059-1062

Alberti, K. G. and P. Z. Zimmet. 1998. 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. **15**: 539-553

Altshuler, D., J. N. Hirschhorn, M. Klannemark, C. M. Lindgren, M. C. Vohl, J. Nemesh, C. R. Lane, S. F. Schaffner, S. Bolk, C. Brewer, T. Tuomi, D. Gaudet, T. J. Hudson, M. Daly, L. Groop, and E. S. Lander. 2000. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat.Genet. **26**: 76-80

Anderson, E. A., R. P. Hoffman, T. W. Balon, C. A. Sinkey, and A. L. Mark. 1991. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J.Clin.Invest **87**: 2246-2252

Apelqvist, A., U. Ahlgren, and H. Edlund. 1997. Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. Curr.Biol. **7**: 801-804

Argiles, J. M., F. J. Lopez-Soriano, R. D. Evans, and D. H. Williamson. 1989. Interleukin-1 and lipid metabolism in the rat. Biochem.J. **259**: 673-678

Arner, P. 2005. Resistin: yet another adipokine tells us that men are not mice. Diabetologia **48**: 2203-2205

Arya, R., J. Blangero, K. Williams, L. Almasy, T. D. Dyer, R. J. Leach, P. O'Connell, M. P. Stern, and R. Duggirala. 2002. Factors of insulin resistance syndrome--related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic mexican-americans. Diabetes **51**: 841-847

Asanuma, Y., C. P. Chung, A. Oeser, A. Shintani, E. Stanley, P. Raggi, and C. M. Stein. 2006. Increased concentration of proatherogenic inflammatory cytokines in systemic lupus erythematosus: relationship to cardiovascular risk factors. J.Rheumatol. **33**: 539-545

Ashida, N., H. Arai, M. Yamasaki, and T. Kita. 2001. Distinct signaling pathways for MCP-1-dependent integrin activation and chemotaxis. J.Biol.Chem. **276**: 16555-16560

Ayyobi, A. F. and J. D. Brunzell. 2003. Lipoprotein distribution in the metabolic syndrome, type 2 diabetes mellitus, and familial combined hyperlipidemia. Am.J.Cardiol. **92**: 27J-33J

Azizi, F., P. Salehi, A. Etemadi, and S. Zahedi-Asl. 2003. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. Diabetes Res.Clin.Pract. **61**: 29-37

Azuma, K., S. Oguchi, Y. Matsubara, T. Mamizuka, M. Murata, H. Kikuchi, K. Watanabe, F. Katsukawa, H. Yamazaki, A. Shimada, and T. Saruta. 2004. Novel resistin promoter polymorphisms: association with serum resistin level in Japanese obese individuals. Horm.Metab Res. **36**: 564-570

Baggiolini, M. 2001. Chemokines in pathology and medicine. J.Intern.Med. **250**: 91-104

Baier, L. J., P. A. Permana, X. Yang, R. E. Pratley, R. L. Hanson, G. Q. Shen, D. Mott, W. C. Knowler, N. J. Cox, Y. Horikawa, N. Oda, G. I. Bell, and C. Bogardus. 2000. A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. J.Clin.Invest **106**: R69-R73

Balkau, B. and M. A. Charles. 1999. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet.Med. **16**: 442-443

Balkau, B., M. Vernay, L. Mhamdi, M. Novak, D. Arondel, S. Vol, J. Tichet, and E. Eschwege. 2003. The incidence and persistence of the NCEP (National Cholesterol Education Program) metabolic syndrome. The French D.E.S.I.R. study. Diabetes Metab **29**: 526-532

Barbieri, M., M. R. Rizzo, M. Papa, R. Acampora, L. De Angelis, F. Olivieri, F. Marchegiani, C. Franceschi, and G. Paolisso. 2005. Role of interaction between variants in the PPARG and interleukin-6 genes on obesity related metabolic risk factors. Exp.Gerontol. **40**: 599-604

Barnett, A. H., C. Eff, R. D. Leslie, and D. A. Pyke. 1981. Diabetes in identical twins. A study of 200 pairs. Diabetologia **20**: 87-93

Barroso, I., J. Luan, R. P. Middelberg, A. H. Harding, P. W. Franks, R. W. Jakes, D. Clayton, A. J. Schafer, S. O'Rahilly, and N. J. Wareham. 2003. Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. PLoS.Biol. **1**: E20

Bays, H., L. Mandarino, and R. A. DeFronzo. 2004. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J.Clin.Endocrinol.Metab **89**: 463-478

Bennermo, M., C. Held, S. Stemme, C. G. Ericsson, A. Silveira, F. Green, and P. Tornvall. 2004. Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? Clin.Chem. **50**: 2136-2140

Bennet, A. M., J. A. Prince, G. Z. Fei, L. Lyrenas, Y. Huang, B. Wiman, J. Frostegard, and U. Faire. 2003. Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction. Atherosclerosis **171**: 359-367

Bennett, R. G., F. G. Hamel, and W. C. Duckworth. 2003. An insulin-degrading enzyme inhibitor decreases amylin degradation, increases amylin-induced cytotoxicity, and increases amyloid formation in insulinoma cell cultures. Diabetes **52**: 2315-2320

Bennett, S. T. and J. A. Todd. 1996. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. Annu.Rev.Genet. **30**: 343-370

Berthier, M. T., A. M. Paradis, A. Tchernof, J. Bergeron, D. Prud'homme, J. P. Despres, and M. C. Vohl. 2003. The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. J.Hum.Genet. **48**: 14-19

Bjorntorp, P. and R. Rosmond. 2000. The metabolic syndrome--a neuroendocrine disorder? Br.J.Nutr. **83 Suppl 1**: S49-S57

Bonafe, M., F. Olivieri, L. Cavallone, S. Giovagnetti, F. Mayegiani, M. Cardelli, C. Pieri, M. Marra, R. Antonicelli, R. Lisa, M. R. Rizzo, G. Paolisso, D. Monti, and C. Franceschi. 2001. A gender--dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur.J.Immunol. **31**: 2357-2361

Boras-Granic, K., R. Grosschedl, and P. A. Hamel. 2006. Genetic interaction between Lef1 and Alx4 is required for early embryonic development. Int.J.Dev.Biol. **50**: 601-610

Borboni, P., L. Condorelli, P. De Stefanis, G. Sesti, and R. Lauro. 1991. Modulation of insulin secretion by diazepam binding inhibitor and its processing products. Neuropharmacology **30**: 1399-1403

Bort, R., J. P. Martinez-Barbera, R. S. Beddington, and K. S. Zaret. 2004. Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. Development **131**: 797-806

Bort, R., M. Signore, K. Tremblay, J. P. Martinez Barbera, and K. S. Zaret. 2006. Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Dev.Biol. **290**: 44-56

Bothig, S. 1989. WHO MONICA Project: objectives and design. Int.J Epidemiol. **18**: S29-S37

Bowden, D. W., M. Sale, T. D. Howard, A. Qadri, B. J. Spray, C. B. Rothschild, G. Akots, S. S. Rich, and B. I. Freedman. 1997. Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes **46**: 882-886

Brand, E., U. Schorr, I. Kunz, E. Kertmen, J. Ringel, A. Distler, and A. M. Sharma. 2001. Tumor necrosis factor-alpha--308 G/A polymorphism in obese Caucasians. Int.J.Obes.Relat Metab Disord. **25**: 581-585

Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1991. Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein-cholesterol levels with or without hypertriglyceridemia. J.Clin.Invest **87**: 536-544

Broedl, U. C., M. Lehrke, E. Fleischer-Brielmaier, A. B. Tietz, J. M. Nagel, B. Goke, P. Lohse, and K. G. Parhofer. 2006. Genetic variants of adiponectin receptor 2 are associated with increased adiponectin levels and decreased triglyceride/VLDL levels in patients with metabolic syndrome. Cardiovasc.Diabetol. **5**: 11

Brull, D. J., H. E. Montgomery, J. Sanders, S. Dhamrait, L. Luong, A. Rumley, G. D. Lowe, and S. E. Humphries. 2001. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler.Thromb.Vasc.Biol. **21**: 1458-1463

Bruun, J. M., A. S. Lihn, S. B. Pedersen, and B. Richelsen. 2005. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J.Clin.Endocrinol.Metab **90**: 2282-2289

Bruunsgaard, H., A. N. Pedersen, M. Schroll, P. Skinhoj, and B. K. Pedersen. 1999. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. Clin.Exp.Immunol. **118**: 235-241

Buetow, K. H., M. Edmonson, R. MacDonald, R. Clifford, P. Yip, J. Kelley, D. P. Little, R. Strausberg, H. Koester, C. R. Cantor, and A. Braun. 2001. High-throughput

development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proc.Natl.Acad.Sci.U.S.A **98**: 581-584

Busfield, F., D. L. Duffy, J. B. Kesting, S. M. Walker, P. K. Lovelock, D. Good, H. Tate, D. Watego, M. Marczak, N. Hayman, and J. T. Shaw. 2002. A genomewide search for type 2 diabetes-susceptibility genes in indigenous Australians. Am.J.Hum.Genet. **70**: 349-357

Cameron, A. J., J. E. Shaw, and P. Z. Zimmet. 2004. The metabolic syndrome: prevalence in worldwide populations. Endocrinol.Metab Clin.North Am. **33**: 351-75, table

Carmelli, D., L. R. Cardon, and R. Fabsitz. 1994. Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments? Am.J.Hum.Genet. **55**: 566-573

Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc.Natl.Acad.Sci.U.S.A **72**: 3666-3670

Cases, J. A., I. Gabriely, X. H. Ma, X. M. Yang, T. Michaeli, N. Fleischer, L. Rossetti, and N. Barzilai. 2001. Physiological increase in plasma leptin markedly inhibits insulin secretion in vivo. Diabetes **50**: 348-352

Cauchi, S., Y. El Achhab, H. Choquet, C. Dina, F. Krempler, R. Weitgasser, C. Nejjari, W. Patsch, M. Chikri, D. Meyre, and P. Froguel. 2007. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J.Mol.Med. **85**: 777-782

Cauchi, S., D. Meyre, C. Dina, H. Choquet, C. Samson, S. Gallina, B. Balkau, G. Charpentier, F. Pattou, V. Stetsyuk, R. Scharfmann, B. Staels, G. Fruhbeck, and P. Froguel. 2006. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes **55**: 2903-2908

Cermakova, Z., J. Petrkova, A. Arakelyan, J. Drabek, F. Mrazek, J. Lukl, and M. Petrek. 2005. The MCP-1 -2518 (A to G) single nucleotide polymorphism is not associated with myocardial infarction in the Czech population. Int.J.Immunogenet. **32**: 315-318

Chaaba, R., N. Attia, S. Hammami, M. Smaoui, S. Mahjoub, M. Hammami, and A. S. Masmoudi. 2005. Association of SNP3 polymorphism in the apolipoprotein A-V gene with plasma triglyceride level in Tunisian type 2 diabetes. Lipids Health Dis. **4**: 1

Chan, D. C., G. F. Watts, T. W. Ng, Y. Uchida, N. Sakai, S. Yamashita, and P. H. Barrett. 2005. Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Clin.Chem. **51**: 578-585

Chaturvedi, N., P. M. McKeigue, and M. G. Marmot. 1993. Resting and ambulatory blood pressure differences in Afro-Caribbeans and Europeans. Hypertension **22**: 90-96

Chaves, F. J., D. Corella, J. V. Sorli, P. Marin-Garcia, M. Guillen, and J. Redon. 2004. Polymorphisms of the renin-angiotensin system influence height in normotensive women in a Spanish population. J.Clin.Endocrinol.Metab **89**: 2301-2305

Chen, W., S. R. Srinivasan, A. Elkasabany, and G. S. Berenson. 1999. The association of cardiovascular risk factor clustering related to insulin resistance syndrome (Syndrome X) between young parents and their offspring: the Bogalusa Heart Study. Atherosclerosis **145**: 197-205

Chen, Z. W., B. Agerberth, K. Gell, M. Andersson, V. Mutt, C. G. Ostenson, S. Efendic, J. Barros-Soderling, B. Persson, and H. Jornvall. 1988. Isolation and characterization of porcine diazepam-binding inhibitor, a polypeptide not only of cerebral occurrence but also common in intestinal tissues and with effects on regulation of insulin release. Eur.J.Biochem. **174**: 239-245

Chimienti, F., S. Devergnas, F. Pattou, F. Schuit, R. Garcia-Cuenca, B. Vandewalle, J. Kerr-Conte, L. Van Lommel, D. Grunwald, A. Favier, and M. Seve. 2006. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J.Cell Sci. **119**: 4199-4206

Christiansen, T., B. Richelsen, and J. M. Bruun. 2005. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. Int.J.Obes.(Lond) **29**: 146-150

Civitarese, A. E., C. P. Jenkinson, D. Richardson, M. Bajaj, K. Cusi, S. Kashyap, R. Berria, R. Belfort, R. A. DeFronzo, L. J. Mandarino, and E. Ravussin. 2004. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. Diabetologia **47**: 816-820

Civitarese, A. E., B. Ukropcova, S. Carling, M. Hulver, R. A. DeFronzo, L. Mandarino, E. Ravussin, and S. R. Smith. 2006. Role of adiponectin in human skeletal muscle bioenergetics. Cell Metab **4**: 75-87

Combs, T. P., A. H. Berg, S. Obici, P. E. Scherer, and L. Rossetti. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest **108**: 1875-1881

Committee DT. 1988. Diabetes mellitus in twins: a cooperative study in Japan. Committee on Diabetic Twins, Japan Diabetes Society. Diabetes Res.Clin.Pract. **5**: 271-280

Cox, N. J. 2001. Challenges in identifying genetic variation affecting susceptibility to type 2 diabetes: examples from studies of the calpain-10 gene. Hum.Mol.Genet. **10**: 2301-2305

Crepaldi, G. and E. Manzato. 1995. [Polymetabolic syndrome]. Minerva Endocrinol **20**: 155-160

d'Alesio, A., M. Garabedian, J. P. Sabatier, G. Guaydier-Souquieres, C. Marcelli, A. Lemacon, O. Walrant-Debray, and F. Jehan. 2005. Two single-nucleotide polymorphisms in the human vitamin D receptor promoter change protein-DNA complex formation and are associated with height and vitamin D status in adolescent girls. Hum.Mol.Genet. **14**: 3539-3548

da Sliva, B., S. M. Gapstur, Y. H. Achenbach, T. S. Schuh, T. J. Kotlar, K. Liu, and W. L. Lowe, Jr. 2000. Lack of association between the -308 polymorphism of the tumor necrosis factor-alpha gene and the insulin resistance syndrome. J.Investig.Med. **48**: 236-244

Daimon, M., T. Oizumi, T. Saitoh, W. Kameda, H. Yamaguchi, H. Ohnuma, M. Igarashi, H. Manaka, and T. Kato. 2002. Calpain 10 gene polymorphisms are related, not to type 2 diabetes, but to increased serum cholesterol in Japanese. Diabetes Res.Clin.Pract. **56**: 147-152

Dalziel, B., A. K. Gosby, R. M. Richman, J. M. Bryson, and I. D. Caterson. 2002. Association of the TNF-alpha -308 G/A promoter polymorphism with insulin resistance in obesity. Obes.Res. **10**: 401-407

Damcott, C. M., S. H. Ott, T. I. Pollin, L. J. Reinhart, J. Wang, J. R. O'connell, B. D. Mitchell, and A. R. Shuldiner. 2005. Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. Diabetes **54**: 2245-2250

Damcott, C. M., T. I. Pollin, L. J. Reinhart, S. H. Ott, H. Shen, K. D. Silver, B. D. Mitchell, and A. R. Shuldiner. 2006. Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. Diabetes **55**: 2654-2659

Dandona, P., A. Aljada, and A. Bandyopadhyay. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol. **25**: 4-7

Day, C. P., J. Grove, A. K. Daly, M. W. Stewart, P. J. Avery, and M. Walker. 1998. Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. Diabetologia **41**: 430-434

de Graaf, J., J. C. Hendriks, P. N. Demacker, and A. F. Stalenhoef. 1993. Identification of multiple dense LDL subfractions with enhanced susceptibility to in vitro oxidation among hypertriglyceridemic subjects. Normalization after clofibrate treatment. Arterioscler.Thromb. **13**: 712-719

DeFronzo, R. A. and E. Ferrannini. 1991. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care **14**: 173-194

Deo, R., A. Khera, D. K. McGuire, S. A. Murphy, J. P. Meo Neto, D. A. Morrow, and J. A. de Lemos. 2004. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. J.Am.Coll.Cardiol. **44**: 1812-1818

DeRijk, R. H., M. Schaaf, and E. R. de Kloet. 2002. Glucocorticoid receptor variants: clinical implications. J.Steroid Biochem.Mol.Biol. **81**: 103-122

