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Genetic Determinants of the Metabolic Syndrome: Searching for candidate genes by reviewing genome-wide association studies

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Ich möchte mich auf das Herzlichste bei Herrn Univ. Prof. Dr. König für die motivierende und kollegiale Betreuung meiner Diplomarbeit bedanken!

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AACE/ACE	American Association of Clinical Endocrinologists/the American College of Endocrinology
ABCA1	ATB-Binding cassette, subfamily A, member 1
ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11
ADAMTS9	a disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 9
ADIPOQ	C1Q and Collagen Domain Containing
ANGPTL3	angiopoietin-like 3
APOA1-APOC3-APOA4-APOA5	apolipoprotein A1-C3-A4-A5
APOB	apolipoprotein B
APOE	apolipoprotein E
AROC	area under the received operating characteristic curve
BMI	body mass index
CAMK1D	calcium/calmodulin-dependent protein kinase i-delta
CDC123	cell division cycle protein 123 homolog
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1
CDKN2	cyclin-dependent kinase inhibitor 2A
CELSR2	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)
CETP	cholesteryl ester transfer protein, plasma
CGS	candidate gene study
CILP2	cartilage intermediate layer protein 2
CTNNBL1	catenin, beta like 1
CVD	cardiovascular disease
DGI	Diabetes Genetics Initiative
EGIR	European Group for the Study of Insulin Resistance
FPR	false positive rate
FTO	fat mass and obesity associated

FUSION	Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics
G6PC2	glucose-6 phosphatase catalytic subunit 2
GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2
GCKR	glucokinase regulatory protein
GDAP1	ganglioside-induced differentiation-associated protein 1
HDL-C	high density lipoprotein-cholesterol
HHEX	haematopoietically expressed homeobox
HNF1A	HNF1 homeobox A
HOMA-IR	homeostasis model assessment of insulin resistance
HR	hazard ratio
IDE	insulin-degrading enzyme
IGF2BP2	insulinlike growth factor 2 mRNA binding protein 2
IGT	impaired glucose tolerance
IL6R	interleukin 6 receptor
INSIG2	insulin-induced gene 2
IR	insulin resistance
IRS	insulin resistance syndrome
JAZF1	juxtaposed with another zinc finger gene 1
KCNJ11	potassium inwardly-rectifying channel, subfamily J, member 11
KIF11	kinesin-interacting factor 11
LD	linkage disequilibrium
LDL-C	low density lipoprotein-cholesterol
LDLR	low density lipoprotein receptor
LGR5	leucine-rich repeat-containing G protein-coupled receptor 5
LIPC	lipase, hepatic
LPL	lipoprotein lipase
MAF	minor allele frequency
MC4R	melanocortin-4 receptor gene
MetSyn	metabolic syndrome
MLXIPL	MLX interacting protein-like

MMAB	methylmalonic aciduria (cobalamin deficiency) cblB type
MVK	mevalonate kinase
MYBPHL	myosin binding protein h-like
NCAN	neurocan
NCEP-ATP III	National Cholesterol Education Program-Adult Treatment Panel III
NOTCH2	notch, drosophila, homolog of, 2
OR	odds ratio
PBX4	pre-b-cell leukemia transcription factor 4
PCSK9	proprotein convertase subtilisin/kexin type 9
PPARG	peroxisome proliferator-activated receptor-gamma
PSRC1	proline/serine-rich coiled-coil 1
RAF	risk allele frequency
RR	relative risk
SBP	systolic blood pressure
SLC30A8	solute carrier family 30 (zinc transporter), member 8
SORT	sortilin
T2DM	type 2 diabetes mellitus
TCF2	transcription factor 2, hepatic
TCF7L2	transcription factor 7-like 2 gene
TG	triglyceride
THADA	thyroid adenoma-associated gene
TRIB1	tribbles, drosophila, homolog of, 1
TSPAN8	tetraspanin 8
WC	waist circumference
WFS1	wolfram syndrome 1
WHO	World Health Organization
WHR	waist to hip ratio
Wnt	wingless-type
WTCCC/UKT2D	Wellcome Trust Case Control Consortium UKT2D United Kingdom T2D Genetics Consortium/United Kingdom T2D Genetics Consortium

1 Introduction

The concept of the metabolic syndrome (MetSyn) - also known as insulin resistance syndrome (IRS) [BALKAU and CHARLES, 1999], syndrome X [REAVEN, 1988] and cardiometabolic syndrome [SOOKOIAN and PIROLA, 2007] - is defined as a cluster of metabolic abnormalities in an individual. Which metabolic abnormalities and which corresponding threshold levels are to consider is still in a debate; however, in general major scientific associations agree on the essential components of the syndrome including obesity, glucose/insulin disturbance, dyslipidemia and hypertension.

The MetSyn is predictive of an increased risk of cardiovascular disease (CVD) and of type 2 diabetes mellitus (T2DM) [LORENZO et al., 2007], itself an important risk factor for CVD. Consequently, the increasing prevalence is an important public health issue; at least for those scientists who believe in the existence of a MetSyn. For others, the MetSyn is not more than the sum of its components; therefore, they question the usefulness to diagnose somebody with the MetSyn at all.

However, the MetSyn is not a new condition. Its history goes back to 1923 when Kylin described it first as an association of hypertension, hyperglycaemia, and gout [KYLIN, 1923]. In 1988, Reaven termed the condition in his Banting Lecture “syndrome X”. He described it as a syndrome comprising a dysregulated glucose and insulin homeostasis, hypertension, and dyslipidemia [REAVEN, 1988]. In the course of time scientific associations have proposed several MetSyn definitions and obesity was added as an essential component. As aforementioned, all agree on the essential components, but the definitions either require obesity or insulin resistance (IR) or no special criteria for the diagnosis of the MetSyn. Furthermore, they distinguish themselves by the different proposed threshold values.

If one assumes, that there really is a MetSyn and it is necessary to fight against the increasing prevalence, two targets are indicated, along to the treatment of the disease: environmental factors and genetic factors. The interplay of those two factors seems to be causative for the MetSyn. The question arising is where to start? Should we try to change the environmental factors in general and so prevent other diseases too? Sadly, this is not so easy, if so, we would not have the epidemic obesity. Other ways have to be found to prevent the MetSyn. Hence, one has to look at the second target, the genetic

factors. It is assumed that when someone is discovered by genetic screening to be a person at risk for the development of the MetSyn, he or she is more willing to change or to improve environmental factors such as dietary fat intake. In this respect, the genetic assessment followed by individualised healthy lifestyle recommendations for at-risk persons is a useful way to prevent the MetSyn.

Preventing a common disease than just treating a common disease is most important. In addition to the public health perspective, that it is more cost effective to prevent than to treat a common disease, one has to notice the individual perspective too. CVD and T2DM influence the quality of life of affected individuals and their families. A healthy person is (mostly) a happier and more satisfied person and that - in turn to the public health perspective improves - among other things - manpower productivity.

For that reasons this thesis is to investigate the genetically susceptibility for the MetSyn along with other questions such as whether the diagnosis of the MetSyn is more predictive than the diagnosis of its single components or whether there is a MetSyn definition that is better than the others; but principally it is investigated which genes or gene variants are associated with the MetSyn or its components by reviewing gene-wide association studies (GWASs).

Who’s at risk?

Source: http://www.nature.com/nrg/journal/v8/n12/images/nrg2280-i1.jpg

2 Literature survey

2.1 Definitions of the MetSyn

Many definitions of the MetSyn have been proposed by scientific associations in the last years. They all agree on the essential components of the syndrome including obesity, glucose/insulin disturbance, dyslipidemia, and hypertension, but they also have distinct differences (Table 1). In the following part, the most important definitions are reviewed.