DeWan, A. T., D. K. Arnett, L. D. Atwood, M. A. Province, C. E. Lewis, S. C. Hunt, and J. Eckfeldt. 2001. A genome scan for renal function among hypertensives: the HyperGEN study. Am.J.Hum.Genet. **68**: 136-144

Dina, C., D. Meyre, S. Gallina, E. Durand, A. Korner, P. Jacobson, L. M. Carlsson, W. Kiess, V. Vatin, C. Lecoeur, J. Delplanque, E. Vaillant, F. Pattou, J. Ruiz, J. Weill, C. Levy-Marchal, F. Horber, N. Potoczna, S. Hercberg, C. Le Stunff, P. Bougneres, P. Kovacs, M. Marre, B. Balkau, S. Cauchi, J. C. Chevre, and P. Froguel. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat.Genet. **39**: 724-726

Dorfmeister, B., J. A. Cooper, J. W. Stephens, H. Ireland, S. J. Hurel, S. E. Humphries, and P. J. Talmud. 2007. The effect of APOA5 and APOC3 variants on lipid parameters in

European Whites, Indian Asians and Afro-Caribbeans with type 2 diabetes. Biochim.Biophys.Acta **1772**: 355-363

Dovio, A. and A. Angeli. 2001. Cytokines and type 2 diabetes mellitus. JAMA **286**: 2233

Draper, N., S. M. Echwald, G. G. Lavery, E. A. Walker, R. Fraser, E. Davies, T. I. Sorensen, A. Astrup, J. Adamski, M. Hewison, J. M. Connell, O. Pedersen, and P. M. Stewart. 2002. Association studies between microsatellite markers within the gene encoding human 11beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. J.Clin.Endocrinol.Metab **87**: 4984-4990

Duggirala, R., J. Blangero, L. Almasy, R. Arya, T. D. Dyer, K. L. Williams, R. J. Leach, P. O'Connell, and M. P. Stern. 2001. A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. Am.J.Hum.Genet. **68**: 1149-1164

Duggirala, R., J. Blangero, L. Almasy, T. D. Dyer, K. L. Williams, R. J. Leach, P. O'Connell, and M. P. Stern. 1999. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. Am.J.Hum.Genet. **64**: 1127-1140

Duncan, B. B. and M. I. Schmidt. 2001. Chronic activation of the innate immune system may underlie the metabolic syndrome. Sao Paulo Med.J. **119**: 122-127

Eckel, R. H. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N.Engl.J.Med. **320**: 1060-1068

Eckel, R. H., S. M. Grundy, and P. Z. Zimmet. 2005b. The metabolic syndrome. Lancet **365**: 1415-1428

Eckel, R. H., S. M. Grundy, and P. Z. Zimmet. 2005a. The metabolic syndrome. Lancet **365**: 1415-1428

Eckel, R. H., T. J. Yost, and D. R. Jensen. 1995. Alterations in lipoprotein lipase in insulin resistance. Int.J.Obes.Relat Metab Disord. **19 Suppl 1**: S16-S21

Edwards, K. L., B. Newman, E. Mayer, J. V. Selby, R. M. Krauss, and M. A. Austin. 1997. Heritability of factors of the insulin resistance syndrome in women twins. Genet.Epidemiol. **14**: 241-253

Egan, B. M. 2003. Insulin resistance and the sympathetic nervous system. Curr.Hypertens.Rep. **5**: 247-254

Ehm, M. G., M. C. Karnoub, H. Sakul, K. Gottschalk, D. C. Holt, J. L. Weber, D. Vaske, D. Briley, L. Briley, J. Kopf, P. McMillen, Q. Nguyen, M. Reisman, E. H. Lai, G. Joslyn, N. S. Shepherd, C. Bell, M. J. Wagner, and D. K. Burns. 2000. Genomewide search for type 2 diabetes susceptibility genes in four American populations. Am.J.Hum.Genet. **66**: 1871-1881

Eichenbaum-Voline, S., M. Olivier, E. L. Jones, R. P. Naoumova, B. Jones, B. Gau, H. N. Patel, M. Seed, D. J. Betteridge, D. J. Galton, E. M. Rubin, J. Scott, C. C. Shoulders, and L. A. Pennacchio. 2004. Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. Arterioscler.Thromb.Vasc.Biol. **24**: 167-174

Einhorn, D., G. M. Reaven, R. H. Cobin, E. Ford, O. P. Ganda, Y. Handelsman, R. Hellman, P. S. Jellinger, D. Kendall, R. M. Krauss, N. D. Neufeld, S. M. Petak, H. W. Rodbard, J. A. Seibel, D. A. Smith, and P. W. Wilson. 2003. American College of Endocrinology position statement on the insulin resistance syndrome. Endocr.Pract. **9**: 237-252

Elbein, S. C., K. C. Chiu, M. D. Hoffman, R. A. Mayorga, K. L. Bragg, and M. F. Leppert. 1995. Linkage analysis of 19 candidate regions for insulin resistance in familial NIDDM. Diabetes **44**: 1259-1265

Elbein, S. C., W. Chu, Q. Ren, C. Hemphill, J. Schay, N. J. Cox, C. L. Hanis, and S. J. Hasstedt. 2002. Role of calpain-10 gene variants in familial type 2 diabetes in Caucasians. J.Clin.Endocrinol.Metab **87**: 650-654

Elbein, S. C., M. D. Hoffman, K. Teng, M. F. Leppert, and S. J. Hasstedt. 1999. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. Diabetes **48**: 1175-1182

Ellis, B. A., A. Poynten, A. J. Lowy, S. M. Furler, D. J. Chisholm, E. W. Kraegen, and G. J. Cooney. 2000. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. Am.J.Physiol Endocrinol.Metab **279**: E554-E560

Ellis, J. A., M. Stebbing, and S. B. Harrap. 2001. Significant population variation in adult male height associated with the Y chromosome and the aromatase gene. J.Clin.Endocrinol.Metab **86**: 4147-4150

Ershler, W. B. 1993. Interleukin-6: a cytokine for gerontologists. J.Am.Geriatr.Soc. **41**: 176-181

Esterbauer, H., C. Schneitler, H. Oberkofler, C. Ebenbichler, B. Paulweber, F. Sandhofer, G. Ladurner, E. Hell, A. D. Strosberg, J. R. Patsch, F. Krempler, and W. Patsch. 2001. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. Nat.Genet. **28**: 178-183

Evans, J. C., T. M. Frayling, P. G. Cassell, P. J. Saker, G. A. Hitman, M. Walker, J. C. Levy, S. O'Rahilly, P. V. Rao, A. J. Bennett, E. C. Jones, S. Menzel, P. Prestwich, N. Simecek, M. Wishart, R. Dhillon, C. Fletcher, A. Millward, A. Demaine, T. Wilkin, Y. Horikawa, N. J. Cox, G. I. Bell, S. Ellard, M. I. McCarthy, and A. T. Hattersley. 2001. Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. Am.J.Hum.Genet. **69**: 544-552

Evans, K. D., S. T. Lau, A. M. Oberbauer, and R. B. Martin. 2003. Alendronate affects long bone length and growth plate morphology in the oim mouse model for Osteogenesis Imperfecta. Bone **32**: 268-274

Fantuzzi, G. and R. Faggioni. 2000. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J.Leukoc.Biol. **68**: 437-446

Farris, W., S. Mansourian, Y. Chang, L. Lindsley, E. A. Eckman, M. P. Frosch, C. B. Eckman, R. E. Tanzi, D. J. Selkoe, and S. Guenette. 2003. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc.Natl.Acad.Sci.U.S.A **100**: 4162-4167

Farvid, M. S., T. W. Ng, D. C. Chan, P. H. Barrett, and G. F. Watts. 2005. Association of adiponectin and resistin with adipose tissue compartments, insulin resistance and dyslipidaemia. Diabetes Obes.Metab **7**: 406-413

Fernandez-Real, J. M., M. Broch, J. Vendrell, C. Gutierrez, R. Casamitjana, M. Pugeat, C. Richart, and W. Ricart. 2000. Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes **49**: 517-520

Fernandez-Real, J. M., C. Gutierrez, W. Ricart, R. Casamitjana, M. Fernandez-Castaner, J. Vendrell, C. Richart, and J. Soler. 1997. The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes **46**: 1468-1472

Fernandez-Real, J. M. and W. Ricart. 2003. Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr.Rev. **24**: 278-301

Ferrannini, E., G. Buzzigoli, R. Bonadonna, M. A. Giorico, M. Oleggini, L. Graziadei, R. Pedrinelli, L. Brandi, and S. Bevilacqua. 1987. Insulin resistance in essential hypertension. N.Engl.J.Med. **317**: 350-357

Ferrucci, L., T. B. Harris, J. M. Guralnik, R. P. Tracy, M. C. Corti, H. J. Cohen, B. Penninx, M. Pahor, R. Wallace, and R. J. Havlik. 1999. Serum IL-6 level and the development of disability in older persons. J.Am.Geriatr.Soc. **47**: 639-646

Festa, A., R. D'Agostino, Jr., G. Howard, L. Mykkanen, R. P. Tracy, and S. M. Haffner. 2000. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation **102**: 42-47

Fey, G. H. and J. Gauldie. 1990. The acute phase response of the liver in inflammation. Prog.Liver Dis. **9**: 89-116

Fisher, E., I. Nitz, I. Lindner, D. Rubin, H. Boeing, M. Mohlig, J. Hampe, S. Schreiber, J. Schrezenmeir, and F. Doring. 2007. Candidate gene association study of type 2 diabetes in a nested case-control study of the EPIC-Potsdam cohort - role of fat assimilation. Mol.Nutr.Food Res. **51**: 185-191

Fishman, D., G. Faulds, R. Jeffery, V. Mohamed-Ali, J. S. Yudkin, S. Humphries, and P. Woo. 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J.Clin.Invest **102**: 1369-1376

Florez, J. C. 2007. The new type 2 diabetes gene TCF7L2. Curr.Opin.Clin.Nutr.Metab Care **10**: 391-396

Florez, J. C., K. A. Jablonski, N. Bayley, T. I. Pollin, P. I. de Bakker, A. R. Shuldiner, W. C. Knowler, D. M. Nathan, and D. Altshuler. 2006a. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N.Engl.J.Med. **355**: 241-250

Florez, J. C., S. Wiltshire, C. M. Agapakis, N. P. Burtt, P. I. de Bakker, P. Almgren, B. K. Bengtsson, T. Tuomi, D. Gaudet, M. J. Daly, J. N. Hirschhorn, M. I. McCarthy, D. Altshuler, and L. Groop. 2006b. High-density haplotype structure and association testing of the insulin-degrading enzyme (IDE) gene with type 2 diabetes in 4,206 people. Diabetes **55**: 128-135

Foley, A. C. and M. Mercola. 2005. Heart induction by Wnt antagonists depends on the homeodomain transcription factor Hex. Genes Dev. **19**: 387-396

Ford, E. S., W. H. Giles, and W. H. Dietz. 2002. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA **287**: 356-359

Franco-Lopez, E., M. F. Gonzalez-Escribano, I. Aguilera, J. M. Pascasio, F. Pareja, A. Bernardos, and A. Nunez-Roldan. 2005. Lack of association of recipient MCP-1 gene promoter polymorphism with acute graft rejection after orthotopic liver transplantation. Transplant.Proc. **37**: 1496-1498

Franks, P. W., W. C. Knowler, S. Nair, J. Koska, Y. H. Lee, R. S. Lindsay, B. R. Walker, H. C. Looker, P. A. Permana, P. A. Tataranni, and R. L. Hanson. 2004. Interaction between an 11betaHSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. Hypertension **44**: 681-688

Frayling, T. M., N. J. Timpson, M. N. Weedon, E. Zeggini, R. M. Freathy, C. M. Lindgren, J. R. Perry, K. S. Elliott, H. Lango, N. W. Rayner, B. Shields, L. W. Harries, J. C. Barrett, S. Ellard, C. J. Groves, B. Knight, A. M. Patch, A. R. Ness, S. Ebrahim, D. A. Lawlor, S. M. Ring, Y. Ben Shlomo, M. R. Jarvelin, U. Sovio, A. J. Bennett, D. Melzer, L. Ferrucci, R. J. Loos, I. Barroso, N. J. Wareham, F. Karpe, K. R. Owen, L. R. Cardon, M. Walker, G. A. Hitman, C. N. Palmer, A. S. Doney, A. D. Morris, G. D. Smith, A. T. Hattersley, and M. I. McCarthy. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science **316**: 889-894

Frederich, R. C., A. Hamann, S. Anderson, B. Lollmann, B. B. Lowell, and J. S. Flier. 1995. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat.Med. **1**: 1311-1314

Frederiksen, L., K. Brodbaek, M. Fenger, T. Jorgensen, K. Borch-Johnsen, S. Madsbad, and S. A. Urhammer. 2002. Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome. J.Clin.Endocrinol.Metab **87**: 3989-3992

Frittitta, L., S. Camastra, R. Baratta, B. V. Costanzo, M. D'Adamo, S. Graci, D. Spampinato, B. A. Maddux, R. Vigneri, E. Ferrannini, and V. Trischitta. 1999. A soluble PC-1 circulates in human plasma: relationship with insulin resistance and associated abnormalities. J.Clin.Endocrinol.Metab **84**: 3620-3625

Frittitta, L., D. Spampinato, A. Solini, R. Nosadini, I. D. Goldfine, R. Vigneri, and V. Trischitta. 1998. Elevated PC-1 content in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects: evidence that PC-1 may be an intrinsic factor in impaired insulin receptor signaling. Diabetes **47**: 1095-1100

Furuta, M., Y. Yano, K. Ito, E. C. Gabazza, A. Katsuki, T. Tanaka, K. Ohtake, N. Hirata, Y. Hori, R. Araki-Sasaki, Y. Sumida, and Y. Adachi. 2002. Relationship of the tumor necrosis factor-alpha -308 A/G promoter polymorphism with insulin sensitivity and abdominal fat distribution in Japanese patients with type 2 diabetes mellitus. Diabetes Res.Clin.Pract. **56**: 141-145

Garant, M. J., W. H. Kao, F. Brancati, J. Coresh, T. M. Rami, C. L. Hanis, E. Boerwinkle, and A. R. Shuldiner. 2002. SNP43 of CAPN10 and the risk of type 2 Diabetes in African-Americans: the Atherosclerosis Risk in Communities Study. Diabetes **51**: 231-237

Gaudino, M., F. Andreotti, R. Zamparelli, A. Di Castelnuovo, G. Nasso, F. Burzotta, L. Iacoviello, M. B. Donati, R. Schiavello, A. Maseri, and G. Possati. 2003. The -174G/C

interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation **108 Suppl 1**: II195-II199

Georges, J. L., V. Loukaci, O. Poirier, A. Evans, G. Luc, D. Arveiler, J. B. Ruidavets, F. Cambien, and L. Tiret. 2001. Interleukin-6 gene polymorphisms and susceptibility to myocardial infarction: the ECTIM study. Etude Cas-Temoin de l'Infarctus du Myocarde. J.Mol.Med. **79**: 300-305

Gerhardt, C. C., I. A. Romero, R. Cancello, L. Camoin, and A. D. Strosberg. 2001. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Mol.Cell Endocrinol. **175**: 81-92

Gerszten, R. E., E. A. Garcia-Zepeda, Y. C. Lim, M. Yoshida, H. A. Ding, M. A. Gimbrone, Jr., A. D. Luster, F. W. Luscinskas, and A. Rosenzweig. 1999. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature **398**: 718-723

Ghosh, S. and N. J. Schork. 1996. Genetic analysis of NIDDM. The study of quantitative traits. Diabetes **45**: 1-14

Ghosh, S., R. M. Watanabe, T. T. Valle, E. R. Hauser, V. L. Magnuson, C. D. Langefeld, D. S. Ally, K. L. Mohlke, K. Silander, K. Kohtamaki, P. Chines, J. J. Balow, G. Birznieks, J. Chang, W. Eldridge, M. R. Erdos, Z. E. Karanjawala, J. I. Knapp, K. Kudelko, C. Martin, A. Morales-Mena, A. Musick, T. Musick, C. Pfahl, R. Porter, and J. B. Rayman. 2000. The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. Am.J.Hum.Genet. **67**: 1174-1185

Gloyn, A. L., Y. Hashim, S. J. Ashcroft, R. Ashfield, S. Wiltshire, and R. C. Turner. 2001. Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with Type 2 diabetes mellitus (UKPDS 53). Diabet.Med. **18**: 206-212

Gloyn, A. L., M. N. Weedon, K. R. Owen, M. J. Turner, B. A. Knight, G. Hitman, M. Walker, J. C. Levy, M. Sampson, S. Halford, M. I. McCarthy, A. T. Hattersley, and T. M. Frayling. 2003. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes **52**: 568-572

Glund, S., A. Deshmukh, Y. C. Long, T. Moller, H. A. Koistinen, K. Caidahl, J. R. Zierath, and A. Krook. 2007. Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. Diabetes **56**: 1630-1637

Gonzalez Sanchez, J. L., R. M. Serrano, P. C. Fernandez, M. Laakso, and M. T. Martinez Larrad. 2002. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. Eur.J.Endocrinol. **147**: 495-501

Gosling, J., S. Slaymaker, L. Gu, S. Tseng, C. H. Zlot, S. G. Young, B. J. Rollins, and I. F. Charo. 1999. MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. J.Clin.Invest **103**: 773-778

Grant, S. F., G. Thorleifsson, I. Reynisdottir, R. Benediktsson, A. Manolescu, J. Sainz, A. Helgason, H. Stefansson, V. Emilsson, A. Helgadottir, U. Styrkarsdottir, K. P.