2.1.1 WHO

In 1999, the World Health Organization (WHO) published a definition of the MetSyn with focus on IR. The definition was suggested to be a working definition that has to be improved in due course. Glucose intolerance, impaired glucose tolerance (IGT) or diabetes mellitus and/or IR together with at least two of the following criteria are required for a positive diagnosis: IGT or diabetes, IR, impaired fasting glucose, central obesity (assessed by waist to hip ratio or by Body Mass Index), dyslipidemia, raised arterial pressure, and microalbuminuria (Table 1). They had noticed that several other components of the MetSyn have been described (e.g. coagulation disorders, hyperuricaemia); still, they believed that these components are not necessary for the recognition of the condition [WHO CONSULATION, 1999].

2.1.2 EGIR

Few months after the WHO definition was published, the European Group for the Study of Insulin Resistance (EGIR) modified the definition and suggested that insulin resistance syndrome (IRS) would be a more appropriate name for the syndrome. They had focused in their definition on IR (IR as defined as “the 25% of the non-diabetic-population with the highest insulin resistance or the highest fasting insulin concentration”) as well, but they dropped the criterion for microalbuminuria and included treatments for hypertension and dyslipidemia as criteria (Table 1). IR and two other criteria are required for the MetSyn diagnosis. Interestingly, EGIR excluded patients with T2DM from their MetSyn definition because they believed that there is no

simple way to measure IR in individuals with diabetes [BALKAU and CHARLES, 1999].

2.1.3 NCEP-ATP III

The Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III-ATP III) of the National Cholesterol Education Program (NCEP) published their definition in 2001 and revised it in 2004. At least three of the following five criteria are required to be present: abdominal obesity, hypertriglyceridemia, reduced HDL-C levels, hypertension, and impaired fasting glucose (Table 1). In contrast to the former definitions, this definition neither requires the presence of IR nor obesity [GRUNDY et al., 2004].

2.1.4 AACE/ACE

In 2003, the American College of Endocrinology Task Force published a MetSyn definition, or as they prefer to say an IRS definition, in a position statement. This position statement reflected a commitment of the American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE). AACE/ACE adopted the blood pressure and lipid criteria from the NCEP-ATP III definition, but suggested modifications on other parts of the definition. Interestingly, they did not include any criteria for obesity in their definition (Table 1). They also did not indicate a number of risk factors that have to be found in a person for the diagnosis of the MetSyn. AACE-ACE stated that “the diagnosis of the MetSyn should be considered in any individual with risk factors and abnormalities” (plasma glucose disturbances, raised triglycerides, lowered HDL cholesterol and hypertension). For epidemiological purposes, however, they concluded that the MetSyn affects an individual when two or more abnormalities are present at the same time. Additionally, AACE/ACE encouraged the use of an elevated 2-hour post-challenge glucose test when individuals at risk (list below) do not sufficiently meet other criteria for MetSyn and a more sensitive test may be needed [EINHORN, 2003].

Risk factors that increase the likelihood of having the MetSyn:

- Overweight:
    - BMI: >25 kg/m^2 or
    - Waist circumference: >102 cm (men), >88 cm (women)

  In each case 10-15% lower for non-Caucasians
- Sedentary lifestyle
- Age: >40 years
- Non-Caucasian ethnicity: e.g. Latino/Hispanic American, African American
- Family history of T2DM, hypertension or CVD
- Family history of glucose intolerance or gestational diabetes
- Acanthosis nigricans
- Polycystic ovary syndrome
- Nonalcoholic fatty liver disease

[EINHORN, 2003]

2.1.5 IDF

The International Diabetes Federation 2005 had focused in their definition of the MetSyn on central obesity. This criterion and at least two others are required for the diagnosis of the MetSyn (Table 1). IR has not to be present. Interestingly, they established several ethnicity specific cut-off points for high waist circumference. For example, in Europids a waist circumference ≥ 94 cm (male) is seen as high, whereas ≥ 90 cm (male) is the risk threshold for South Asians [IDF, 2006].

2.1.6 AHA/NHLBI

In “Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart Lung, and Blood Institute Scientific Statement” a further

definition was published in 2005. For the diagnosis of the MetSyn, at least three criteria are required (Table 1). Like the NCEP-ATPIII definition, this definition does not require the presence of one special criterion. Ethnic cut-off points for waist circumference are not defined, but they recommend lower cut-off-points for Asian Americans (80 cm in women, 90 cm in men) [GRUNDY et. al, 2005].

Table 1. Diagnostic criteria for the MetSyn

Criteria	WHO (1999)	EGIR (1999)	NCEP-ATPIII (2001/04)	AACE/ ACE (2003)	IDF (2005)	AHA/ NHLBI (2005)
IR	Presence	Presence				
IFG (FPG) or IGT (2h PG) mmol/L	≥ 6.1 ≥ 7.8 or DM	≥ 6.1, but nondiabetic	≥ 5.6	61-6.9 7.7-11.0	≥ 5.6 or T2DM	≥ 5.6 or RX
Waist (cm) or WHR	> 0.9 (> 0.85)	≥ 94 (≥ 80)	> 102 (>88)		≥ 94 (≥80)*	≥ 102 (≥88)*
BMI (kg/m2)	> 30					
BP (mmHG)	≥ 140/90	≥ 140/90 or RX	≥ 130/85	> 130/85	≥ 130/85 or RX	≥ 130/85 or RX
TG (mmol/L)	≥ 1.7	≥ 2.0 or RX	≥ 1.7	> 1.7	≥ 1.7 or RX	≥ 1.7 or RX
HDL-C (mmol/L)	< 0.9 (<1.0)	<1.0 or RX	<1.03 (<1.29)	<1.03 (<1.29)	≤1.03 (≤1.29) or RX	≤1.03 (≤1.3) or RX
UAER (µg/min) or A/C (mg/g)	≥ 20 ≥ 30					

WHO World Health Organization; EGIR European Group for the Study of Insulin Resistance; NCEP-ATPIII National Cholesterol Education Program-Adult Treatment Panel III; AACE/ACE American Association of Clinical Endocrinologists/American College of Endocrinology; IDF International Diabetes Federation; AHA/NHLBI American Heart Association/National Heart Lung, and Blood Institute

A/C albumin/creatinine ratio; BMI body mass index; BP blood pressure; DM diabetes mellitus; FPG fasting plasma glucose; HDL high-density lipoprotein cholesterol; IFG impaired fasting glucose; IGT impaired glucose tolerance; IR insulin resistance; PG plasma glucose; RX receiving treatment; TG triglycerides; UAER urinary albumin excretion rate; WHR waist:hip ratio

* in Europids; values in brackets for females

[DAY, 2007] modified

2.2 Prevalence of the MetSyn

At the first glance the MetSyn-definitions do not seem to be extremely different, nevertheless, on closer examination distinct differences appear [DAY, 2007]. Only 33.9% of a population, which individuals were diagnosed with the MetSyn at least by one definition, fulfilled all three investigated definitions (EGIR, WHO, NCEP) (Figure 1). Furthermore to the rather small overlapping of the diagnosed individuals, it is to notice that the assessed prevalence rates differ considerably. The DECODE Study Group compared different definitions of the MetSyn in relation to cardiovascular mortality in European men (n = 4,715) and women (n = 5,554). They showed that the prevalence of the MetSyn according to definitions of WHO, NCEP-ATPIII, NCEP-ATPIII revised (FPG cut-off value was lowered from 6.1mmol/l to 5.6 mmol/l) and IDF was 27.0%, 25.9%, 32,2% and 35.9% respectively in men and 19.7%, 23.4%, 28,5% and 34.1% respectively in women [THE DECODE STUDY GROUP, 2006]. Other studies also showed that the IDF definition tends to higher prevalence rates in Europeans compared to previously established definitions [ATHYROS et al. 2005; LAWLOR et al., 2006]. The lower threshold for high blood pressure and obesity could explain this effect [LAWLOR et al., 2006]. In contrast, the comparison of the IDF definition with the AHA/NHLBI definition showed that the IDF definition tends to smaller prevalence rates as it was shown in a study conducted in Singapore (n = 4,723). The prevalence of the MetSyn by the IDF definition was 20,2 % and 26,9% by the AHA/NHLBI definition [KHOO et al., 2007].