Magnusson, G. B. Walters, E. Palsdottir, T. Jonsdottir, T. Gudmundsdottir, A. Gylfason, J. Saemundsdottir, R. L. Wilensky, M. P. Reilly, D. J. Rader, Y. Bagger, C. Christiansen, V. Gudnason, G. Sigurdsson, U. Thorsteinsdottir, J. R. Gulcher, A. Kong, and K. Stefansson. 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat.Genet. **38**: 320-323

Greenberg, A. S., W. J. Shen, K. Muliro, S. Patel, S. C. Souza, R. A. Roth, and F. B. Kraemer. 2001. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. J.Biol.Chem. **276**: 45456-45461

Greenhalgh, P. M. 1997. Diabetes in British south Asians: nature, nurture, and culture. Diabet.Med. **14**: 10-18

Griffin, T. J. and L. M. Smith. 2000. Single-nucleotide polymorphism analysis by MALDI-TOF mass spectrometry. Trends Biotechnol. **18**: 77-84

Groop, L. C. and T. Tuomi. 1997. Non-insulin-dependent diabetes mellitus--a collision between thrifty genes and an affluent society. Ann.Med. **29**: 37-53

Groves, C. J., S. Wiltshire, D. Smedley, K. R. Owen, T. M. Frayling, M. Walker, G. A. Hitman, J. C. Levy, S. O'Rahilly, S. Menzel, A. T. Hattersley, and M. I. McCarthy. 2003. Association and haplotype analysis of the insulin-degrading enzyme (IDE) gene, a strong positional and biological candidate for type 2 diabetes susceptibility. Diabetes **52**: 1300-1305

Groves, C. J., E. Zeggini, J. Minton, T. M. Frayling, M. N. Weedon, N. W. Rayner, G. A. Hitman, M. Walker, S. Wiltshire, A. T. Hattersley, and M. I. McCarthy. 2006. Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes **55**: 2640-2644

Grundy, S. M., J. I. Cleeman, S. R. Daniels, K. A. Donato, R. H. Eckel, B. A. Franklin, D. J. Gordon, R. M. Krauss, P. J. Savage, S. C. Smith, Jr., J. A. Spertus, and F. Costa. 2005. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation **112**: 2735-2752

Grunfeld, C., S. Adi, M. Soued, A. Moser, W. Fiers, and K. R. Feingold. 1990. Search for mediators of the lipogenic effects of tumor necrosis factor: potential role for interleukin 6. Cancer Res. **50**: 4233-4238

Gudis, K., A. Tatsuguchi, K. Wada, S. Futagami, K. Nagata, T. Hiratsuka, Y. Shinji, K. Miyake, T. Tsukui, Y. Fukuda, and C. Sakamoto. 2005. Microsomal prostaglandin E synthase (mPGES)-1, mPGES-2 and cytosolic PGES expression in human gastritis and gastric ulcer tissue. Lab Invest **85**: 225-236

Gupta, A., R. Gupta, M. Sarna, S. Rastogi, V. P. Gupta, and K. Kothari. 2003. Prevalence of diabetes, impaired fasting glucose and insulin resistance syndrome in an urban Indian population. Diabetes Res.Clin.Pract. **61**: 69-76

Gut, I. G. 2001. Automation in genotyping of single nucleotide polymorphisms. Hum.Mutat. **17**: 475-492

Hak, A. E., C. D. Stehouwer, M. L. Bots, K. H. Polderman, C. G. Schalkwijk, I. C. Westendorp, A. Hofman, and J. C. Witteman. 1999. Associations of C-reactive protein with

measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. Arterioscler.Thromb.Vasc.Biol. **19**: 1986-1991

Hallaq, H., E. Pinter, J. Enciso, J. McGrath, C. Zeiss, M. Brueckner, J. Madri, H. C. Jacobs, C. M. Wilson, H. Vasavada, X. Jiang, and C. W. Bogue. 2004. A null mutation of Hhex results in abnormal cardiac development, defective vasculogenesis and elevated Vegfa levels. Development **131**: 5197-5209

Halldorsson, B. V., S. Istrail, and F. M. De La Vega. 2004. Optimal selection of SNP markers for disease association studies. Hum.Hered. **58**: 190-202

Halle, M., A. Berg, M. W. Baumstark, D. Konig, M. Huonker, and J. Keul. 1999. Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels. Atherosclerosis **143**: 185-192

Haluzik, M. and D. Haluzikova. 2006. The role of resistin in obesity-induced insulin resistance. Curr.Opin.Investig.Drugs **7**: 306-311

Hamid, Y. H., C. S. Rose, S. A. Urhammer, C. Glumer, R. Nolsoe, O. P. Kristiansen, T. Mandrup-Poulsen, K. Borch-Johnsen, T. Jorgensen, T. Hansen, and O. Pedersen. 2005. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia **48**: 251-260

Hani, E. H., P. Boutin, E. Durand, H. Inoue, M. A. Permutt, G. Velho, and P. Froguel. 1998. Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia **41**: 1511-1515

Hanis, C. L., E. Boerwinkle, R. Chakraborty, D. L. Ellsworth, P. Concannon, B. Stirling, V. A. Morrison, B. Wapelhorst, R. S. Spielman, K. J. Gogolin-Ewens, J. M. Shepard, S. R. Williams, N. Risch, D. Hinds, N. Iwasaki, M. Ogata, Y. Omori, C. Petzold, H. Rietzch, H. E. Schroder, J. Schulze, N. J. Cox, S. Menzel, V. V. Boriraj, X. Chen, L. R. Lim, T. Lindner, L. E. Mereu, Y. Q. Wang, K. Xiang, K. Yamagata, Y. Yang, and G. I. Bell. 1996. A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nat.Genet. **13**: 161-166

Hanley, A. J., A. J. Karter, K. Williams, A. Festa, R. B. D'Agostino, Jr., L. E. Wagenknecht, and S. M. Haffner. 2005. Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study. Circulation **112**: 3713-3721

Hansen, T., L. Ambye, N. Grarup, L. Hansen, S. M. Echwald, J. Ferrer, and O. Pedersen. 2001. Genetic variability of the SUR1 promoter in relation to beta-cell function and Type II diabetes mellitus. Diabetologia **44**: 1330-1334

Hanson, R. L., M. G. Ehm, D. J. Pettitt, M. Prochazka, D. B. Thompson, D. Timberlake, T. Foroud, S. Kobes, L. Baier, D. K. Burns, L. Almasy, J. Blangero, W. T. Garvey, P. H. Bennett, and W. C. Knowler. 1998. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am.J.Hum.Genet. **63**: 1130-1138

Hansson, G. K., L. Jonasson, P. S. Seifert, and S. Stemme. 1989. Immune mechanisms in atherosclerosis. Arteriosclerosis **9**: 567-578

Hardy, G. H. 2003. Mendelian proportions in a mixed population. 1908. Yale J.Biol.Med. **76**: 79-80

Harris, T. B., L. Ferrucci, R. P. Tracy, M. C. Corti, S. Wacholder, W. H. Ettinger, Jr., H. Heimovitz, H. J. Cohen, and R. Wallace. 1999. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am.J.Med. **106**: 506-512

Hegele, R. A., S. B. Harris, B. Zinman, A. J. Hanley, and H. Cao. 2001. Absence of association of type 2 diabetes with CAPN10 and PC-1 polymorphisms in Oji-Cree. Diabetes Care **24**: 1498-1499

Heid, I. M., S. A. Wagner, H. Gohlke, B. Iglseder, J. C. Mueller, P. Cip, G. Ladurner, R. Reiter, A. Stadlmayr, V. Mackevics, T. Illig, F. Kronenberg, and B. Paulweber. 2006b. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes **55**: 375-384

Heid, I. M., S. A. Wagner, H. Gohlke, B. Iglseder, J. C. Mueller, P. Cip, G. Ladurner, R. Reiter, A. Stadlmayr, V. Mackevics, T. Illig, F. Kronenberg, and B. Paulweber. 2006a. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes **55**: 375-384

Herbert, A., C. Liu, S. Karamohamed, J. Liu, A. Manning, C. S. Fox, J. B. Meigs, and L. A. Cupples. 2006. BMI modifies associations of IL-6 genotypes with insulin resistance: the Framingham Study. Obesity.(Silver.Spring) **14**: 1454-1461

Herder, C., J. Baumert, B. Thorand, W. Koenig, W. de Jager, C. Meisinger, T. Illig, S. Martin, and H. Kolb. 2006a. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetologia **49**: 921-929

Herder, C., T. Illig, W. Rathmann, S. Martin, B. Haastert, S. Muller-Scholze, R. Holle, B. Thorand, W. Koenig, H. E. Wichmann, and H. Kolb. 2005. Inflammation and type 2 diabetes: results from KORA Augsburg. Gesundheitswesen **67 Suppl 1**: S115-S121

Herder, C., S. Muller-Scholze, P. Rating, W. Koenig, B. Thorand, B. Haastert, R. Holle, T. Illig, W. Rathmann, J. Seissler, H. E. Wichmann, and H. Kolb. 2006b. Systemic monocyte chemoattractant protein-1 concentrations are independent of type 2 diabetes or parameters of obesity: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Eur.J.Endocrinol. **154**: 311-317

Herrmann, S. M., S. Ricard, V. Nicaud, C. Mallet, D. Arveiler, A. Evans, J. B. Ruidavets, G. Luc, L. Bara, H. J. Parra, O. Poirier, and F. Cambien. 1998. Polymorphisms of the tumour necrosis factor-alpha gene, coronary heart disease and obesity. Eur.J.Clin.Invest **28**: 59-66

Hillenkamp, F., M. Karas, R. C. Beavis, and B. T. Chait. 1991. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. Anal.Chem. **63**: 1193A-1203A

Hoffstedt, J., P. Eriksson, L. Hellstrom, S. Rossner, M. Ryden, and P. Arner. 2000. Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. Diabetologia **43**: 117-120

Holle, R., M. Happich, H. Lowel, and H. E. Wichmann. 2005. KORA--a research platform for population based health research. Gesundheitswesen **67 Suppl 1**: S19-S25

Hong, Y., N. L. Pedersen, K. Brismar, and U. de Faire. 1997. Genetic and environmental architecture of the features of the insulin-resistance syndrome. Am.J.Hum.Genet. **60**: 143-152

Hong, Y., T. Rice, J. Gagnon, J. P. Despres, A. Nadeau, L. Perusse, C. Bouchard, A. S. Leon, J. S. Skinner, J. H. Wilmore, and D. C. Rao. 1998. Familial clustering of insulin and abdominal visceral fat: the HERITAGE Family Study. J.Clin.Endocrinol.Metab **83**: 4239-4245

Horikawa, Y., N. Oda, N. J. Cox, X. Li, M. Orho-Melander, M. Hara, Y. Hinokio, T. H. Lindner, H. Mashima, P. E. Schwarz, L. Bosque-Plata, Y. Horikawa, Y. Oda, I. Yoshiuchi, S. Colilla, K. S. Polonsky, S. Wei, P. Concannon, N. Iwasaki, J. Schulze, L. J. Baier, C. Bogardus, L. Groop, E. Boerwinkle, C. L. Hanis, and G. I. Bell. 2000. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat.Genet. **26**: 163-175

Hotamisligil, G. S. 2006. Inflammation and metabolic disorders. Nature **444**: 860-867

Hsueh, W. C., S. A. Cole, A. R. Shuldiner, B. A. Beamer, J. Blangero, J. E. Hixson, J. W. MacCluer, and B. D. Mitchell. 2001. Interactions between variants in the beta3-adrenergic receptor and peroxisome proliferator-activated receptor-gamma2 genes and obesity. Diabetes Care **24**: 672-677

Hu, F. B., J. B. Meigs, T. Y. Li, N. Rifai, and J. E. Manson. 2004. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes **53**: 693-700

Humphries, S. E., D. Gable, J. A. Cooper, H. Ireland, J. W. Stephens, S. J. Hurel, K. W. Li, J. Palmen, M. A. Miller, F. P. Cappuccio, R. Elkeles, I. Godsland, G. J. Miller, and P. J. Talmud. 2006. Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. J.Mol.Med. **84**: 1-10

Hurlstone, A. and H. Clevers. 2002. T-cell factors: turn-ons and turn-offs. EMBO J. **21**: 2303-2311

Hutley, L. and J. B. Prins. 2005. Fat as an endocrine organ: relationship to the metabolic syndrome. Am.J.Med.Sci. **330**: 280-289

Huxtable, S. J., P. J. Saker, L. Haddad, M. Walker, T. M. Frayling, J. C. Levy, G. A. Hitman, S. O'Rahilly, A. T. Hattersley, and M. I. McCarthy. 2000. Analysis of parent-offspring trios provides evidence for linkage and association between the insulin gene and type 2 diabetes mediated exclusively through paternally transmitted class III variable number tandem repeat alleles. Diabetes **49**: 126-130

IDF. 2003. Diabetes Atlas 2003.

Inagaki, N., T. Gonoi, J. P. Clement, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino, and J. Bryan. 1995. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science **270**: 1166-1170

Ioannidis, J. P., E. E. Ntzani, T. A. Trikalinos, and D. G. Contopoulos-Ioannidis. 2001. Replication validity of genetic association studies. Nat.Genet. **29**: 306-309

Isomaa, B., P. Almgren, T. Tuomi, B. Forsen, K. Lahti, M. Nissen, M. R. Taskinen, and L. Groop. 2001. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care **24**: 683-689

Jakel, H., M. Nowak, A. Helleboid-Chapman, J. Fruchart-Najib, and J. C. Fruchart. 2006. Is apolipoprotein A5 a novel regulator of triglyceride-rich lipoproteins? Ann.Med. **38**: 2-10

Jakobsson, P. J., S. Thoren, R. Morgenstern, and B. Samuelsson. 1999. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc.Natl.Acad.Sci.U.S.A **96**: 7220-7225

Jang, Y., J. H. Lee, O. Y. Kim, S. J. Koh, J. S. Chae, J. H. Woo, H. Cho, J. E. Lee, and J. M. Ordovas. 2006. The SNP276G>T polymorphism in the adiponectin (ACDC) gene is more strongly associated with insulin resistance and cardiovascular disease risk than SNP45T>G in nonobese/nondiabetic Korean men independent of abdominal adiposity and circulating plasma adiponectin. Metabolism **55**: 59-66

Jellema, A., M. P. Zeegers, E. J. Feskens, P. C. Dagnelie, and R. P. Mensink. 2003. Gly972Arg variant in the insulin receptor substrate-1 gene and association with Type 2 diabetes: a meta-analysis of 27 studies. Diabetologia **46**: 990-995

Jensen, M. D., M. Caruso, V. Heiling, and J. M. Miles. 1989. Insulin regulation of lipolysis in nondiabetic and IDDM subjects. Diabetes **38**: 1595-1601

Johnson, G. C., L. Esposito, B. J. Barratt, A. N. Smith, J. Heward, G. Di Genova, H. Ueda, H. J. Cordell, I. A. Eaves, F. Dudbridge, R. C. Twells, F. Payne, W. Hughes, S. Nutland, H. Stevens, P. Carr, E. Tuomilehto-Wolf, J. Tuomilehto, S. C. Gough, D. G. Clayton, and J. A. Todd. 2001. Haplotype tagging for the identification of common disease genes. Nat.Genet. **29**: 233-237

Jones, K. G., D. J. Brull, L. C. Brown, M. Sian, R. M. Greenhalgh, S. E. Humphries, and J. T. Powell. 2001. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation **103**: 2260-2265

Joven, J., B. Coll, M. Tous, N. Ferre, C. Alonso-Villaverde, S. Parra, and J. Camps. 2006. The influence of HIV infection on the correlation between plasma concentrations of monocyte chemoattractant protein-1 and carotid atherosclerosis. Clin.Chim.Acta **368**: 114-119