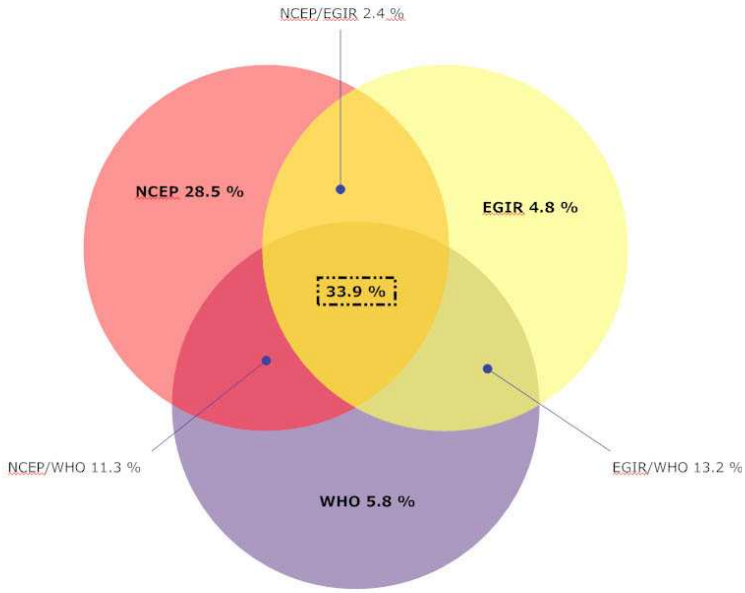


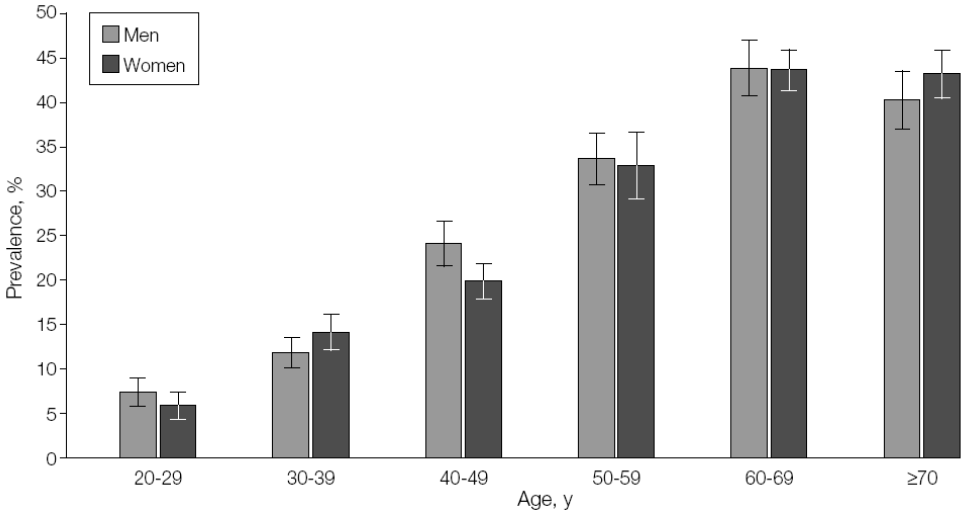
Figure 1. Agreement and disparity in the diagnosis of the MetSyn

using the modified WHO definition, the EGIR definition, and the NCEP definition among 1,503 women who qualified for the diagnosis of the MetSyn by at least one of these definitions.[THE DECODE STUDY GROUP, 2005] modified

As pointed out above, the assessed prevalence rates vary substantially by using different definitions in addition to the variation caused by different study populations and different study design. As a result, the comparison of studies is more complicated than usual.

Independently from the definitions, it has been shown that the worldwide prevalence of the MetSyn is increasing [LEVESQUE and LAMARCHE, 2008], largely because of the rise in obesity rates, and that the MetSyn is age-dependent [FORD et al., 2002; THE DECODE STUDY GROUP, 2005]. That means: the older the individuals, the higher the MetSyn prevalence (Figure 2).

Figure 2. Age-specific prevalence of the MetSyn among 8,814 US adults



[FORD et al., 2002] reproduced

2.3 Clinical relevance of the MetSyn

The MetSyn is predictive of an increased risk of CVD and T2DM [LORENZO et al., 2007], itself an important risk factor for CVD; still, it is not yet clear which MetSyn-definition predicts best. It is even not clear whether there is a need to predict the risk by diagnosing the MetSyn, because there are already established risk predicting models for CVD (Framingham Risk Score) and T2DM (Diabetes Risk Score). Finally, the existence of a MetSyn is in question, because some think that it is not more than the sum of its components. In the next part, this will be discussed in more detail.

2.3.1 Prediction of CVD and T2DM

In the beginning, it seems that the definitions do not have comparable power to predict CVD or T2DM.

The IDF definition appears to be a less powerful CVD predictor [LEVESQUE and LAMARCHE, 2008; KHOO et al., 2007], but a good predictor of increased T2DM risk [KHOO et al., 2007].

The WHO definition has been shown to predict T2DM better than the NCEP-ATPIII definition [STERN et. al, 2004]. In addition, it was shown by two meta-analyses that the WHO definition tends to stronger associations between the MetSyn and CVD than the NCEP-ATPIII definition [GAMI et al., 2007; GALASSI et al., 2006]. The more recent and larger meta-analysis showed that the RR of incident cardiovascular events and death was 2.06, 1.67, and 1.35 for the WHO definition, the NCEP-ATPIII definition and for other definitions respectively. Noteworthy, the association was stronger in women and in studies including lower risk (<10%) individuals. However, significant heterogeneity between the pooled studies existed and, therefore, the results are to question [GAMI et al., 2007]. The second meta-analysis reported 1.54 (95% CI 1.30-1.81; $P < 0.001$) as overall RR of CVD associated with the MetSyn (WHO and NCEP-ATPIII definition) when analysing the 14 studies that controlled for important confounders (age, sex, race, cigarette smoking). The unadjusted CVD RR was 1.61 (95 % CI 1.42-1.83) and 1.82 (1.27-2.61) for the ATPIII definition and the WHO definition respectively [GALASSI et al., 2006].

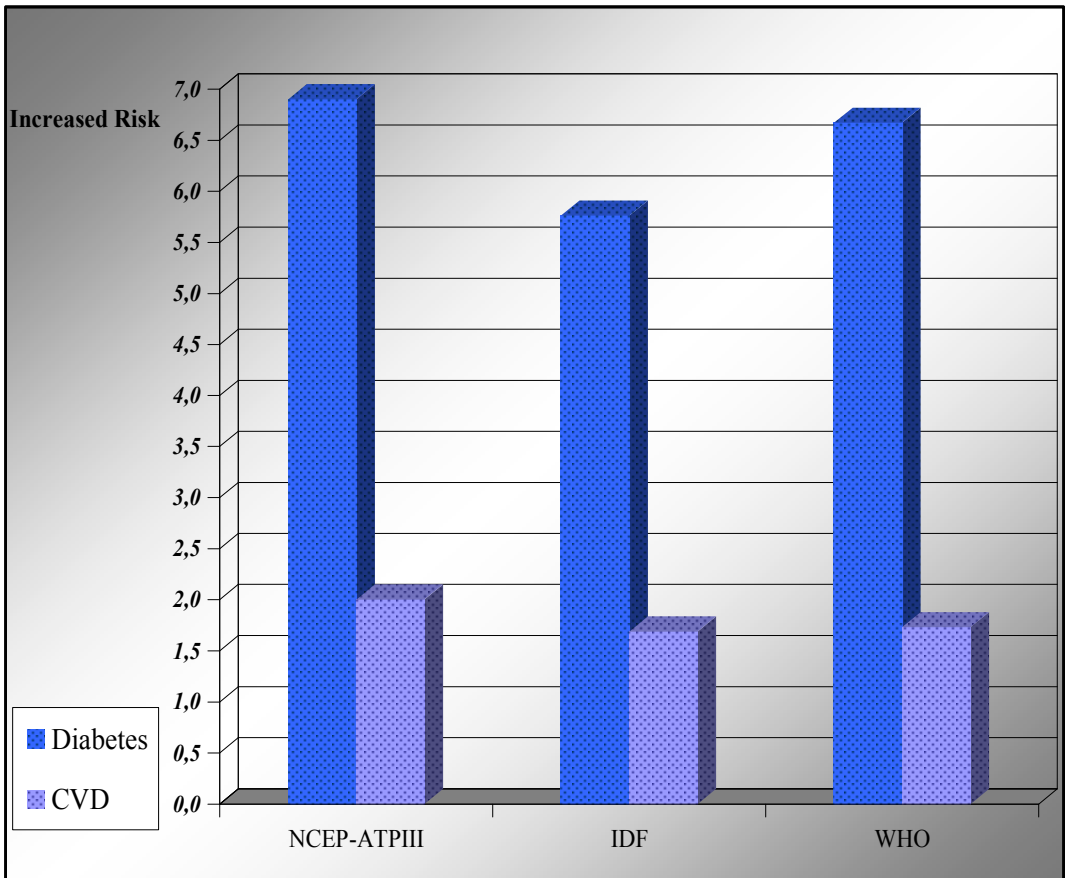
The opposite was shown by the San Antonio Heart Study (SAHS, n = 2,559), because here the NECP-ATPIII definition showed the strongest association for incident CVD risk followed by the WHO and the IDF definition, particularly in men aged ≥ 45 years and women aged ≥55 years. The power of the MetSyn to predict diabetes was larger than the power to predict CVD (Figure 3) and was again strongest for the NCEP-ATPIII definition, followed by the WHO and the IDF definition [LORENZO et al., 2007].

Figure 3. MetSyn predicts incident diabetes and incident CVD

Data from Lorenzo et al.

Diabetes: NCEP-ATPIII (OR 6.90; 95% CI 4.97–9.58), IDF (OR 5.76; 95% CI 4.11–9.07), WHO (OR 6.67; 95% CI 4.75–9.35), adjusted for age, sex, ethnic origin, history of CVD and T2DM, non-HDL cholesterol, smoking status, and family history of heart attack;

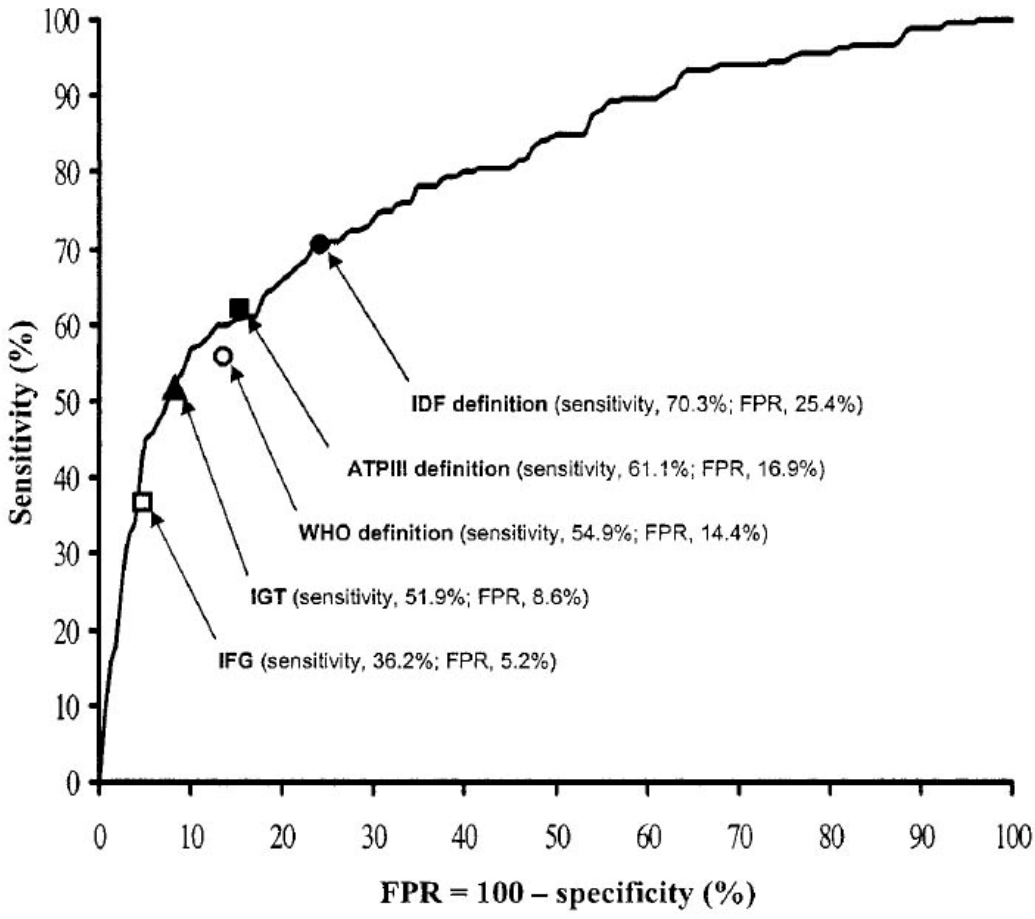
CVD: NCEP-ATPIII (OR 2.00; 95% CI 1.33–3.01), IDF (OR 1.69; 95% CI 1.13–2.54), WHO (OR 1.73; 95% CI 1.12–2.67) adjusted for age, sex, ethnic origin, and family history of diabetes.



Moreover, Lorenzo et al. indicated the importance to assess sensitivities and false positive rates, when comparing the different definitions. They found that the three definitions (WHO, IDF, NCEP-ATPIII) predict T2DM and CVD rather similarly; however, they predict the diseases with different sensitivities and FPRs (Figure 4) [LORENZO et al., 2007]. Sensitivities, false-positive rates, and aROCs (area under the received operating characteristic curve) are seen as better methods, than ORs (RRs or HRs), to assess the significance of a potential new risk factor [STERN et al., 2004].

Figure 4. ROC for predicting diabetes for the 2-h glucose value and sensitivity and FPR of IGT, IFG, and MetSyn

The ATPIII definition was less sensitive ($P < 0.001$) and more specific ($P < 0.001$) compared to the IDF definition. The difference in sensitivity between ATPIII and WHO definitions was close to significant ($P = 0.058$), but the WHO definition was more specific ($P = 0.014$).



[LORENZO et al., 2007], reproduced

2.3.2 Diabetes Predicting Model and Framingham Risk Score

Years before Lorenzo et al. encouraged the use of sensitivities, false-positive rates and aROCs, Stern et al. called for the same in a publication that shaded a rather negative light on the benefit of the MetSyn. They compared the MetSyn, defined by the NCEP-ATP III, to the Diabetes Predicting Model and the Framingham Risk Score as predictors of T2DM and CVD respectively, and found that even though the MetSyn can predict T2DM and CVD, it predicts less effectively (lower sensitivity and higher false-positive rate) than the Diabetes Risk Score and the Framingham Risk Score. Moreover, the combination of these predicting models with the MetSyn does not significantly improve the prediction value of the risk scores [STERN et. al 2004].