Julius, U. 2003. Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Exp.Clin.Endocrinol.Diabetes **111**: 246-250

Kahn, R., J. Buse, E. Ferrannini, and M. Stern. 2005. The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia **48**: 1684-1699

Kakuma, T., Y. Lee, M. Higa, Z. Wang, W. Pan, I. Shimomura, and R. H. Unger. 2000. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. Proc.Natl.Acad.Sci.U.S.A **97**: 8536-8541

Kamimura, D., K. Ishihara, and T. Hirano. 2003. IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev.Physiol Biochem.Pharmacol. **149**: 1-38

Kanda, H., S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, and M. Kasuga. 2006. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J.Clin.Invest **116**: 1494-1505

Kantartzis, K., A. Fritsche, F. Machicao, H. U. Haring, and N. Stefan. 2006. The -8503 G/A polymorphism of the adiponectin receptor 1 gene is associated with insulin sensitivity dependent on adiposity. Diabetes Care **29**: 464

Kaplan, N. M. 1989. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch.Intern.Med. **149**: 1514-1520

Kaprio, J., J. Tuomilehto, M. Koskenvuo, K. Romanov, A. Reunanen, J. Eriksson, J. Stengard, and Y. A. Kesaniemi. 1992. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia **35**: 1060-1067

Karamohamed, S., S. Demissie, J. Volcjak, C. Liu, N. Heard-Costa, J. Liu, C. M. Shoemaker, C. I. Panhuysen, J. B. Meigs, P. Wilson, L. D. Atwood, L. A. Cupples, and A. Herbert. 2003. Polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes in men from the NHLBI Framingham Heart Study. Diabetes **52**: 1562-1567

Karas, M. and F. Hillenkamp. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal.Chem. **60**: 2299-2301

Kawamura, T., G. Egusa, M. Okubo, M. Imazu, and M. Yamakido. 1999. Association of beta3-adrenergic receptor gene polymorphism with insulin resistance in Japanese-American men. Metabolism **48**: 1367-1370

Keil, U., A. D. Liese, H. W. Hense, B. Filipiak, A. Doring, J. Stieber, and H. Lowel. 1998. Classical risk factors and their impact on incident non-fatal and fatal myocardial infarction and all-cause mortality in southern Germany. Results from the MONICA Augsburg cohort study 1984-1992. Monitoring Trends and Determinants in Cardiovascular Diseases. Eur Heart J **19**: 1197-1207

Kern, P. A., G. B. Di Gregorio, T. Lu, N. Rassouli, and G. Ranganathan. 2003. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes **52**: 1779-1785

Kikuchi, A., S. Kishida, and H. Yamamoto. 2006. Regulation of Wnt signaling by protein-protein interaction and post-translational modifications. Exp.Mol.Med. **38**: 1-10

Kim, K. H., K. Lee, Y. S. Moon, and H. S. Sul. 2001. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol.Chem. **276**: 11252-11256

Kim, M. S., C. J. Day, and N. A. Morrison. 2005. MCP-1 is induced by receptor activator of nuclear factor-{kappa}B ligand, promotes human osteoclast fusion, and rescues granulocyte macrophage colony-stimulating factor suppression of osteoclast formation. J.Biol.Chem. **280**: 16163-16169

Kim-Motoyama, H., K. Yasuda, T. Yamaguchi, N. Yamada, T. Katakura, A. R. Shuldiner, Y. Akanuma, Y. Ohashi, Y. Yazaki, and T. Kadowaki. 1997. A mutation of the beta 3-adrenergic receptor is associated with visceral obesity but decreased serum triglyceride. Diabetologia **40**: 469-472

King, H., R. E. Aubert, and W. H. Herman. 1998. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care **21**: 1414-1431

King, H. and M. Rewers. 1993. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. Diabetes Care **16**: 157-177

Kirpekar, F., E. Nordhoff, L. K. Larsen, K. Kristiansen, P. Roepstorff, and F. Hillenkamp. 1998. DNA sequence analysis by MALDI mass spectrometry. Nucleic Acids Res. **26**: 2554-2559

Kissebah, A. H., G. E. Sonnenberg, J. Myklebust, M. Goldstein, K. Broman, R. G. James, J. A. Marks, G. R. Krakower, H. J. Jacob, J. Weber, L. Martin, J. Blangero, and A. G. Comuzzie. 2000. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc.Natl.Acad.Sci.U.S.A **97**: 14478-14483

Klipstein-Grobusch, K., M. Mohlig, J. Spranger, K. Hoffmann, F. U. Rodrigues, A. M. Sharma, S. Klaus, A. F. Pfeiffer, and H. Boeing. 2006. Interleukin-6 g.-174G>C promoter polymorphism is associated with obesity in the EPIC-Potsdam Study. Obesity.(Silver.Spring) **14**: 14-18

Knowler, W. C., R. C. Williams, D. J. Pettitt, and A. G. Steinberg. 1988. Gm3;5,13,14 and type 2 diabetes mellitus: an association in American Indians with genetic admixture. Am.J.Hum.Genet. **43**: 520-526

Koebnick, C., K. Wagner, A. L. Garcia, S. Gruendel, P. H. Lahmann, M. O. Weickert, M. Mohlig, I. A. Harsch, C. Einig, M. Speth, N. Katz, U. Trippo, and H. J. Zunft. 2006. Increase in serum resistin during weight loss in overweight subjects is related to lipid metabolism. Int.J.Obes.(Lond) **30**: 1097-1103

Kowalski, J., B. Okopien, A. Madej, K. Makowiecka, M. Zielinski, Z. Kalina, and Z. S. Herman. 2001. Levels of sICAM-1, sVCAM-1 and MCP-1 in patients with hyperlipoproteinemia IIa and -IIb. Int.J.Clin.Pharmacol.Ther. **39**: 48-52

Kozma, G. T., A. Falus, A. Bojszko, D. Krikovszky, T. Szabo, A. Nagy, and C. Szalai. 2002. Lack of association between atopic eczema/dermatitis syndrome and polymorphisms in the promoter region of RANTES and regulatory region of MCP-1. Allergy **57**: 160-163

Kragelund, B. B., J. Knudsen, and F. M. Poulsen. 1999. Acyl-coenzyme A binding protein (ACBP). Biochim.Biophys.Acta **1441**: 150-161

Kraja, A. T., S. C. Hunt, J. S. Pankow, R. H. Myers, G. Heiss, C. E. Lewis, D. Rao, and M. A. Province. 2005a. An evaluation of the metabolic syndrome in the HyperGEN study. Nutr.Metab (Lond) **2**: 2

Kraja, A. T., D. C. Rao, A. B. Weder, R. Cooper, J. D. Curb, C. L. Hanis, S. T. Turner, M. de Andrade, C. A. Hsiung, T. Quertermous, X. Zhu, and M. A. Province. 2005b. Two major QTLs and several others relate to factors of metabolic syndrome in the family blood pressure program. Hypertension **46**: 751-757

Krief, S., F. Lonnqvist, S. Raimbault, B. Baude, A. Van Spronsen, P. Arner, A. D. Strosberg, D. Ricquier, and L. J. Emorine. 1993. Tissue distribution of beta 3-adrenergic receptor mRNA in man. J.Clin.Invest **91**: 344-349

Kristiansen, O. P. and T. Mandrup-Poulsen. 2005. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes **54 Suppl 2**: S114-S124

Kruglyak, L. and D. A. Nickerson. 2001. Variation is the spice of life. Nat.Genet. **27**: 234-236

Kunnari, A., O. Ukkola, and Y. A. Kesaniemi. 2005. Resistin polymorphisms are associated with cerebrovascular disease in Finnish Type 2 diabetic patients. Diabet.Med. **22**: 583-589

Kuroda, S., T. Uzu, T. Fujii, M. Nishimura, S. Nakamura, T. Inenaga, and G. Kimura. 1999. Role of insulin resistance in the genesis of sodium sensitivity in essential hypertension. J.Hum.Hypertens. **13**: 257-262

Kusminski, C. M., P. G. McTernan, and S. Kumar. 2005. Role of resistin in obesity, insulin resistance and Type II diabetes. Clin.Sci.(Lond) **109**: 243-256

Kwiterovich, P. O., Jr. 2002. Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity. Am.J.Cardiol. **90**: 30i-47i

Laaksonen, D. E., H. M. Lakka, L. K. Niskanen, G. A. Kaplan, J. T. Salonen, and T. A. Lakka. 2002. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. Am.J.Epidemiol. **156**: 1070-1077

Laclaustra, M., D. Corella, and J. M. Ordovas. 2007. Metabolic syndrome pathophysiology: the role of adipose tissue. Nutr.Metab Cardiovasc.Dis. **17**: 125-139

Lafontan, M. 1994. Differential recruitment and differential regulation by physiological amines of fat cell beta-1, beta-2 and beta-3 adrenergic receptors expressed in native fat cells and in transfected cell lines. Cell Signal. **6**: 363-392

Lakka, H. M., L. Oksanen, T. P. Tuomainen, K. Kontula, and J. T. Salonen. 2000. The common pentanucleotide polymorphism of the 3'-untranslated region of the leptin receptor gene is associated with serum insulin levels and the risk of type 2 diabetes in non-diabetic men: a prospective case-control study. J.Intern.Med. **248**: 77-83

Lee, S. C., Y. B. Pu, G. N. Thomas, Z. S. Lee, B. Tomlinson, C. S. Cockram, J. A. Critchley, and J. C. Chan. 2000. Tumor necrosis factor alpha gene G-308A polymorphism in the metabolic syndrome. Metabolism **49**: 1021-1024

Lee, W. L., W. J. Lee, Y. T. Chen, T. J. Liu, K. W. Liang, C. T. Ting, and S. W. Huey-Herng. 2006. The presence of metabolic syndrome is independently associated with elevated serum CD40 ligand and disease severity in patients with symptomatic coronary artery disease. Metabolism **55**: 1029-1034

Lee, Y., H. Hirose, M. Ohneda, J. H. Johnson, J. D. McGarry, and R. H. Unger. 1994. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. Proc.Natl.Acad.Sci.U.S.A **91**: 10878-10882

Legro, R. S., V. D. Castracane, and R. P. Kauffman. 2004. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstet.Gynecol.Surv. **59**: 141-154

Lehman, D. M., R. Arya, J. Blangero, L. Almasy, S. Puppala, T. D. Dyer, R. J. Leach, P. O'Connell, M. P. Stern, and R. Duggirala. 2005. Bivariate linkage analysis of the insulin resistance syndrome phenotypes on chromosome 7q. Hum.Biol. **77**: 231-246

Lesage, S., E. H. Hani, A. Philippi, M. Vaxillaire, J. Hager, P. Passa, F. Demenais, P. Froguel, and N. Vionnet. 1995. Linkage analyses of the MODY3 locus on chromosome 12q with late-onset NIDDM. Diabetes **44**: 1243-1247

Leushner, J. 2001. MALDI TOF mass spectrometry: an emerging platform for genomics and diagnostics. Expert.Rev.Mol.Diagn. **1**: 11-18

Lewis, G. F. 2002. Lipid metabolism. Curr.Opin.Lipidol. **13**: 97-99

Lewis, G. F. and G. Steiner. 1996. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. Diabetes Care **19**: 390-393

Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J.Clin.Invest **95**: 158-166

Li, J. K., M. C. Ng, W. Y. So, C. K. Chiu, R. Ozaki, P. C. Tong, C. S. Cockram, and J. C. Chan. 2006. Phenotypic and genetic clustering of diabetes and metabolic syndrome in Chinese families with type 2 diabetes mellitus. Diabetes Metab Res.Rev. **22**: 46-52

Libra, M., S. S. Signorelli, Y. Bevelacqua, P. M. Navolanic, V. Bevelacqua, J. Polesel, R. Talamini, F. Stivala, M. C. Mazzarino, and G. Malaponte. 2006. Analysis of G(-174)C IL-6 polymorphism and plasma concentrations of inflammatory markers in patients with type 2 diabetes and peripheral arterial disease. J.Clin.Pathol. **59**: 211-215

Lichten, M. and A. S. Goldman. 1995. Meiotic recombination hotspots. Annu.Rev.Genet. **29**: 423-444

Liese, A. D., E. J. Mayer-Davis, H. A. Tyroler, C. E. Davis, U. Keil, M. I. Schmidt, F. L. Brancati, and G. Heiss. 1997. Familial components of the multiple metabolic syndrome: the ARIC study. Diabetologia **40**: 963-970

Lindgren, C. M., M. M. Mahtani, E. Widen, M. I. McCarthy, M. J. Daly, A. Kirby, M. P. Reeve, L. Kruglyak, A. Parker, J. Meyer, P. Almgren, M. Lehto, T. Kanninen, T. Tuomi, L. C. Groop, and E. S. Lander. 2002. Genomewide search for type 2 diabetes mellitus susceptibility loci in Finnish families: the Botnia study. Am.J.Hum.Genet. **70**: 509-516

Lindsay, R. S., D. J. Wake, S. Nair, J. Bunt, D. E. Livingstone, P. A. Permana, P. A. Tataranni, and B. R. Walker. 2003. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. J.Clin.Endocrinol.Metab **88**: 2738-2744

Lissner, L., C. Bjorkelund, B. L. Heitmann, J. C. Seidell, and C. Bengtsson. 2001. Larger hip circumference independently predicts health and longevity in a Swedish female cohort. Obes.Res. **9**: 644-646

Little, D. P., A. Braun, B. Darnhofer-Demar, A. Frilling, Y. Li, R. T. McIver, Jr., and H. Koster. 1997a. Detection of RET proto-oncogene codon 634 mutations using mass spectrometry. J.Mol.Med. **75**: 745-750

Little, D. P., A. Braun, B. Darnhofer-Demar, and H. Koster. 1997b. Identification of apolipoprotein E polymorphisms using temperature cycled primer oligo base extension and mass spectrometry. Eur.J.Clin.Chem.Clin.Biochem. **35**: 545-548

Liu, M. Y., A. M. Xydakis, R. C. Hoogeveen, P. H. Jones, E. O. Smith, K. W. Nelson, and C. M. Ballantyne. 2005b. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. Clin Chem. **51**: 1102-1109

Liu, M. Y., A. M. Xydakis, R. C. Hoogeveen, P. H. Jones, E. O. Smith, K. W. Nelson, and C. M. Ballantyne. 2005a. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. Clin.Chem. **51**: 1102-1109

Loos, R. J., P. T. Katzmarzyk, D. C. Rao, T. Rice, A. S. Leon, J. S. Skinner, J. H. Wilmore, T. Rankinen, and C. Bouchard. 2003. Genome-wide linkage scan for the metabolic syndrome in the HERITAGE Family Study. J.Clin.Endocrinol.Metab **88**: 5935-5943

Love-Gregory, L., J. Wasson, J. Lin, G. Skolnick, B. Suarez, and M. A. Permutt. 2003. E23K single nucleotide polymorphism in the islet ATP-sensitive potassium channel gene (Kir6.2) contributes as much to the risk of Type II diabetes in Caucasians as the PPARgamma Pro12Ala variant. Diabetologia **46**: 136-137

Love-Gregory, L. D., J. Wasson, J. Ma, C. H. Jin, B. Glaser, B. K. Suarez, and M. A. Permutt. 2004. A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 alpha gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an ashkenazi jewish population. Diabetes **53**: 1134-1140

Lu, H. L., H. W. Wang, Y. Wen, M. X. Zhang, and H. H. Lin. 2006. Roles of adipocyte derived hormone adiponectin and resistin in insulin resistance of type 2 diabetes. World J.Gastroenterol. **12**: 1747-1751

Luo, T. H., Y. Zhao, G. Li, W. T. Yuan, J. J. Zhao, J. L. Chen, W. Huang, and M. Luo. 2001. A genome-wide search for type II diabetes susceptibility genes in Chinese Hans. Diabetologia **44**: 501-506

Lyngso, D., L. Simonsen, and J. Bulow. 2002. Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. J.Physiol **543**: 379-386

Maddux, B. A. and I. D. Goldfine. 2000. Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha-subunit. Diabetes **49**: 13-19

Mahtani, M. M., E. Widen, M. Lehto, J. Thomas, M. McCarthy, J. Brayer, B. Bryant, G. Chan, M. Daly, C. Forsblom, T. Kanninen, A. Kirby, L. Kruglyak, K. Munnelly, M. Parkkonen, M. P. Reeve-Daly, A. Weaver, T. Brettin, G. Duyk, E. S. Lander, and L. C. Groop. 1996. Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat.Genet. **14**: 90-94