The opposite was shown by the DECODE Study Group; they justified in men the use of the MetSyn in clinical practice. The study purpose was to investigate if the MetSyn detect further men at risk of cardiovascular death beyond those identified by a risk score and the MetSyn did. Men with a low cardiovascular risk score and the MetSyn had a significantly higher risk of fatal CVD than those without the syndrome. Anyhow, the diagnostic tool waist circumference is easier to assess and showed a similar fatal CVD risk. A waist circumference larger than 102 cm was related to an OR of 2.24 (1.05-4.76) whereas the significant HR for fatal CVD, after adjusting for age and study centre was 2.71 (1.33-5.51) for men affected by the MetSyn [THE DECODE STUDY GROUP, 2007].

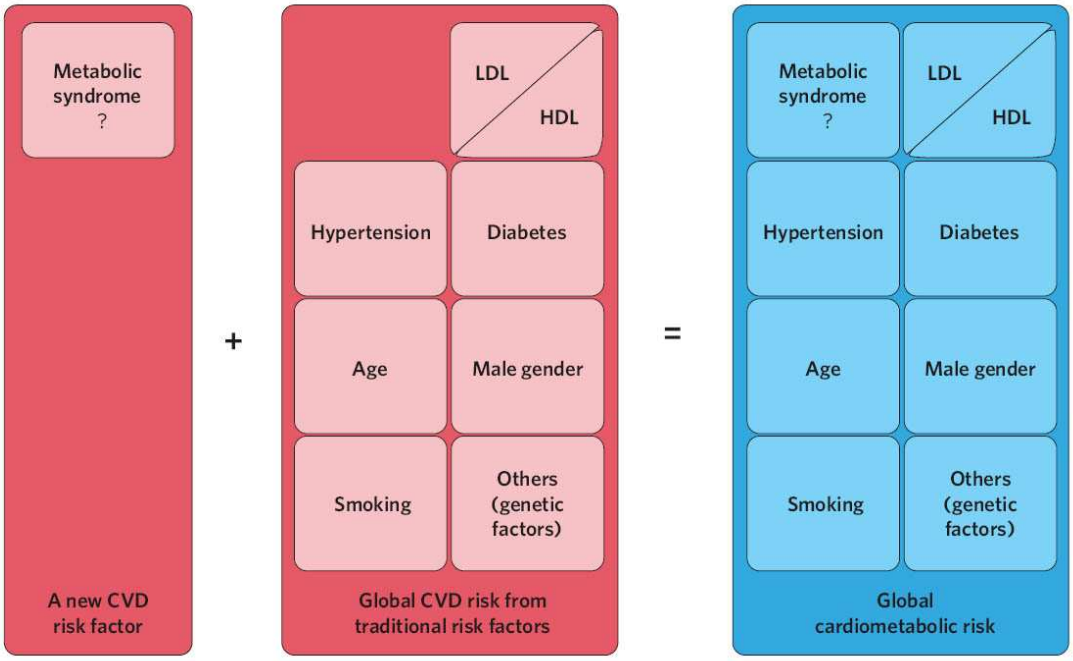
Positive aspects regarding the MetSyn were also shown by Lorenzo et al. Their study results lead to the assumption that the MetSyn may complement Framingham scoring for men aged $\geq$ 45 years and women aged $\geq$ 55 years. Furthermore, they suggested that the MetSyn is particularly useful for predicting diabetes. This was due to the reason that the MetSyn added to a predicting model including 2h- and fasting glucose levels. It follows that the T2DM prediction is not only by glucose intolerance [LORENZO et al., 2007].

In the joint AHA-NHLBI statement, it was already previously (in 2005) noticed that the MetSyn per se is not a sufficient tool to estimate the risk for CVD, but the diagnosis of the syndrome is seen as one part of the overall risk assessment. In MetSyn patients without atherosclerotic cardiovascular disease or diabetes, the Framingham Risk Score

should be applied to estimate 10-year risk for CVD. The diagnosis of the MetSyn should, in contrast, predict a long-term risk for CVD [GRUNDY et al., 2005]. For individuals with long-term or higher lifetime risk lifestyle modifications (weight loss, increased physical activity, healthy diet) should be emphasized, because those individuals could particularly benefit from early prevention. If the short-term risk of CVD or diabetes is high, as assessed with the Framingham Risk Score or the Diabetes Risk Score, the altering of specific risk factors due medication should be considered [LEVESQUE and LAMARCHE 2008], but lifestyle modifications should also be emphasized for those individuals.

Regarding to the overall risk assessment of cardiovascular risk researchers defined the summation of risks conferred by the MetSyn and traditional risk factors as global cardiometabolic risk; however, they also pointed out that the additive value of the MetSyn to such a global cardiometabolic risk is unclear [DESPRÉS and LEMIEUX, 2006].

Figure 5. The global cardiometabolic risk and its contributing factors



[DESPRES and LEMIEUX, 2006] reproduced

2.3.3 Is the MetSyn more than the sum of its components?

The inferiority to the Framingham risk score to predict short time risk is not the only criticism passed on the MetSyn. Studies found that the HRs (CVD) for the individual components of the syndrome were similar or lager than the HRs for the whole syndrome [THE DECODE STUDY GROUP, 2006; LAWLOR et al., 2006].

Opposite to this findings the authors of a recent meta-analysis of 37 longitudinal studies (n = 172,573), which assessed the association between the MetSyn and cardiovascular events and mortality, reported that the cardiovascular risk, which is related to the MetSyn as a whole syndrome is beyond that, which is associated with the risks conferred by the single components. The pooled results of three studies that simultaneously adjusted for MetSyn and some of its components showed an increased risk of CVD or death in patients with MetSyn (RR 1.54, 95% CI 1.32-1.79) [GAMI et al., 2007].

2.3.4 MetSyn as a reminder

Last but not least, the term MetSyn serves in a very practical way. It reminds physicians to investigate whether there are additional risk factors due to the presence of one risk factor. That the further investigation is important was, for instance, shown by a small study conducted in Scotland (n = 356). The researchers investigated whether undiagnosed IGT/IFG and T2DM is common amongst patients with ischaemic heart disease and patients with hypertension. They found that in primary care patients (diagnosed with hypertension) 2% have undiagnosed T2DM and 18.5% have IFG and/or IGT. In other words, testing 50 patients identifies one new case of T2DM and testing six patients identifies one new case of IFG or IGT. The results were very similar in patients diagnosed with ischaemic heart disease [SAVAGE et al., 2003].

2.4 Driving and underlying factors of the MetSyn

It is currently unknown which components of MetSyn are primary and which are secondary, but visceral obesity is believed to be a driving factor for the syndrome (Table 2). In consequence of visceral obesity, one can develop IR and T2DM [GURI et al., 2008; SONG et al., 2006]. The term IR refers to a state of insulin action dysfunction. This state is followed by hyperinsulinaemia (increased insulin secretion by pancreatic beta cells) to uphold normal glucose tolerance. If the elevated rate of insulin secretion cannot be held, a decrease of the fasting insulin concentration occurs. This subsequently results in impaired glucose tolerance and finally in T2DM [KASHYAP and DEFRONZO, 2007].

Obesity also contributes to other risk factors clustered in the MetSyn (hypertension, increased TG level, decreased HDL-C level). Moreover, adipose tissue is an endocrine organ. It secretes adipocytokines into the circulation and it influences inflammatory markers, such as C-reactive protein, leading to a proinflammatory state.