Manolagas, S. C. 1998. The role of IL-6 type cytokines and their receptors in bone. Ann.N.Y.Acad.Sci. **840**: 194-204

Manzato, E., S. Zambon, A. Zambon, A. Cortella, G. Sartore, and G. Crepaldi. 1993. Levels and physicochemical properties of lipoprotein subclasses in moderate hypertriglyceridemia. Clin.Chim.Acta **219**: 57-65

Mar, R., P. Pajukanta, H. Allayee, M. Groenendijk, G. Dallinga-Thie, R. M. Krauss, J. S. Sinsheimer, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2004. Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. Circ.Res. **94**: 993-999

Marso, S. P., J. W. Murphy, J. A. House, D. M. Safley, and W. S. Harris. 2005. Metabolic syndrome-mediated inflammation following elective percutaneous coronary intervention. Diab.Vasc.Dis.Res. **2**: 31-36

Martin, S., V. Nicaud, S. E. Humphries, and P. J. Talmud. 2003. Contribution of APOA5 gene variants to plasma triglyceride determination and to the response to both fat and glucose tolerance challenges. Biochim.Biophys.Acta **1637**: 217-225

Mather, H. M. and H. Keen. 1985. The Southall Diabetes Survey: prevalence of known diabetes in Asians and Europeans. Br.Med.J.(Clin.Res.Ed) **291**: 1081-1084

Matsuda, A. and T. Kuzuya. 1994. Diabetic twins in Japan. Diabetes Res.Clin.Pract. **24 Suppl**: S63-S67

Mayer, E. J., B. Newman, M. A. Austin, D. Zhang, C. P. Quesenberry, Jr., K. Edwards, and J. V. Selby. 1996. Genetic and environmental influences on insulin levels and the insulin resistance syndrome: an analysis of women twins. Am.J.Epidemiol. **143**: 323-332

McCarthy, M. 1998. Weighing in on diabetes risk. Nat.Genet. **19**: 209-210

McDermott, D. H., Q. Yang, S. Kathiresan, L. A. Cupples, J. M. Massaro, J. F. Keaney, Jr., M. G. Larson, R. S. Vasan, J. N. Hirschhorn, C. J. O'Donnell, P. M. Murphy, and E. J. Benjamin. 2005. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. Circulation **112**: 1113-1120

Medici, F., M. Hawa, A. Ianari, D. A. Pyke, and R. D. Leslie. 1999. Concordance rate for type II diabetes mellitus in monozygotic twins: actuarial analysis. Diabetologia **42**: 146-150

Medina, J., L. I. Fernandez-Salazar, L. Garcia-Buey, and R. Moreno-Otero. 2004. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes Care **27**: 2057-2066

Meigs, J. B., L. A. Cupples, and P. W. Wilson. 2000. Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes **49**: 2201-2207

Meigs, J. B., R. B. D'Agostino, Sr., P. W. Wilson, L. A. Cupples, D. M. Nathan, and D. E. Singer. 1997. Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. Diabetes **46**: 1594-1600

Meigs, J. B., C. I. Panhuysen, R. H. Myers, P. W. Wilson, and L. A. Cupples. 2002. A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees: The Framingham Offspring Study. Diabetes **51**: 833-840

Meigs, J. B., P. W. Wilson, D. M. Nathan, R. B. D'Agostino, Sr., K. Williams, and S. M. Haffner. 2003. Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring Studies. Diabetes **52**: 2160-2167

Meirhaeghe, A., D. Cottel, P. Amouyel, and J. Dallongeville. 2005. Association between peroxisome proliferator-activated receptor gamma haplotypes and the metabolic syndrome in French men and women. Diabetes **54**: 3043-3048

Meng, Z. X., J. X. Sun, J. J. Ling, J. H. Lv, D. Y. Zhu, Q. Chen, Y. J. Sun, and X. Han. 2006. Prostaglandin E2 regulates Foxo activity via the Akt pathway: implications for pancreatic islet beta cell dysfunction. Diabetologia **49**: 2959-2968

Menzaghi, C., A. Coco, L. Salvemini, R. Thompson, S. De Cosmo, A. Doria, and V. Trischitta. 2006. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. J.Clin.Endocrinol.Metab **91**: 2792-2795

Merkel, M. and J. Heeren. 2005. Give me A5 for lipoprotein hydrolysis! J.Clin.Invest **115**: 2694-2696

Miller, M., J. Rhyne, H. Chen, V. Beach, R. Ericson, K. Luthra, M. Dwivedi, and A. Misra. 2007. APOC3 promoter polymorphisms C-482T and T-455C are associated with the metabolic syndrome. Arch.Med.Res. **38**: 444-451

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. **16**: 1215

Mine, S., Y. Okada, T. Tanikawa, C. Kawahara, T. Tabata, and Y. Tanaka. 2006. Increased expression levels of monocyte CCR2 and monocyte chemoattractant protein-1 in patients with diabetes mellitus. Biochem.Biophys.Res.Commun. **344**: 780-785

Minokoshi, Y. and B. B. Kahn. 2003. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem.Soc.Trans. **31**: 196-201

Mitchell, B. D., C. M. Kammerer, P. O'Connell, C. R. Harrison, M. Manire, P. Shipman, M. P. Moyer, M. P. Stern, and M. L. Frazier. 1995. Evidence for linkage of postchallenge insulin levels with intestinal fatty acid-binding protein (FABP2) in Mexican-Americans. Diabetes **44**: 1046-1053

Molbak, A. G., B. Christau, B. Marner, K. Borch-Johnsen, and J. Nerup. 1994. Incidence of insulin-dependent diabetes mellitus in age groups over 30 years in Denmark. Diabet.Med. **11**: 650-655

Mori, Y., S. Otabe, C. Dina, K. Yasuda, C. Populaire, C. Lecoeur, V. Vatin, E. Durand, K. Hara, T. Okada, K. Tobe, P. Boutin, T. Kadowaki, and P. Froguel. 2002. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes **51**: 1247-1255

Morton, N. E. and A. Collins. 1998. Tests and estimates of allelic association in complex inheritance. Proc.Natl.Acad.Sci.U.S.A **95**: 11389-11393

Murakami, M. and I. Kudo. 2004. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog.Lipid Res. **43**: 3-35

Murakami, M., K. Nakashima, D. Kamei, S. Masuda, Y. Ishikawa, T. Ishii, Y. Ohmiya, K. Watanabe, and I. Kudo. 2003. Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J.Biol.Chem. **278**: 37937-37947

Murakami, M., H. Naraba, T. Tanioka, N. Semmyo, Y. Nakatani, F. Kojima, T. Ikeda, M. Fueki, A. Ueno, S. Oh, and I. Kudo. 2000. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J.Biol.Chem. **275**: 32783-32792

Murakami, T., S. Michelagnoli, R. Longhi, G. Gianfranceschi, F. Pazzucconi, L. Calabresi, C. R. Sirtori, and G. Franceschini. 1995. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. Arterioscler.Thromb.Vasc.Biol. **15**: 1819-1828

Nagaev, I., M. Bokarewa, A. Tarkowski, and U. Smith. 2006. Human Resistin Is a Systemic Immune-Derived Proinflammatory Cytokine Targeting both Leukocytes and Adipocytes. PLoS.ONE. **1**: e31

Nair, S., Y. H. Lee, R. S. Lindsay, B. R. Walker, P. A. Tataranni, C. Bogardus, L. J. Baier, and P. A. Permana. 2004. 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. Diabetologia **47**: 1088-1095

Nakagawa, T., H. Hu, S. Zharikov, K. R. Tuttle, R. A. Short, O. Glushakova, X. Ouyang, D. I. Feig, E. R. Block, J. Herrera-Acosta, J. M. Patel, and R. J. Johnson. 2006. A causal role for uric acid in fructose-induced metabolic syndrome. Am.J.Physiol Renal Physiol **290**: F625-F631

Narayan, K. M., B. A. Bowman, and M. E. Engelgau. 2001. Prevention of type 2 diabetes. BMJ **323**: 63-64

Naukkarinen, J., C. Ehnholm, and L. Peltonen. 2006. Genetics of familial combined hyperlipidemia. Curr.Opin.Lipidol. **17**: 285-290

Nawrocki, A. R. and P. E. Scherer. 2004. The delicate balance between fat and muscle: adipokines in metabolic disease and musculoskeletal inflammation. Curr.Opin.Pharmacol. **4**: 281-289

NCEP. 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA **285**: 2486-2497

NCEP. 2002. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation **106**: 3143-3421

Nelken, N. A., S. R. Coughlin, D. Gordon, and J. N. Wilcox. 1991. Monocyte chemoattractant protein-1 in human atheromatous plaques. J.Clin.Invest **88**: 1121-1127

Nelson, W. J. and R. Nusse. 2004. Convergence of Wnt, beta-catenin, and cadherin pathways. Science **303**: 1483-1487

Neuschwander-Tetri, B. A. and S. H. Caldwell. 2003. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology **37**: 1202-1219

Newman, B., J. V. Selby, M. C. King, C. Slemenda, R. Fabsitz, and G. D. Friedman. 1987. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia **30**: 763-768

Ng, M. C., W. Y. So, V. K. Lam, C. S. Cockram, G. I. Bell, N. J. Cox, and J. C. Chan. 2004. Genome-wide scan for metabolic syndrome and related quantitative traits in Hong Kong Chinese and confirmation of a susceptibility locus on chromosome 1q21-q25. Diabetes **53**: 2676-2683

Ng, T. W., G. F. Watts, M. S. Farvid, D. C. Chan, and P. H. Barrett. 2005. Adipocytokines and VLDL metabolism: independent regulatory effects of adiponectin, insulin resistance, and fat compartments on VLDL apolipoprotein B-100 kinetics? Diabetes **54**: 795-802

Nicaud, V., S. Raoux, O. Poirier, F. Cambien, D. S. O'Reilly, and L. Tiret. 2002. The TNF alpha/G-308A polymorphism influences insulin sensitivity in offspring of patients with coronary heart disease: the European Atherosclerosis Research Study II. Atherosclerosis **161**: 317-325

Nielsen, E. M., L. Hansen, B. Carstensen, S. M. Echwald, T. Drivsholm, C. Glumer, B. Thorsteinsson, K. Borch-Johnsen, T. Hansen, and O. Pedersen. 2003. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes **52**: 573-577

Nitz, I., F. Doring, J. Schrezenmeir, and B. Burwinkel. 2005. Identification of new acyl-CoA binding protein transcripts in human and mouse. Int.J.Biochem.Cell Biol. **37**: 2395-2405

Nyholt, D. R. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am.J.Hum.Genet. **74**: 765-769

O'Brien, P. J., W. E. Alborn, J. H. Sloan, M. Ulmer, A. Boodhoo, M. D. Knierman, A. E. Schultze, and R. J. Konrad. 2005. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low concentrations compared with other apolipoproteins. Clin.Chem. **51**: 351-359

O'Rahilly, S. and N. J. Wareham. 2006. Genetic variants and common diseases--better late than never. N.Engl.J.Med. **355**: 306-308

Oakes, N. D., K. S. Bell, S. M. Furler, S. Camilleri, A. K. Saha, N. B. Ruderman, D. J. Chisholm, and E. W. Kraegen. 1997. Diet-induced muscle insulin resistance in rats is ameliorated by acute dietary lipid withdrawal or a single bout of exercise: parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. Diabetes **46**: 2022-2028

Odermatt, A., B. Dick, P. Arnold, T. Zaehner, V. Plueschke, M. N. Deregibus, H. Repetto, B. M. Frey, F. J. Frey, and P. Ferrari. 2001. A mutation in the cofactor-binding domain of 11beta-hydroxysteroid dehydrogenase type 2 associated with mineralocorticoid hypertension. J.Clin.Endocrinol.Metab **86**: 1247-1252

Olefsky, J. M. and A. R. Saltiel. 2000. PPAR gamma and the treatment of insulin resistance. Trends Endocrinol.Metab **11**: 362-368

Olivier, M., X. Wang, R. Cole, B. Gau, J. Kim, E. M. Rubin, and L. A. Pennacchio. 2004. Haplotype analysis of the apolipoprotein gene cluster on human chromosome 11. Genomics **83**: 912-923

Olivieri, F., M. Bonafe, S. Giovagnetti, R. Stecconi, M. Cardelli, L. Cavallone, L. Spazzafumo, F. Marchegiani, G. Carrieri, E. Mugianesi, C. Giampieri, M. Centurelli, R. Moresi, S. Tesei, R. Lisa, C. Viticchi, L. Falsetti, S. Salvioli, and C. Franceschi. 2003. In vitro IL-6 production by EBV-immortalized B lymphocytes from young and elderly people genotyped for -174 C/G polymorphism in IL-6 gene: a model to study the genetic basis of inflamm-aging. Mech.Ageing Dev. **124**: 549-553

Ong, K. K., D. I. Phillips, C. Fall, J. Poulton, S. T. Bennett, J. Golding, J. A. Todd, and D. B. Dunger. 1999. The insulin gene VNTR, type 2 diabetes and birth weight. Nat.Genet. **21**: 262-263

Ornstein, R. M. and M. S. Jacobson. 2006. Supersize teens: the metabolic syndrome. Adolesc.Med.Clin. **17**: 565-587

Ort, T., A. A. Arjona, J. R. MacDougall, P. J. Nelson, M. E. Rothenberg, F. Wu, A. Eisen, and Y. D. Halvorsen. 2005. Recombinant human FIZZ3/resistin stimulates lipolysis in cultured human adipocytes, mouse adipose explants, and normal mice. Endocrinology **146**: 2200-2209

Osawa, H., K. Yamada, H. Onuma, A. Murakami, M. Ochi, H. Kawata, T. Nishimiya, T. Niiya, I. Shimizu, W. Nishida, M. Hashiramoto, A. Kanatsuka, Y. Fujii, J. Ohashi, and H. Makino. 2004. The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am.J.Hum.Genet. **75**: 678-686

Oshima, H., M. M. Taketo, and M. Oshima. 2006. Destruction of pancreatic beta-cells by transgenic induction of prostaglandin E2 in the islets. J.Biol.Chem. **281**: 29330-29336

Padovani, J. C., A. Pazin-Filho, M. V. Simoes, J. A. Marin-Neto, M. A. Zago, and R. F. Franco. 2000. Gene polymorphisms in the TNF locus and the risk of myocardial infarction. Thromb.Res. **100**: 263-269

Palanivel, R. and G. Sweeney. 2005. Regulation of fatty acid uptake and metabolism in L6 skeletal muscle cells by resistin. FEBS Lett. **579**: 5049-5054

Parker, A., J. Meyer, S. Lewitzky, J. S. Rennich, G. Chan, J. D. Thomas, M. Orho-Melander, M. Lehtovirta, C. Forsblom, A. Hyrkko, M. Carlsson, C. Lindgren, and L. C. Groop. 2001. A gene conferring susceptibility to type 2 diabetes in conjunction with obesity is located on chromosome 18p11. Diabetes **50**: 675-680

Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science **294**: 169-173

Pennacchio, L. A., M. Olivier, J. A. Hubacek, R. M. Krauss, E. M. Rubin, and J. C. Cohen. 2002. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. Hum.Mol.Genet. **11**: 3031-3038

Petrkova, J., J. Szotkowska, Z. Hermanova, J. Lukl, and M. Petrek. 2004. Monocyte chemoattractant protein-1 in patients with peripheral arterial disease. Mediators.Inflamm. **13**: 39-43

Pickup, J. C. 2004. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care **27**: 813-823

Pickup, J. C., G. D. Chusney, S. M. Thomas, and D. Burt. 2000. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci. **67**: 291-300

Pickup, J. C., M. B. Mattock, G. D. Chusney, and D. Burt. 1997. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia **40**: 1286-1292

Pizzuti, A., L. Frittitta, A. Argiolas, R. Baratta, I. D. Goldfine, M. Bozzali, T. Ercolino, G. Scarlato, L. Iacoviello, R. Vigneri, V. Tassi, and V. Trischitta. 1999. A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. Diabetes **48**: 1881-1884

Poch, E., D. Gonzalez, V. Giner, E. Bragulat, A. Coca, and S. A. de La. 2001. Molecular basis of salt sensitivity in human hypertension. Evaluation of renin-angiotensin-aldosterone system gene polymorphisms. Hypertension **38**: 1204-1209

Polonsky, K. S. 2006. Retinol-binding protein 4, insulin resistance, and type 2 diabetes. N.Engl.J.Med. **354**: 2596-2598

Poulsen, P., K. O. Kyvik, A. Vaag, and H. Beck-Nielsen. 1999. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. Diabetologia **42**: 139-145

Pradhan, A. D., J. E. Manson, N. Rifai, J. E. Buring, and P. M. Ridker. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA **286**: 327-334

Pravenec, M., L. Kazdova, M. Cahova, V. Landa, V. Zidek, P. Mlejnek, M. Simakova, J. Wang, N. Qi, and T. W. Kurtz. 2006. Fat-specific transgenic expression of resistin in the spontaneously hypertensive rat impairs fatty acid re-esterification. Int.J.Obes.(Lond) **30**: 1157-1159

Prochazka, M., S. Lillioja, J. F. Tait, W. C. Knowler, D. M. Mott, M. Spraul, P. H. Bennett, and C. Bogardus. 1993. Linkage of chromosomal markers on 4q with a putative gene determining maximal insulin action in Pima Indians. Diabetes **42**: 514-519

Pusch, W., J. H. Wurmbach, H. Thiele, and M. Kostrzewa. 2002. MALDI-TOF mass spectrometry-based SNP genotyping. Pharmacogenomics. **3**: 537-548

Qi, L., R. M. van Dam, J. B. Meigs, J. E. Manson, D. Hunter, and F. B. Hu. 2006. Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum.Mol.Genet. **15**: 1914-1920

Radziuk, J. 2000. Insulin sensitivity and its measurement: structural commonalities among the methods. J.Clin.Endocrinol.Metab **85**: 4426-4433

Rae, C. and A. Graham. 2006. Human resistin promotes macrophage lipid accumulation. Diabetologia **49**: 1112-1114

Rahimi, P., C. Y. Wang, P. Stashenko, S. K. Lee, J. A. Lorenzo, and D. T. Graves. 1995. Monocyte chemoattractant protein-1 expression and monocyte recruitment in osseous inflammation in the mouse. Endocrinology **136**: 2752-2759

Randolph, G. J. and M. B. Furie. 1995. A soluble gradient of endogenous monocyte chemoattractant protein-1 promotes the transendothelial migration of monocytes in vitro. J.Immunol. **155**: 3610-3618

Rasmussen, S. K., S. A. Urhammer, J. N. Jensen, T. Hansen, K. Borch-Johnsen, and O. Pedersen. 2000. The -238 and -308 G-->A polymorphisms of the tumor necrosis factor alpha gene promoter are not associated with features of the insulin resistance syndrome or altered birth weight in Danish Caucasians. J.Clin.Endocrinol.Metab **85**: 1731-1734

Rathmann, W., B. Haastert, A. Icks, G. Giani, R. Holle, W. Koenig, H. Lowel, and C. Meisinger. 2006. Prevalence of the metabolic syndrome in the elderly population according to IDF, WHO, and NCEP definitions and associations with C-reactive protein: the KORA Survey 2000. Diabetes Care **29**: 461

Rathmann, W., B. Haastert, A. Icks, H. Lowel, C. Meisinger, R. Holle, and G. Giani. 2003. High prevalence of undiagnosed diabetes mellitus in Southern Germany: target populations for efficient screening. The KORA survey 2000. Diabetologia **46**: 182-189

Rea, I. M., O. A. Ross, M. Armstrong, S. McNerlan, D. H. Alexander, M. D. Curran, and D. Middleton. 2003. Interleukin-6-gene C/G 174 polymorphism in nonagenarian and octogenarian subjects in the BELFAST study. Reciprocal effects on IL-6, soluble IL-6 receptor and for IL-10 in serum and monocyte supernatants. Mech.Ageing Dev. **124**: 555-561

Reaven, G. M. 1997. Banting Lecture 1988. Role of insulin resistance in human disease. 1988. Nutrition **13**: 65

Reaven, G. M. 1998. Insulin resistance and human disease: a short history. J.Basic Clin.Physiol Pharmacol. **9**: 387-406

Reilly, M. P., M. Lehrke, M. L. Wolfe, A. Rohatgi, M. A. Lazar, and D. J. Rader. 2005. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation **111**: 932-939

Reynisdottir, I., G. Thorleifsson, R. Benediktsson, G. Sigurdsson, V. Emilsson, A. S. Einarsdottir, E. E. Hjorleifsdottir, G. T. Orlygsdottir, G. T. Bjornsdottir, J. Saemundsdottir, S. Halldorsson, S. Hrafnkelsdottir, S. B. Sigurjonsdottir, S. Steinsdottir, M. Martin, J. P. Kochan, B. K. Rhees, S. F. Grant, M. L. Frigge, A. Kong, V. Gudnason, K. Stefansson, and J. R. Gulcher. 2003. Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34-q35.2. Am.J.Hum.Genet. **73**: 323-335

Ribalta, J., L. Figuera, J. Fernandez-Ballart, E. Vilella, C. M. Castro, L. Masana, and J. Joven. 2002. Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia. Clin.Chem. **48**: 1597-1600

Rietveld, I., J. A. Janssen, E. F. van Rossum, J. J. Houwing-Duistermaat, F. Rivadeneira, A. Hofman, H. A. Pols, C. M. van Duijn, and S. W. Lamberts. 2004. A polymorphic CA repeat in the IGF-I gene is associated with gender-specific differences in body height, but has no effect on the secular trend in body height. Clin.Endocrinol.(Oxf) **61**: 195-203

Rivera-Chavez, F. A., D. L. Peters-Hybki, R. C. Barber, and G. E. O'Keefe. 2003. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock **20**: 218-223

Romeo, S., F. Sentinelli, F. Capici, M. Arca, A. Berni, E. Vecci, U. Di Mario, and M. G. Baroni. 2001. The G-308A variant of the Tumor Necrosis Factor-alpha (TNF-alpha) gene is not associated with obesity, insulin resistance and body fat distribution. BMC.Med.Genet. **2**: 10

Ronti, T., G. Lupattelli, and E. Mannarino. 2006. The endocrine function of adipose tissue: an update. Clin.Endocrinol.(Oxf) **64**: 355-365

Rosenbloom, A. L., J. R. Joe, R. S. Young, and W. E. Winter. 1999. Emerging epidemic of type 2 diabetes in youth. Diabetes Care **22**: 345-354

Rosmond, R. 2002. The glucocorticoid receptor gene and its association to metabolic syndrome. Obes.Res. **10**: 1078-1086

Ross, O. A., M. D. Curran, A. Meenagh, F. Williams, Y. A. Barnett, D. Middleton, and I. M. Rea. 2003. Study of age-association with cytokine gene polymorphisms in an aged Irish population. Mech.Ageing Dev. **124**: 199-206

Rovin, B. H., L. Lu, and R. Saxena. 1999. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem.Biophys.Res.Commun. **259**: 344-348

Rudich, A., N. Kozlovsky, R. Potashnik, and N. Bashan. 1997. Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes. Am.J Physiol **272**: E935-E940

Rudich, A., A. Tirosh, R. Potashnik, M. Khamaisi, and N. Bashan. 1999. Lipoic acid protects against oxidative stress induced impairment in insulin stimulation of protein kinase B and glucose transport in 3T3-L1 adipocytes. Diabetologia **42**: 949-957

Sacks, F. M. and H. Campos. 2003. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. J.Clin.Endocrinol.Metab **88**: 4525-4532

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**: 487-491

Sandra, A. and S. J. Marshall. 1986. Arachidonic acid inhibition of insulin action and phosphoinositide turnover in fat cells. Mol.Cell Endocrinol. **45**: 105-111

Sartipy, P. and D. J. Loskutoff. 2003. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc.Natl.Acad.Sci.U.S.A **100**: 7265-7270

Sato, N., K. Kobayashi, T. Inoguchi, N. Sonoda, M. Imamura, N. Sekiguchi, N. Nakashima, and H. Nawata. 2005. Adenovirus-mediated high expression of resistin causes dyslipidemia in mice. Endocrinology **146**: 273-279

Saxena, R., L. Gianniny, N. P. Burtt, V. Lyssenko, C. Giuducci, M. Sjogren, J. C. Florez, P. Almgren, B. Isomaa, M. Orho-Melander, U. Lindblad, M. J. Daly, T. Tuomi, J. N. Hirschhorn, K. G. Ardlie, L. C. Groop, and D. Altshuler. 2006. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes

and reduce the insulin response to glucose in nondiabetic individuals. Diabetes **55**: 2890-2895

Saxena, R., B. F. Voight, V. Lyssenko, N. P. Burtt, P. I. de Bakker, H. Chen, J. J. Roix, S. Kathiresan, J. N. Hirschhorn, M. J. Daly, T. E. Hughes, L. Groop, D. Altshuler, P. Almgren, J. C. Florez, J. Meyer, K. Ardlie, B. K. Bengtsson, B. Isomaa, G. Lettre, U. Lindblad, H. N. Lyon, O. Melander, C. Newton-Cheh, P. Nilsson, M. Orho-Melander, L. Rastam, E. K. Speliotes, M. R. Taskinen, T. Tuomi, C. Guiducci, A. Berglund, J. Carlson, L. Gianniny, R. Hackett, L. Hall, J. Holmkvist, E. Laurila, M. Sjogren, M. Sterner, A. Surti, M. Svensson, M. Svensson, R. Tewhey, B. Blumenstiel, M. Parkin, M. Defelice, R. Barry, W. Brodeur, J. Camarata, N. Chia, M. Fava, J. Gibbons, B. Handsaker, C. Healy, K. Nguyen, C. Gates, C. Sougnez, D. Gage, M. Nizzari, S. B. Gabriel, G. W. Chirn, Q. Ma, H. Parikh, D. Richardson, D. Ricke, and S. Purcell. 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science **316**: 1331-1336

Schaid, D. J., C. M. Rowland, D. E. Tines, R. M. Jacobson, and G. A. Poland. 2002. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am.J.Hum.Genet. **70**: 425-434

Schernthaner, G. H., H. P. Kopp, S. Kriwanek, K. Krzyzanowska, M. Satler, R. Koppensteiner, and G. Schernthaner. 2006. Effect of massive weight loss induced by bariatric surgery on serum levels of interleukin-18 and monocyte-chemoattractant-protein-1 in morbid obesity. Obes.Surg. **16**: 709-715

Schmidt, M. I. and B. B. Duncan. 2003. Diabesity: an inflammatory metabolic condition. Clin.Chem.Lab Med. **41**: 1120-1130

Schuit, S. C., J. B. van Meurs, A. P. Bergink, K. M. van der, Y. Fang, G. Leusink, A. Hofman, J. P. van Leeuwen, A. G. Uitterlinden, and H. A. Pols. 2004. Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. J.Clin.Endocrinol.Metab **89**: 303-309

Scillitani, A., C. Jang, B. Y. Wong, G. N. Hendy, and D. E. Cole. 2006. A functional polymorphism in the PTHR1 promoter region is associated with adult height and BMD measured at the femoral neck in a large cohort of young caucasian women. Hum.Genet. **119**: 416-421

Scott, L. J., L. L. Bonnycastle, C. J. Willer, A. G. Sprau, A. U. Jackson, N. Narisu, W. L. Duren, P. S. Chines, H. M. Stringham, M. R. Erdos, T. T. Valle, J. Tuomilehto, R. N. Bergman, K. L. Mohlke, F. S. Collins, and M. Boehnke. 2006. Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. Diabetes **55**: 2649-2653

Scott, L. J., K. L. Mohlke, L. L. Bonnycastle, C. J. Willer, Y. Li, W. L. Duren, M. R. Erdos, H. M. Stringham, P. S. Chines, A. U. Jackson, L. Prokunina-Olsson, C. J. Ding, A. J. Swift, N. Narisu, T. Hu, R. Pruim, R. Xiao, X. Y. Li, K. N. Conneely, N. L. Riebow, A. G. Sprau, M. Tong, P. P. White, K. N. Hetrick, M. W. Barnhart, C. W. Bark, J. L. Goldstein, L. Watkins, F. Xiang, J. Saramies, T. A. Buchanan, R. M. Watanabe, T. T. Valle, L. Kinnunen, G. R. Abecasis, E. W. Pugh, K. F. Doheny, R. N. Bergman, J. Tuomilehto, F. S. Collins, and M. Boehnke. 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science **316**: 1341-1345

Seckl, J. R., N. M. Morton, K. E. Chapman, and B. R. Walker. 2004. Glucocorticoids and 11beta-hydroxysteroid dehydrogenase in adipose tissue. Recent Prog.Horm.Res. **59**: 359-393

Serjeantson, S. W., D. Owerbach, P. Zimmet, J. Nerup, and K. Thoma. 1983. Genetics of diabetes in Nauru: effects of foreign admixture, HLA antigens and the insulin-gene-linked polymorphism. Diabetologia **25**: 13-17

Sesso, H. D., J. E. Buring, N. Rifai, G. J. Blake, J. M. Gaziano, and P. M. Ridker. 2003. C-reactive protein and the risk of developing hypertension. JAMA **290**: 2945-2951

Seufert, J., T. J. Kieffer, C. A. Leech, G. G. Holz, W. Moritz, C. Ricordi, and J. F. Habener. 1999. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. J.Clin.Endocrinol.Metab **84**: 670-676

Sham, P. C., S. I. Ao, J. S. Kwan, P. Kao, F. Cheung, P. Y. Fong, and M. K. Ng. 2007. Combining functional and linkage disequilibrium information in the selection of tag SNPs. Bioinformatics. **23**: 129-131

Shaw, J. T., P. K. Lovelock, J. B. Kesting, J. Cardinal, D. Duffy, B. Wainwright, and D. P. Cameron. 1998. Novel susceptibility gene for late-onset NIDDM is localized to human chromosome 12q. Diabetes **47**: 1793-1796

Shintani, M., H. Ikegami, T. Fujisawa, Y. Kawaguchi, M. Ohishi, T. Katsuya, J. Higaki, K. Shimamoto, and T. Ogihara. 2002. Leptin gene polymorphism is associated with hypertension independent of obesity. J.Clin.Endocrinol.Metab **87**: 2909-2912

Shoelson, S. E., J. Lee, and A. B. Goldfine. 2006. Inflammation and insulin resistance. J.Clin.Invest **116**: 1793-1801

Shoulders, C. C., E. L. Jones, and R. P. Naoumova. 2004. Genetics of familial combined hyperlipidemia and risk of coronary heart disease. Hum.Mol.Genet. **13 Spec No 1**: R149-R160

Sie, M. P., F. A. Sayed-Tabatabaei, H. H. Oei, A. G. Uitterlinden, H. A. Pols, A. Hofman, C. M. van Duijn, and J. C. Witteman. 2006. Interleukin 6 -174 g/c promoter polymorphism and risk of coronary heart disease: results from the rotterdam study and a meta-analysis. Arterioscler.Thromb.Vasc.Biol. **26**: 212-217

Silander, K., K. L. Mohlke, L. J. Scott, E. C. Peck, P. Hollstein, A. D. Skol, A. U. Jackson, P. Deloukas, S. Hunt, G. Stavrides, P. S. Chines, M. R. Erdos, N. Narisu, K. N. Conneely, C. Li, T. E. Fingerlin, S. K. Dhanjal, T. T. Valle, R. N. Bergman, J. Tuomilehto, R. M. Watanabe, M. Boehnke, and F. S. Collins. 2004. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. Diabetes **53**: 1141-1149

Simeoni, E., M. M. Hoffmann, B. R. Winkelmann, J. Ruiz, S. Fleury, B. O. Boehm, W. Marz, and G. Vassalli. 2004. Association between the A-2518G polymorphism in the monocyte chemoattractant protein-1 gene and insulin resistance and Type 2 diabetes mellitus. Diabetologia **47**: 1574-1580

Simmons, D., D. R. Williams, and M. J. Powell. 1991. The Coventry Diabetes Study: prevalence of diabetes and impaired glucose tolerance in Europids and Asians. Q.J.Med. **81**: 1021-1030

Sinha, R., G. Fisch, B. Teague, W. V. Tamborlane, B. Banyas, K. Allen, M. Savoye, V. Rieger, S. Taksali, G. Barbetta, R. S. Sherwin, and S. Caprio. 2002. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N.Engl.J.Med. **346**: 802-810

Sladek, R., G. Rocheleau, J. Rung, C. Dina, L. Shen, D. Serre, P. Boutin, D. Vincent, A. Belisle, S. Hadjadj, B. Balkau, B. Heude, G. Charpentier, T. J. Hudson, A. Montpetit, A. V. Pshezhetsky, M. Prentki, B. I. Posner, D. J. Balding, D. Meyre, C. Polychronakos, and P. Froguel. 2007. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature **445**: 881-885

Smith, S. R., F. Bai, C. Charbonneau, L. Janderova, and G. Argyropoulos. 2003. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. Diabetes **52**: 1611-1618

Song, Y., T. Niu, J. E. Manson, D. J. Kwiatkowski, and S. Liu. 2004. Are variants in the CAPN10 gene related to risk of type 2 diabetes? A quantitative assessment of population and family-based association studies. Am.J.Hum.Genet. **74**: 208-222