Table 2. The overflow hypothesis

The capacity of adipocytes to store fat is limited; therefore, an overflow of fat (free fatty acids) occurs when excess fat is in the metabolism.

overflow to		**consequence:**
Muscle	→	insulin resistance
Liver	→	increased hepatic glucose production
Pancreas	→	decreased insulin secretion
Arteries	→	arteriosclerosis

[KASHYAP and DEFRONZO, 2007] modified

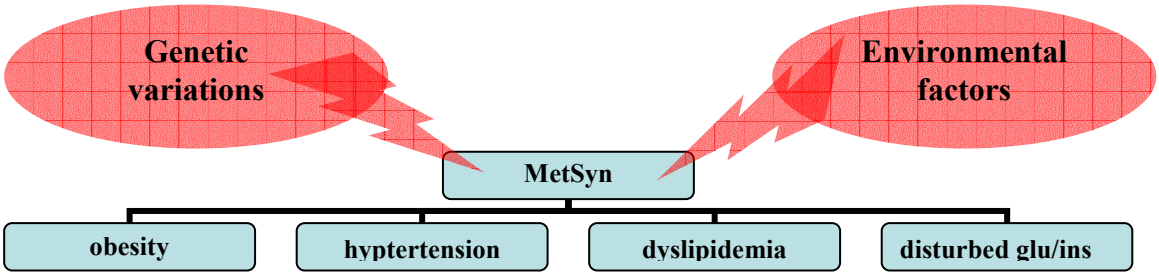
On the other hand, IR is discussed as a driving factor. This is underpinned by the proposed synonym of the MetSyn: insulin resistance syndrome. In an insulin-resistant state, an impairment of insulin to inhibit lipolysis occurs, this results in constantly elevated plasma free fatty acids (FFAs). As shown above FFAs have a central role in the syndrome. It was even shown that IR causes T2DM. This happens through metabolic and cardiovascular changes, which can also lead to obesity, dyslipidemia, CAD, and hypertension [KASHYAP and DEFRONZO, 2007].

Even it is not clear whether visceral obesity or IR is primary, it is believed that the interplay of environmental factors and genetic variations is causative for the MetSyn (Figure 6) [SONG et al., 2006].

That environmental factors are at play is easy to understand, since the human genome has not changed markedly in the last decades, but the prevalence of the MetSyn has drastically increased. Furthermore, it is seen that changing to healthy eating habits improves the most metabolic abnormalities of the MetSyn [PHILLIPS et al., 2008].

That genes play an important role in the development of the MetSyn is pointed out by a simple consideration. There are individuals with the same healthy or unhealthy life circumstances and possibly the same BMI and/or fatmass and/or fat distribution, but not everybody from the "unhealthy" group comes down with the MetSyn and not everybody from the "healthy" group is protected. Those two groups might have a different genetic background, different genetic variations that lead for example to protection through improved insulin sensitivity. Furthermore, heritability estimates for the MetSyn and its components indicate a genetic contribution. This is shown in the next part.

Figure 6. Factors influencing the MetSyn



2.5 Heritability of the MetSyn

That the MetSyn has to have a component of heritability was shown in a number of investigations and is emphasized by the familial nature and the obvious differences in the prevalence of the MetSyn among various populations. Mexican Americans, for example, are particularly at risk to develop the MetSyn [STERN et al., 2004; FORD et al., 2002]. Heritability is defined as "the proportion of phenotypic variation of a trait that can be attributed to genetic variation" [SOUREN et al., 2007].

Several studies have provided estimates for the degree to which the metabolic abnormalities of the MetSyn can be explained by heritability. Freeman et al. conduced a study in 537 adults from 89 families in the UK (white North European population). The population was healthy (defined by absence of DM and overt vascular disease) and not characterized by a high degree of IR. Additionally to the investigation of genetic influences, they were particularly interested in environmental influences of the IRS. The highest significant heritability (adjusted for age, sex, BMI and smoking) was found for HDL-C (43%) and the lowest for systolic blood pressure (14 %) sharply followed by WHR (15%). In between (20-24%) were fasting glucose, insulin, estimated IR (fasting glucose x fasting insulin / 22.5), triglycerides, and LDL-C. Covariates explained 20-25% of the variance of lipids and IR. Most remarkably, when the researchers accounted for the covariates, genetic and household effects, the remaining unexplained variance ranged from 32 % for HDL-C to 59 % for fasting glucose. That indicates that many uncovered factors e.g. additional environmental factors are at play and genes may not contribute that much to the MetSyn [FREEMAN et al., 2002]. Mills et al. reported heritabilities that were not significantly different from the heritabilities (adjusted for age and sex) reported by Freeman et al. except for the fasting glucose when investigating the same population. However, that time the sample had an increased susceptibility to T2DM (UK families: 811 non-diabetic relatives from 278 pedigrees) (Table 3) [MILLS et al. 2004].

That the HDL-C heritability is high was also shown in The Erasmus Rucphen Family study, which consists of 3,000 genealogically documented individuals from a Dutch genetic isolate. They found the highest heritability for HDL-C (42,9%, $P < 0.0001$)

followed by waist circumference (37.8%, P < 0.0001). Additionally, they investigated the heritability of the syndrome itself. They reported a significant heritability (10.6%) of the MetSyn according to IDF definition [HENNEMAN et al., 2008]. Previously an even higher significant heritability estimate (24%; adjusted for age and sex) for the MetSyn as defined by the NCEP-ATP III was reported in a study conducted in 803 subjects from 89 Caribbean-Hispanic families, a high-risk population. This study also showed the highest heritability for HDL-C (60%) and the lowest heritability (16%) for systolic blood pressure after adjusting for age, sex, and medication [LIN et al. 2005].

Table 3. Comparison of the heritability estimates for features of the IRS

measured in the study of Mills et al. and Freeman et al. after adjustment for age and sex

Features	Mills et al. h^2	CoV %[#]	Freeman et al. h^2	CoV %[#]	Difference h^2
BMI	0.52	0.48	0.37	7.00	0.15
WHR	0.31	40.8	0.23	28.0	0.08
Fasting glucose	0.77	14.6	0.21	14.0	0.56*
Fasting insulin	0.30	1.30	0.29	1.00	0.01
Triglycerides	0.40	7.40	0.19	11.0	0.21
Log HDL cholesterol	0.52	10.2	0.44	14.0	0.08
Log LDL cholesterol	0.57	19.9	0.33	20.0	0.24
HOMA %S	0.29	1.50	0.31	3.00	-0.02
Systolic BP	0.28	32.4	0.19	16.0	0.09

CoV Covariates; h^2 heritability estimate; HOMA %S homeostasis model assessment of insulin sensitivity = estimated IR
[#]Percentage of variance explained by covariates; *P < 0.001

[MILLS et al., 2004] modified

Twin studies have considerable advantages compared to the aforementioned studies. They allow the splitting of variations into genetic, shared environmental and unique environmental components. Studies with MZ (monozygotic) twins offer the most valid estimations, because they share their common environment to the same degree and so a higher concordance rate in MZ twins than in DZ (dizygotic) twins reflects a genetic

contribution. Such twin studies came up with even higher heritability rates for metabolic abnormalities seen in the MetSyn than shown by the studies that used “only” relatives.

The recently published “East Flanders Prospective Twin Survey” investigated 240 healthy MZ- and 138 healthy DZ-twin pairs aged 18 to 34 years. The study aimed to determine the genetic contribution to T2DM. Therefore, they estimated the heritabilities of 18 anthropometric and metabolic characteristics related to this disease. They reported for obesity parameters (body mass, BMI, fat mass, sum of four skinfold thicknesses, WHR) adjusted heritabilities in the range of 70% to 85%. In that context, it is to notice that women had significantly lower heritabilities for body mass, BMI and fat mass. The adjusted heritability estimates for men and women were, furthermore, 67% for fasting glucose, 49% for fasting insulin, 48% for the homeostasis model assessment of insulin resistance, 62% for beta cell function, and finally 47% for the heritability of the insulin-like growth factor binding protein-1 levels. The authors stated that this is the first twin study so far which reports a heritability of non-esterified fatty acid levels (37%). The estimated HDL-C was 76% and was thus between that of the heritabilities of total cholesterol, LDL-C, total cholesterol:HDL-C ratio and triglyceride and leptin levels, which ranged from 79 % to 53 % [SOUREN et al, 2007].