Spranger, J., A. Kroke, M. Mohlig, K. Hoffmann, M. M. Bergmann, M. Ristow, H. Boeing, and A. F. Pfeiffer. 2003. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes **52**: 812-817

Steensberg, A., G. van Hall, T. Osada, M. Sacchetti, B. Saltin, and P. B. Klarlund. 2000. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J.Physiol **529 Pt 1**: 237-242

Steinthorsdottir, V., G. Thorleifsson, I. Reynisdottir, R. Benediktsson, T. Jonsdottir, G. B. Walters, U. Styrkarsdottir, S. Gretarsdottir, V. Emilsson, S. Ghosh, A. Baker, S. Snorradottir, H. Bjarnason, M. C. Ng, T. Hansen, Y. Bagger, R. L. Wilensky, M. P. Reilly, A. Adeyemo, Y. Chen, J. Zhou, V. Gudnason, G. Chen, H. Huang, K. Lashley, A. Doumatey, W. Y. So, R. C. Ma, G. Andersen, K. Borch-Johnsen, T. Jorgensen, J. V. Vliet-Ostaptchouk, M. H. Hofker, C. Wijmenga, C. Christiansen, D. J. Rader, C. Rotimi, M. Gurney, J. C. Chan, O. Pedersen, G. Sigurdsson, J. R. Gulcher, U. Thorsteinsdottir, A. Kong, and K. Stefansson. 2007. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat.Genet. **39**: 770-775

Stephens, J. W., S. J. Hurel, J. A. Cooper, J. Acharya, G. J. Miller, and S. E. Humphries. 2004. A common functional variant in the interleukin-6 gene is associated with increased body mass index in subjects with type 2 diabetes mellitus. Mol.Genet.Metab **82**: 180-186

Steppan, C. M. and M. A. Lazar. 2002. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab **13**: 18-23

Stern, M. P., B. D. Mitchell, J. Blangero, L. Reinhart, C. M. Krammerer, C. R. Harrison, P. A. Shipman, P. O'Connell, M. L. Frazier, and J. W. MacCluer. 1996. Evidence for a major gene for type II diabetes and linkage analyses with selected candidate genes in Mexican-Americans. Diabetes **45**: 563-568

Stickens, D., G. Clines, D. Burbee, P. Ramos, S. Thomas, D. Hogue, J. T. Hecht, M. Lovett, and G. A. Evans. 1996. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. Nat.Genet. **14**: 25-32

Storm, N., B. Darnhofer-Patel, B. D. van den, and C. P. Rodi. 2003. MALDI-TOF mass spectrometry-based SNP genotyping. Methods Mol.Biol. **212**: 241-262

Sugiyama, T., N. Kato, Y. Ishinaga, Y. Yamori, and Y. Yazaki. 2001. Evaluation of selected polymorphisms of the Mendelian hypertensive disease genes in the Japanese population. Hypertens.Res. **24**: 515-521

Sung, R. Y., P. C. Tong, C. W. Yu, P. W. Lau, G. T. Mok, M. C. Yam, P. K. Lam, and J. C. Chan. 2003. High prevalence of insulin resistance and metabolic syndrome in overweight/obese preadolescent Hong Kong Chinese children aged 9-12 years. Diabetes Care **26**: 250-251

Sutherland, J., B. McKinnley, and R. H. Eckel. 2004. The metabolic syndrome and Inflammation. Metab.Syndr.Relat.Disord. **2**: 82-104

Tabara, Y., K. Kohara, Y. Yamamoto, M. Igase, J. Nakura, I. Kondo, and T. Miki. 2003. Polymorphism of the monocyte chemoattractant protein (MCP-1) gene is associated with the plasma level of MCP-1 but not with carotid intima-media thickness. Hypertens.Res. **26**: 677-683

Taghibiglou, C., F. Rashid-Kolvear, S. C. Van Iderstine, H. Le Tien, I. G. Fantus, G. F. Lewis, and K. Adeli. 2002. Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructose-fed hamster model of insulin resistance. J.Biol.Chem. **277**: 793-803

Talmud, P. J., E. Hawe, S. Martin, M. Olivier, G. J. Miller, E. M. Rubin, L. A. Pennacchio, and S. E. Humphries. 2002. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. Hum.Mol.Genet. **11**: 3039-3046

Tanikawa, N., Y. Ohmiya, H. Ohkubo, K. Hashimoto, K. Kangawa, M. Kojima, S. Ito, and K. Watanabe. 2002. Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem.Biophys.Res.Commun. **291**: 884-889

Teran-Garcia, M. and C. Bouchard. 2007. Genetics of the metabolic syndrome. Appl.Physiol Nutr.Metab **32**: 89-114

Terry, C. F., V. Loukaci, and F. R. Green. 2000. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J.Biol.Chem. **275**: 18138-18144

Testa, R., F. Olivieri, A. R. Bonfigli, C. Sirolla, M. Boemi, F. Marchegiani, M. Marra, S. Cenerelli, R. Antonicelli, A. Dolci, G. Paolisso, and C. Franceschi. 2005. Interleukin-6-174 G>C polymorphism affects the association between IL-6 plasma levels and insulin resistance in type 2 diabetic patients. Diabetes Res.Clin.Pract.

The Welcome Trust Case Control Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature **447**: 661-678

Thomas, G. N., B. Tomlinson, J. C. Chan, R. P. Young, and J. A. Critchley. 2000a. The Trp64Arg polymorphism of the beta3-adrenergic receptor gene and obesity in Chinese subjects with components of the metabolic syndrome. Int.J.Obes.Relat Metab Disord. **24**: 545-551

Thomas, M. K., N. Rastalsky, J. H. Lee, and J. F. Habener. 2000b. Hedgehog signaling regulation of insulin production by pancreatic beta-cells. Diabetes **49**: 2039-2047

Tirosh, A., A. Rudich, R. Potashnik, and N. Bashan. 2001. Oxidative stress impairs insulin but not platelet-derived growth factor signalling in 3T3-L1 adipocytes. Biochem.J **355**: 757-763

Tooke, J. E. and M. M. Hannemann. 2000. Adverse endothelial function and the insulin resistance syndrome. J.Intern.Med. **247**: 425-431

Tran, P. O., C. E. Gleason, V. Poitout, and R. P. Robertson. 1999. Prostaglandin E(2) mediates inhibition of insulin secretion by interleukin-1beta. J.Biol.Chem. **274**: 31245-31248

Trayhurn, P. 2005. Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiol Scand. **184**: 285-293

Trayhurn, P. and I. S. Wood. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br.J Nutr. **92**: 347-355

Tsai, H. J., G. Sun, D. E. Weeks, R. Kaushal, M. Wolujewicz, S. T. McGarvey, J. Tufa, S. Viali, and R. Deka. 2001. Type 2 diabetes and three calpain-10 gene polymorphisms in Samoans: no evidence of association. Am.J.Hum.Genet. **69**: 1236-1244

Ubeda, M., J. M. Rukstalis, and J. F. Habener. 2006. Inhibition of cyclin-dependent kinase 5 activity protects pancreatic beta cells from glucotoxicity. J.Biol.Chem. **281**: 28858-28864

Unger, R. H. 2003. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. Trends Endocrinol.Metab **14**: 398-403

Utzschneider, K. M., D. B. Carr, J. Tong, T. M. Wallace, R. L. Hull, S. Zraika, Q. Xiao, J. S. Mistry, B. M. Retzlaff, R. H. Knopp, and S. E. Kahn. 2005. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. Diabetologia **48**: 2330-2333

Valenti, L., A. L. Fracanzani, P. Dongiovanni, G. Santorelli, A. Branchi, E. Taioli, G. Fiorelli, and S. Fargion. 2002. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology **122**: 274-280

van der Vleuten, G. M., A. Isaacs, W. W. Zeng, E. ter Avest, P. J. Talmud, G. M. Dallinga-Thie, C. M. van Duijn, A. F. Stalenhoef, and J. de Graaf. 2007. Haplotype analyses of the APOA5 gene in patients with familial combined hyperlipidemia. Biochim.Biophys.Acta **1772**: 81-88

van der Vliet, H. N., M. G. Sammels, A. C. Leegwater, J. H. Levels, P. H. Reitsma, W. Boers, and R. A. Chamuleau. 2001. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. J.Biol.Chem. **276**: 44512-44520

van Dijk, K. W., P. C. Rensen, P. J. Voshol, and L. M. Havekes. 2004. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? Curr.Opin.Lipidol. **15**: 239-246

van Hall, G., A. Steensberg, M. Sacchetti, C. Fischer, C. Keller, P. Schjerling, N. Hiscock, K. Moller, B. Saltin, M. A. Febbraio, and B. K. Pedersen. 2003. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J.Clin.Endocrinol.Metab **88**: 3005-3010

van Oijen, M., P. P. Arp, F. J. de Jong, A. Hofman, P. J. Koudstaal, A. G. Uitterlinden, and M. M. Breteler. 2006. Polymorphisms in the interleukin 6 and transforming growth factor beta1 gene and risk of dementia. The Rotterdam Study. Neurosci.Lett. **402**: 113-117

van Rossum, E. F. and S. W. Lamberts. 2004. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. Recent Prog.Horm.Res. **59**: 333-357

Vassaux, G., D. Gaillard, C. Darimont, G. Ailhaud, and R. Negrel. 1992. Differential response of preadipocytes and adipocytes to prostacyclin and prostaglandin E2: physiological implications. Endocrinology **131**: 2393-2398

Vaxillaire, M., A. Dechaume, V. Vasseur-Delannoy, S. Lahmidi, V. Vatin, F. Lepretre, P. Boutin, S. Hercberg, G. Charpentier, C. Dina, and P. Froguel. 2006. Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. Diabetes **55**: 856-861

Venkatesan, S., P. Cullen, P. Pacy, D. Halliday, and J. Scott. 1993. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. Arterioscler.Thromb. **13**: 1110-1118

Verma, S., S. H. Li, C. H. Wang, P. W. Fedak, R. K. Li, R. D. Weisel, and D. A. Mickle. 2003. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation **108**: 736-740

Viedt, C., J. Vogel, T. Athanasiou, W. Shen, S. R. Orth, W. Kubler, and J. Kreuzer. 2002. Monocyte chemoattractant protein-1 induces proliferation and interleukin-6 production in human smooth muscle cells by differential activation of nuclear factor-kappaB and activator protein-1. Arterioscler.Thromb.Vasc.Biol. **22**: 914-920

Vionnet, N., E. H. Hani, S. Dupont, S. Gallina, S. Francke, S. Dotte, F. De Matos, E. Durand, F. Lepretre, C. Lecoeur, P. Gallina, L. Zekiri, C. Dina, and P. Froguel. 2000. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am.J.Hum.Genet. **67**: 1470-1480

Vliet-Ostaptchouk, J. V., R. Shiri-Sverdlov, A. Zhernakova, E. Strengman, T. W. van Haeften, M. H. Hofker, and C. Wijmenga. 2007. Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. Diabetologia **50**: 59-62

Vozarova, B., J. M. Fernandez-Real, W. C. Knowler, L. Gallart, R. L. Hanson, J. D. Gruber, W. Ricart, J. Vendrell, C. Richart, P. A. Tataranni, and J. K. Wolford. 2003. The interleukin-6 (-174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. Hum.Genet. **112**: 409-413

Vrablik, M., A. Horinek, R. Ceska, V. Adamkova, R. Poledne, and J. A. Hubacek. 2003. Ser19->Trp polymorphism within the apolipoprotein AV gene in hypertriglyceridaemic people. J.Med.Genet. **40**: e105

Wake, D. J. and B. R. Walker. 2006. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 in obesity. Endocrine. **29**: 101-108

Wallenius, K., J. O. Jansson, and V. Wallenius. 2003. The therapeutic potential of interleukin-6 in treating obesity. Expert.Opin.Biol.Ther. **3**: 1061-1070

Wallenius, V., K. Wallenius, B. Ahren, M. Rudling, H. Carlsten, S. L. Dickson, C. Ohlsson, and J. O. Jansson. 2002. Interleukin-6-deficient mice develop mature-onset obesity. Nat.Med. **8**: 75-79

Wang, H., P. Maechler, P. A. Antinozzi, K. A. Hagenfeldt, and C. B. Wollheim. 2000. Hepatocyte nuclear factor 4alpha regulates the expression of pancreatic beta -cell genes implicated in glucose metabolism and nutrient-induced insulin secretion. J.Biol.Chem. **275**: 35953-35959

Wang, H., H. Zhang, Y. Jia, Z. Zhang, R. Craig, X. Wang, and S. C. Elbein. 2004. Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. Diabetes **53**: 2132-2136

Wang, X. Y., M. Hurme, M. Jylha, and A. Hervonen. 2001. Lack of association between human longevity and polymorphisms of IL-1 cluster, IL-6, IL-10 and TNF-alpha genes in Finnish nonagenarians. Mech.Ageing Dev. **123**: 29-38

Weedon, M. N., P. E. Schwarz, Y. Horikawa, N. Iwasaki, T. Illig, R. Holle, W. Rathmann, T. Selisko, J. Schulze, K. R. Owen, J. Evans, L. Bosque-Plata, G. Hitman, M. Walker, J. C. Levy, M. Sampson, G. I. Bell, M. I. McCarthy, A. T. Hattersley, and T. M. Frayling. 2003. Meta-analysis and a large association study confirm a role for calpain-10 variation in type 2 diabetes susceptibility. Am.J.Hum.Genet. **73**: 1208-1212

Wei, J. N., F. C. Sung, C. C. Lin, R. S. Lin, C. C. Chiang, and L. M. Chuang. 2003. National surveillance for type 2 diabetes mellitus in Taiwanese children. JAMA **290**: 1345-1350

Weinberg, W. 1908. Über den Nachweis der Vererbung beim Menschen. Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg **64**: 368-382

Weir, B. S. and S. R. Wilson. 1986. Log-linear models for linked loci. Biometrics **42**: 665-670

Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest **112**: 1796-1808

Weiss, R., J. Dziura, T. S. Burgert, W. V. Tamborlane, S. E. Taksali, C. W. Yeckel, K. Allen, M. Lopes, M. Savoye, J. Morrison, R. S. Sherwin, and S. Caprio. 2004. Obesity and the metabolic syndrome in children and adolescents. N.Engl.J.Med. **350**: 2362-2374

Wellen, K. E. and G. S. Hotamisligil. 2005. Inflammation, stress, and diabetes. J.Clin.Invest **115**: 1111-1119

Wichmann, H. E., C. Gieger, and T. Illig. 2005. KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen **67 Suppl 1**: S26-S30

Wild, S., G. Roglic, A. Green, R. Sicree, and H. King. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care **27**: 1047-1053

Wilkin, T. J. 2001. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. Diabetologia **44**: 914-922

Wilson, A. F., R. C. Elston, L. D. Tran, and R. M. Siervogel. 1991. Use of the robust sib-pair method to screen for single-locus, multiple-locus, and pleiotropic effects: application to traits related to hypertension. Am.J.Hum.Genet. **48**: 862-872

Wiltshire, S., A. T. Hattersley, G. A. Hitman, M. Walker, J. C. Levy, M. Sampson, S. O'Rahilly, T. M. Frayling, J. I. Bell, G. M. Lathrop, A. Bennett, R. Dhillon, C. Fletcher, C. J. Groves, E. Jones, P. Prestwich, N. Simecek, P. V. Rao, M. Wishart, G. F. Bottazzo, R. Foxon, S. Howell, D. Smedley, L. R. Cardon, S. Menzel, and M. I. McCarthy. 2001. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. Am.J.Hum.Genet. **69**: 553-569

Wjst, M. 2004. Target SNP selection in complex disease association studies. BMC.Bioinformatics. **5**: 92

Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A. Tartaglia, and H. Chen. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest **112**: 1821-1830

Yamauchi, T., K. Hara, N. Kubota, Y. Terauchi, K. Tobe, P. Froguel, R. Nagai, and T. Kadowaki. 2003a. Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. Curr.Drug Targets.Immune.Endocr.Metabol.Disord. **3**: 243-254

Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, and T. Kadowaki. 2003b. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature **423**: 762-769

Yen, C. J., B. A. Beamer, C. Negri, K. Silver, K. A. Brown, D. P. Yarnall, D. K. Burns, J. Roth, and A. R. Shuldiner. 1997. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochem.Biophys.Res.Commun. **241**: 270-274

Yi, F., P. L. Brubaker, and T. Jin. 2005. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J.Biol.Chem. **280**: 1457-1464

Yki-Jarvinen, H. 2002. Ectopic fat accumulation: an important cause of insulin resistance in humans. J.R.Soc.Med. **95 Suppl 42**: 39-45