2.6 Genome-wide association studies

2.6.1 What are genome-wide association studies?

That question was univocal answered by the National Institutes of Health. They defined the genome-wide association study (GWAS) as

> "any study of genetic variation across the entire human genome that is designed to identify genetic associations with observable traits (such as blood pressure or weight), or the presence or absence of a disease or condition. To meet the definition of a GWAS, the density of genetic markers and the extent of linkage disequilibrium should be sufficient to capture (by the r^2 parameter) a large proportion of the common variation in the genome of the population under study, and the number of samples (in a case-control or trio design) should provide sufficient power to detect variants of modest effect" [NIH, 2007].

2.6.2 What are SNPs?

Single nucleotide polymorphisms (SNPs) are the most common form of variation in the human genome. As many as 12 million have a reference SNP (rs) number in the National Centre for Biotechnology Information's dbSNP database [NCBI, 2008a]. A SNP is the replacement of one nucleotide with another nucleotide. That changes the DNA sequence, for example from CTA to TTA (Figure 7).

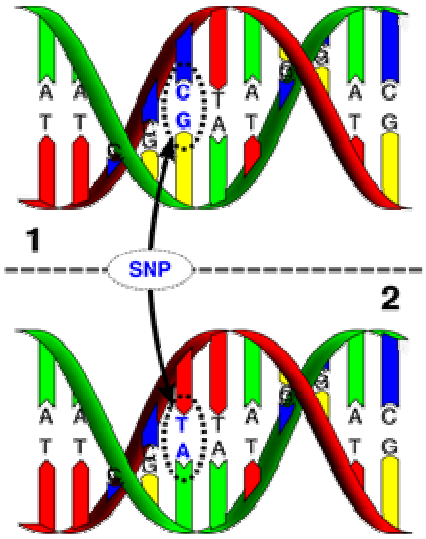


Figure 7. SNP

DNA strand 1 differs from DNA strand 2 at a single base-pair location (a C/T polymorphism).

Source: http://commons.wikimedia.org/wiki/Image:Dna-SNP.svg

The SNP is a non-synonymous one, when the changing of a single nucleotide leads to another amino acid and subsequently to another polypeptide sequence. If not so, the SNP is synonymous.

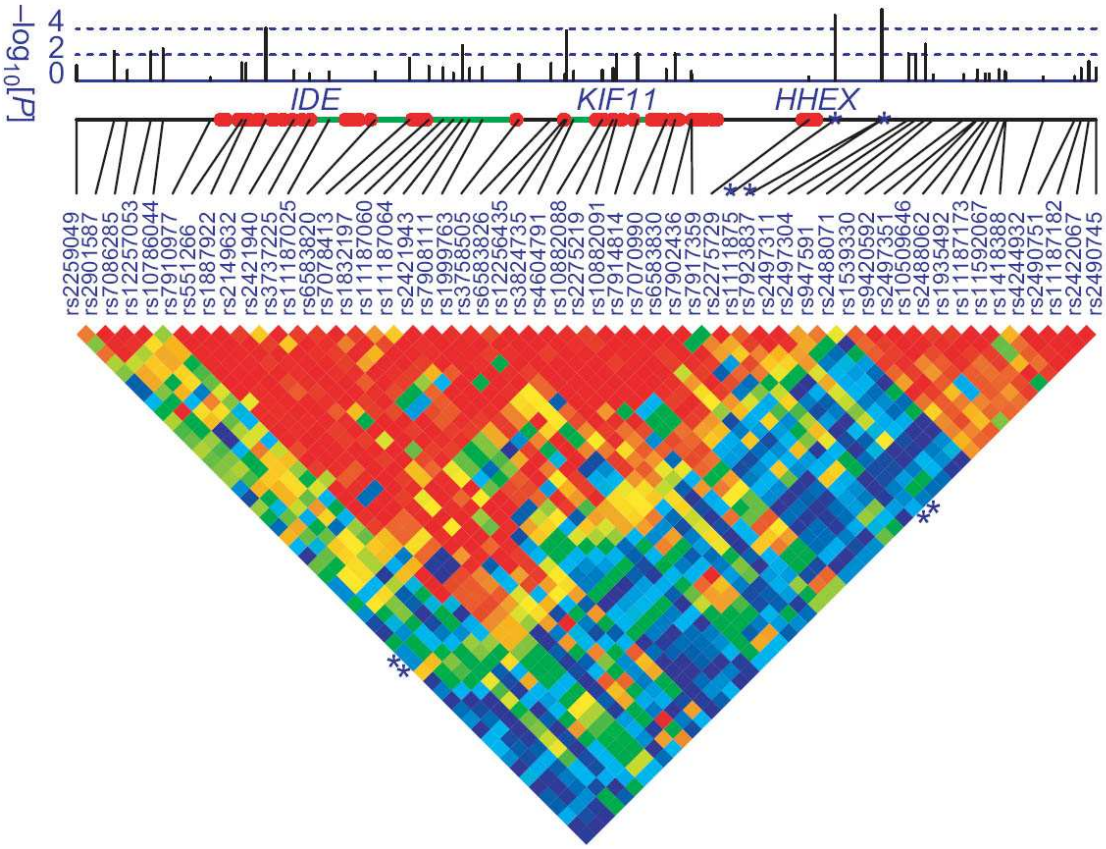
In GWASs, hundreds of thousands of SNPs can be assayed by high-throughput genotyping technologies and then they are related to "observable traits or the presence or absence of a disease or condition" [NIH, 2007]. SNPs can increase or decrease the risk of disease; nevertheless, not all SNPs are functional. Functional SNPs, including missense coding SNPs and nonexonic regulatory SNPs, are assumed to contribute more likely to common diseases than non-functional SNPs [SONG et al., 2006].

The degree of linkage disequilibrium (LD) measures whether a SNP tends to be inherited together with another SNP more often than expected by chance. If LD is present, there is an association between alleles at different loci or in other words, there is a correlation between SNPs. A standard way to quantify the degree of that correlation is r^2, showing the best correlation when $r^2 = 1$ [FRAYLING, 2007b]. That correlation is important when one GWAS identifies SNP rsXX and another SNP rsXY to be associated with a disease. In that case, they can assume that they have found the same signal when rsXX and rsXY are in a perfect correlation ($r^2 =1$). The HapMap (haplotype map of the human genome) catalogue provides a map of the correlation structure for common genetic variants, like SNPs. Moreover, their distribution among people within population and among populations in different parts of the world is described.

Although LD helps to identify highly correlated SNPs in a chromosomal region, it is difficult to determine which SNP within the group is more likely to be the causative variant. It is also notable that the SNP with the best association within the group has not to be the causal variant. Another unknown (not genotyped) SNP in LD can be the causative one [FRAYLING, 2007b]. For that reason further gene investigations have to be carried out after an association via GWAS was found.

Figure 8. Pairwise LD diagram for the IDE-KIF11-HHEX

The bar graph indicates the $-\log_{10}$ p values for association with T2DM for each SNP genotyped in the region; those reaching a prespecified value of $P > 10^{-4}$ or greater are presumed to show association with disease. The region contains 1 "triangle" or "block" of LD. Pairwise LD estimates between SNPS are plotted for the region. The asterisks mark the SNPs chosen for confirmatory studies.



[SLADEK et al. 2007] reproduced

2.6.3 Advantages of GWASs

GWASs are a powerful method to identify totally novel susceptibility genes for common diseases – so non-Mendelian diseases -, because they permit a comprehensive scan of the genome without a hypothesis [PEARSON and MANOLIO, 2008]. There is no need for knowledge about gene function, pathophysiological pathways or molecular pathways of the investigated disease, but a need for a justified assumption that genes play a role in the development of the investigated disease. In addition to the identification of disease susceptibility genes in known gene regions, GWASs identify loci for common diseases and traits even in genomic regions containing no known genes [PEARSON and MANOLIO, 2008].