Yudkin, J. S., M. Kumari, S. E. Humphries, and V. Mohamed-Ali. 2000. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis **148**: 209-214

Yudkin, J. S., C. D. Stehouwer, J. J. Emeis, and S. W. Coppack. 1999. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler.Thromb.Vasc.Biol. **19**: 972-978

Zeggini, E., M. N. Weedon, C. M. Lindgren, T. M. Frayling, K. S. Elliott, H. Lango, N. J. Timpson, J. R. Perry, N. W. Rayner, R. M. Freathy, J. C. Barrett, B. Shields, A. P. Morris, S. Ellard, C. J. Groves, L. W. Harries, J. L. Marchini, K. R. Owen, B. Knight, L. R. Cardon, M. Walker, G. A. Hitman, A. D. Morris, A. S. Doney, P. R. Burton, D. G. Clayton, N. Craddock, P. Deloukas, A. Duncanson, D. P. Kwiatkowski, W. H. Ouwehand, N. J. Samani, J. A. Todd, P. Donnelly, D. Davison, D. Easton, D. Evans, H. T. Leung, C. C. Spencer, M. D. Tobin, A. P. Attwood, J. P. Boorman, B. Cant, U. Everson, J. M. Hussey, J. D. Jolley, A. S. Knight, K. Koch, E. Meech, S. Nutland, C. V. Prowse, H. E. Stevens, N. C. Taylor, G. R. Walters, N. M. Walker, N. A. Watkins, T. Winzer, R. W. Jones, W. L. McArdle, S. M. Ring, D. P. Strachan, M. Pembrey, G. Breen, D. St Clair, S. Caesar, K. Gordon-Smith, L. Jones, C. Fraser, E. K. Green, D. Grozeva, M. L. Hamshere, P. A. Holmans, I. R. Jones, G. Kirov, V. Moskvina, I. Nikolov, M. C. O'donovan, M. J. Owen, D. A. Collier, A. Elkin, A. Farmer, R. Williamson, P. McGuffin, A. H. Young, I. N. Ferrier, S. G. Ball, A. J. Balmforth, J. H. Barrett, D. T. Bishop, M. M. Iles, A. Maqbool, N. Yuldasheva, A. S. Hall, P. S. Braund, R. J. Dixon, M. Mangino, S. Stevens, J. R. Thompson, F. Bredin, M. Tremelling, M. Parkes, H. Drummond, C. W. Lees, E. R. Nimmo, J. Satsangi, S. A. Fisher, A. Forbes, C. M. Lewis, C. M. Onnie, N. J. Prescott, J. Sanderson, C. G. Mathew, J. Barbour, M. K. Mohiuddin, C. E. Todhunter, J. C. Mansfield, T. Ahmad, F. R. Cummings, D. P. Jewell, J. Webster, M. J. Brown, G. M. Lathrop, J. Connell, A. Dominiczak, C. A. Braga Marcano, B. Burke, R. Dobson, J. Gungadoo, K. L. Lee, P. B. Munroe, S. J. Newhouse, A. Onipinla, C. Wallace, M. Xue, M. Caulfield, M. Farrall, A. Barton, I. N. Bruce, H. Donovan, S. Eyre, P. D. Gilbert, S. L. Hider, A. M. Hinks, S. L. John, C. Potter, A. J. Silman, D. P. Symmons, W. Thomson, J. Worthington, D. B. Dunger, B. Widmer, M. Newport, G. Sirugo, E. Lyons, F. Vannberg, A. V. Hill, L. A. Bradbury, C. Farrar, J. J. Pointon, P. Wordsworth, M. A. Brown, J. A. Franklyn, J. M. Heward, M. J. Simmonds, S. C. Gough, S. Seal, M. R. Stratton, N. Rahman, M. Ban, A. Goris, S. J. Sawcer, A. Compston, D. Conway, M. Jallow, K. A. Rockett, S. J. Bumpstead, A. Chaney, K. Downes, M. J. Ghori, R. Gwilliam, S. E. Hunt, M. Inouye, A. Keniry, E. King, R. McGinnis, S. Potter, R. Ravindrarajah, P. Whittaker, C. Widden, D. Withers, N. J. Cardin, T. Ferreira, J. Pereira-Gale, I. B. Hallgrimsdottir, B. N. Howie, Z. Su, Y. Y. Teo, D. Vukcevic, D. Bentley, A. Compston, N. J. Ouwehand, M. R. Samani, J. D. Isaacs, A. W. Morgan, G. D. Wilson, A. Ardern-Jones, J. Berg, A. Brady, N. Bradshaw, C. Brewer, G. Brice, B. Bullman, J. Campbell, B. Castle, R. Cetnarsryj, C. Chapman, C. Chu, N. Coates, T. Cole, R. Davidson, A. Donaldson, H. Dorkins, F. Douglas, D. Eccles, R. Eeles, F. Elmslie, D. G. Evans, S. Goff, S. Goodman, D. Goudie, J. Gray, L. Greenhalgh, H. Gregory, S. V. Hodgson, T. Homfray, R. S. Houlston, L. Izatt, L. Jackson, L. Jeffers, V. Johnson-Roffey, F. Kavalier, C. Kirk, F. Lalloo, C. Langman, I. Locke, M. Longmuir, J. Mackay, A. Magee, S. Mansour, Z. Miedzybrodzka, J. Miller, P. Morrison, V. Murday, J. Paterson, and G. Pichert. 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science **316**: 1336-1341

Zhang, C., L. Qi, D. J. Hunter, J. B. Meigs, J. E. Manson, R. M. van Dam, and F. B. Hu. 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. Diabetes **55**: 2645-2648

Zhu, Q., K. Yamagata, A. Miura, N. Shihara, Y. Horikawa, J. Takeda, J. Miyagawa, and Y. Matsuzawa. 2003. T130I mutation in HNF-4alpha gene is a loss-of-function mutation in hepatocytes and is associated with late-onset Type 2 diabetes mellitus in Japanese subjects. Diabetologia **46**: 567-573

Zietz, B., C. Buchler, H. Herfarth, U. Muller-Ladner, D. Spiegel, J. Scholmerich, and A. Schaffler. 2005. Caucasian patients with type 2 diabetes mellitus have elevated levels

of monocyte chemoattractant protein-1 that are not influenced by the -2518 A-->G promoter polymorphism. Diabetes Obes.Metab **7**: 570-578

Zimmet, P. 2000. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted? J.Intern.Med. **247**: 301-310

Zimmet, P., K. G. Alberti, and J. Shaw. 2001. Global and societal implications of the diabetes epidemic. Nature **414**: 782-787

Appendix 1

Appendix 1a: PCR Primer used for genotyping with hME or i-Plex method.

Gene	SNP ID	Primer 1	Primer 2
IL-6	rs1800796	ACGTTGGATGACGCCTTGAAGTAACTGCAC	ACGTTGGATGTCTTCTGTGTTCTGGCTCTC
	rs1800795	ACGTTGGATGGATTGTGCAATGTGACGTCC	ACGTTGGATGAGCCTCAATGACGACCTAAG
APOA5	rs3135506	ACGTTGGATGCTGGTCTGGCTGAAGTAGTC	ACGTTGGATGTGATTACCTAGTCCCTCTCC
	rs3135507	ACGTTGGATGAACTGGGCCTTGGTGTCTTC	ACGTTGGATGACACGATGGATCTGATGGAG
	rs2075291	ACGTTGGATGATGGGTGGAAGAGCTCTTTG	ACGTTGGATGACGAGGCTTGGGCTTTGCTG
	rs2266788	ACGTTGGATGGGTGAATGTAATGCATCCAG	ACGTTGGATGAGGACAGGGAGGCCACCAAA
	rs2072560	ACGTTGGATGATCAGTGCGCGATGACTTGG	ACGTTGGATGACAGATATCCAGGCCGTCAG
	rs2542061	ACGTTGGATGACCTCCATTAAGGTCTGGTG	ACGTTGGATGTGGACTCCATCTCAGAACGC
	rs633867	ACGTTGGATGGCACAAGTTTTTGTGCAGATG	ACGTTGGATGTGGAGGAGTTCTCCTATGAC
	rs662799	ACGTTGGATGTTTGGGCTTGCTCTCCTCAG	ACGTTGGATGACTCTGAGCCCCAGGAACTG
	rs1729411	ACGTTGGATGAATCCTGGAACAAGCAAGGG	ACGTTGGATGACTTTCGCTCCAGTTCCTG
	rs651821	ACGTTGGATGCTCCTTCTTCCCCTAACCAG	ACGTTGGATGTGAAAGAAGAGCCAGAGCCC
	rs619054	ACGTTGGATGACCCCTGAGGATCTACCTGC	ACGTTGGATGAGGACTGAACCATGCTAGAG
MCP-1	rs1860188	ACGTTGGATGATGCTCAGGAGTATTCTTGG	ACGTTGGATGCCAAGCTTCCAGCATCTAAG
	rs1024610	ACGTTGGATGGAAGTTTTCCTCATATCAGGG	ACGTTGGATGATTGAATGCGGTCCACCAAG
	rs3760396	ACGTTGGATGTGCCCATTTGGAAGATGCT	ACGTTGGATGGAAACGTGTACAAGTCCTCC
	rs13306748	ACGTTGGATGGTGAATGAAGCACAGACCAG	ACGTTGGATGCAGGGTAATCAAAGAGAGGG
	rs2857657	ACGTTGGATGTGAGGTATAGGCAGAAGCAC	ACGTTGGATGAGCAGAAAAGAGTCATGAGG
	rs4586	ACGTTGGATGATGCAATCAATGCCCCAGTC	ACGTTGGATGTCTTCTATAGCTCGCGAGCC
	rs3917888	ACGTTGGATGAGGTTTATGAGGCTTGTCCC	ACGTTGGATGTGATGTGAGTTCAGCACACC
	rs13900	ACGTTGGATGGGCATAATGTTTCACATCAAC	ACGTTGGATGATTTTCCCCTAGCTTTCCCC
	rs3917900	ACGTTGGATGCTGGCTACAAGGATGTGAGC	ACGTTGGATGTGTAAATCTTCACCTGGCTC
	rs991804	ACGTTGGATGAGGCTAGGTCAGAACAAAGG	ACGTTGGATGTTCAGGTCATTGGAGCCAAC
Resistin	rs4804762	ACGTTGGATGTTGCGGTGAGCCAAGGTCGT	ACGTTGGATGCAGTTTGCTACCCCCTTTTT
	rs3760678	ACGTTGGATGTGTTGCAGGTGATGCCATTG	ACGTTGGATGTCCTGGATGACTAGCTTGGG
	rs1862513	ACGTTGGATGACCACAGCCCCTGGCATTAT	ACGTTGGATGAGCCTTCCCACTTCCAACAG
	rs3219176	ACGTTGGATGAGGGTAAGTGACAGCTGCTC	ACGTTGGATGGATGGAGGCTCAGCCTTGAC
	rs3219177	ACGTTGGATGTCTGTGCCAGGGATCAGTGAG	ACGTTGGATGATGAGAGGATCCAGGAGGTC
	rs3745367	ACGTTGGATGGAAGGTTTGGAGTGAGAGCG	ACGTTGGATGATTCAACCCCAACTCCACTC
	rs3219178	ACGTTGGATGTCCAACCACTGAATGGGCAC	ACGTTGGATGCTAGGATCTTGGTCCTGACT
	rs12981326	ACGTTGGATGGAGGCTGAGGGTGGGAGCG	ACGTTGGATGCATTCAGTGGTTGGAGCCTC
	rs3745369	ACGTTGGATGTTCAGGCCCTATTTTCGGAG	ACGTTGGATGGACACCGCGACTTTCTACTG
	rs1423096	ACGTTGGATGCGTCCCTTTCATAGGGTAAC	ACGTTGGATGGTCCACTGTAAACCAATCCC
TCF7L2	rs12255372	ACGTTGGATGGCCTCAAAACCTAGCACAGC	ACGTTGGATGTGCAAATCCAGCAGGTTAGC
	rs7903146	ACGTTGGATGCAGAGGCCTGAGTAATTATC	ACGTTGGATGAACTAAGGGTGCCTCATACG
ACBP	rs2084202	ACGTTGGATGAGTGGGGAAGCGAGGAGTC	ACGTTGGATGATTCTCCTCGTCCCGCACTC
	rs8192506	ACGTTGGATGTCCTTACAGGGACTTCCAAG	ACGTTGGATGCAAATCCAGTCTCTCATATCC
PTGES2	rs13283456	ACGTTGGATGCTGAGGGGCTTTTCCCTTCC	ACGTTGGATGACTTGTCAGCAGCCTCATAG
	rs13283456	ACGTTGGATGCTGAGGGGCTTTTCCCTTCC	ACGTTGGATGACTTGTCAGCAGCCTCATAG

Appendix 1b: Extension Primers used for genotyping with hME or i-Plex method.

Gene	SNP ID	Extension Primer	method
IL-6	rs1800796	GCAGTTCTACAACAGCC	hME
	rs1800795	AATGTGACGTCCTTTAGCAT	hME
APOA5	rs3135506	CTTTCCGTGCCTGGGTGGCC	hME
	rs3135507	GGTGTCTTCCCCCACCA	hME
	rs2075291	GAGCTCTTTGAAGCGGC	hME
	rs2266788	GTCGCAGGAGGCTGGAT	hME
	rs2072560	GGACAAAGGAGATGATGG	hME
	rs2542061	TAGCCACTCTACATTGTTTCT	i-Plex
	rs633867	CCTATGACTCAGCAATTCTA	i-Plex
	rs662799	GCGGAACTGGAGCGAAAGT	i-Plex
	rs1729411	GGTTCCTGGGGCTCAGAGTC	i-Plex
	rs651821	GCCATGCTTGCCATTA	i-Plex
	rs619054	AAAGGCTCCCCAGACAAG	i-Plex
MCP-1	rs1860188	ATTGGATCTGCCCCAG	i-Plex
	rs1024610	GGTAAAGGATGCACTAAC	i-Plex
	rs3760396	AGTCCTCCAACTAGTTGC	i-Plex
	rs13306748	TTAGGCTGTTTCCAGACACG	i-Plex
	rs2857657	CAAACGGCAGGCAGGAGAAGA	i-Plex
	rs4586	TTAGATCTTCCTATTGGTGAAGTTATA	i-Plex
	rs3917888	AGCACACCAACCTTCC	i-Plex
	rs13900	TAGCTTTCCCCAGACACC	i-Plex
	rs3917900	TGATTGTCCCAGTAGTG	i-Plex
	rs991804	CAGCCAGTCCTGGTAA	i-Plex
Resistin	rs4804762	CAGGTTGCTACCCCCTTTTTTTTGAGA	i-Plex
	rs3760678	CACCATAGCAAGACTCCA	i-Plex
	rs1862513	TTCCCACTTCCAACAGGGCCTCC	i-Plex
	rs3219176	AAGCCTTGACCCCAGCCTCCCC	i-Plex
	rs3219177	CCCCAAGCTCCCCAAGGGTCT	i-Plex
	rs3745367	CTCCGACTGTCCCCACCTTATCCAC	i-Plex
	rs3219178	GGGTGTCCACCCTCACT	i-Plex
	rs12981326	CCCGTCCCCACCCCCG	i-Plex
	rs3745369	TTTCTACTGCCCCATC	i-Plex
	rs1423096	GGGCGCTTATGGTTTGCTGGA	i-Plex
TCF7L2	rs12255372	GGAATATCCAGGCAAGAAT	i-Plex
	rs7903146	TCCTCATACGGCAATTAAATTATATA	i-Plex
ACBP	rs2084202	AAGCGAGGAGTCCGTGGCCG	hME
	rs8192506	GGACTTCCAAGGAAGATGCC	hME
PTGES2	rs13283456	TCCCCGCCCAGGCACC	i-Plex
	rs13283456	TTCCCCGCCCAGGCACC	hME

CURRICULUM VITAE

Harald Grallert

Personal data

Date of birth	1978-03-21
Place of birth	Munich
Nationality	German
Marital Status	single

Education

Jul. 2004 – Oct. 2007	PhD in Biology (Dr. rer. nat.) Institute of Epidemiology, GSF – National Research Center for Environment and Health
Apr. 2004 – Jun. 2004	Student Assistant at the Department of Mikrobiology, Zentralinstitut für Ernährungs und Lebensmittelforschung Weihenstephan (ZIEL), TU München
Apr. 2004	Diploma thesis: Sequencing and characterisation of the genetic locus responsible for emetic toxin production in *Bacillus cereus.*
Oct. 1998 – Apr. 2004	Diploma studies of biology TU München
Aug. 1997 – Sept. 1998	community service (social care service; Caritas)
Jun. 1997	Abitur (general qualification for university entrance) Gymnasium Unterhaching