As mentioned before GWASs identify genes that play a role in common diseases (or complex disorders). In common diseases not only one gene is responsible for the disease, several genes contribute to the disease development. Consequently, the responsible gene variants show, mostly, only modest increases in risk (moderate RR, OR or HR). GWASs are powerful enough to identify such modest increases [KEITH, 2007].

2.6.4 Candidate gene studies and linkage studies

Candidate gene studies and linkage studies are two other well-known approaches to identify disease susceptibility genes.

Candidate gene studies (CGSs) assay only a limited number of variants. Therefore, they need a hypotheses regarding genetic association with a disease. Related or unrelated individuals with and without disease are investigated. By contrast, linkage studies are always family-based, meaning family members with and without disease are investigated [PEARSON and MANOLIO, 2008]. This approach is valuable in finding genes for Mendelian conditions; however, it has only limited power to detect genes of modest or small effect underlying complex disorders. Thousands of sib-pairs would be required to identify such genes [HINNEY and HEBEBRAND, 2008]. To note is that in linkage studies logarithmic odds (LOD) are calculated. LOD is “a statistical estimate of whether 2 loci are likely to be located near each other on a chromosome; therefore, they

are likely to be inherited together. An LOD score of 3 means the odds are 1000 to 1 in support of genetic linkage" [SONG et al., 2006].

2.6.5 Carrying out GWASs

To carry out a GWAS, researchers require DNA samples from thousands of participants (mostly case-control but also cohorts or family trios). Therefore, they take a blood sample or rub a cotton swab along the inside of the mouth to harvest cells. The DNA is then purified from the blood or cells and genotyped with microarrays, which can contain more than 500,000 SNPs. For that purpose, the DNA is placed on those microarrays and scanned on laboratory machines. Then the frequencies of each allele are compared among the study groups. If the allele frequencies differ statistically significant, a clue is given for the location of a disease-causing problem [NHGRI, 2008b].

The association of an allele with a disease can then be calculated with OR or RR, including different genetic models (dominant, recessive and additive). The most common genetic model seems to be the additive one. Here, each copy of the risk allele leads to a risk increase by the same amount. Last but not least the population attributable risk can be calculated, which can be seen as the "proportion of a disease or trait in the population that is due to a specific cause, such as a genetic variant" [PEARSON and MANOLIO, 2008].

To ensure that the region of the human genome or the SNP identified in a GWAS is associated with the disease and reduce false positive rates, replication in independent, but very similar population, is necessary. Many studies are using for that reason multistage designs (Table 4) [PEARSON and MANOLIO, 2008].

Table 4. Example of a hypothetical multistage design in GWAS: 3-stage study

Stage	Case/Control	SNPs Analysed
1	400/400	500,000
2	4,000/4,000	25,000
3	20,000/20,000	25

[PEARSON and MANOLIO, 2008] modified

2.6.6 Power to detect associations via GWAS

Several factors influence the power to detect an association between a genetic variation and a disease:

- Frequency of the risk allele or genotype

The less frequent the allele, the more improbable it is to find an association.

- RR or OR conferred by disease-associated allele or genotype

The smaller the RR or OR, the more improbable it is to find an association.

- Correlation between the genotyped marker and the risk allele

The better the correlation, the more likely it is to find an association.

- Disease prevalence

The higher the prevalence, the more likely it is to find an association.

- Genetic heterogeneity of the sample population

The smaller the genetic heterogeneity, the more likely it is to find an association.

It is clear that case and control populations should be as homogeneous as possible and that researcher must ensure that there is no misclassification. Cases have to be really affected by the disease and controls have to be truly disease free. This is particularly crucial for diseases that are difficult to diagnose reliably.

- Sample size

The bigger the sample size, the more likely it is to find an association.

A sufficient sample size is – as power studies showed - at least 2,000 to 5,000 samples for both cases and controls when using general populations. In less heterogeneous populations – like the Quebec Founder Population – only 1,000 to 1,500 cases and controls are needed for a well-powered GWAS. The sample size challenges the recruitment of individuals with a similar phenotype and the matching of cases and controls with respect to genetic background.

[KEITH, 2007].

Additional key components to ensure a high study power are:

- A large numbers of markers
- Comprehensive maps, accurate high-throughput genotyping technologies
- Sophisticated IT infrastructure (a GWAS produces terabytes of data), rapid algorithms for data analysis

[KEITH, 2007]

Furthermore, it should be checked for genotyping errors:

- The SNP call rate (the proportion of samples or SNPs for which a specific allele SNP can be reliably identified by a genotyping method) should be > 95%.
- The minor allele frequency (= MAF, the frequency of the less common allele of a polymorphism) should be > 1% (in both cases and controls).
- No severe violations of Hardy-Weinberg equilibrium should occur.
- Mendelian inheritance errors in trio studies should be avoided.
- The concordance rates in duplicate samples should be > 99.5%.

[PEARSON and MANOLIO, 2008]

The numerousness of points stated above indicate that GWASs are prone to potential errors and therefore, one has to pay them attention. Otherwise, false-positive and false-negative results occur. For instance, significant differences in population structure can lead to strong deviations from the null hypotheses (no SNP associated with the trait) and so to the misinterpretation that a SNP is highly associated with the trait [PEARSON and MANOLIO, 2008].

To avoid or better to reduce false-positive and false-negative results further, the replication of the findings is essential as well as the use of stringent levels of statistical significance. The importance of stringent levels is shown by a simple calculation. At the traditional $P < 0.05$ level of significance, an association study of 1 million SNPs will show 50,000 SNPs to be “associated” with disease just by chance. To reduce this high

false-positive rate, particularly when a multiple testing is performed, one can apply the Bonferroni correction, in which the traditional $P < 0.05$ value is divided by the number of tests performed. So for a GWAS testing 1,000,000 SNPs a P-value of $< 5 \times 10^{-8}$ (0.05/1,000,000) would be suitable to confirm significant association [YANG et al., 2005]. That would be certainly a very conservative correction, but that is not the only critic passed on the Bonferroni correction. It is further criticised because it relies on the assumption that every SNP association is independent although LD between individual SNPs exists [PEARSON and MANOLIO et al., 2008]. Another way to define a significance threshold was shown by Frayling. He stated in his review that a P-value of approximately $< 5 \times 10^{-7}$ should be the significance level for GWASs investigating T2DM. He based this threshold on the so far found genes, for which a replication was successful [FRAYLING et al., 2007a].

2.7 Associated candidate genes

In that chapter, an insight into the existing evidence linking gene variants, which protect or predispose individuals to the development of the MetSyn by reviewing GWASs is given. Unfortunately, no GWAS was published so far - to my knowledge - that investigated the association of gene variants with the MetSyn directly. Therefore, GWASs are reviewed that investigated gene associations with components of the MetSyn, C-reactive protein or with known outcomes, like T2DM, first. The most important associations are underpinned with results from candidate gene studies. Noteworthy is, that the focus is on studies conducted in Europeans or in populations of European ancestry. However, as the topic of this thesis is the MetSyn itself, direct gene associations were also investigated. That is the reason for the brief review of linkage studies at the end of this chapter that directly investigated the MetSyn.

Table 5. Reviewed GWASs - Overview

Study	Disease/ Trait	Initial Sample Size	Replication Sample Size	associated Gene – important examples	P-value	OR per copy or B-coefficient for HetZ and [95% CI]
Bouatia-Naji et al., 2008	FPG	654 normoglycemic individuals	9,353 individuals	G6PC2	4 x 10-23	0.06 [0.05-0.08] mmol/l decrease
Chambers et al., 2008	WC and related PT	2,684 Asian Indian men	11,955 Asian Indian / European individuals	MC4R	2 x 10-9	0.88 [0.59-1.17] cm increase in WC
Chen et al., 2008	FPG	5,088 nondiabetic individuals	18,436 nondiabetic individuals	G6PC2, ABCB11	4 x 10-7	NR
Fox et al. 2007	BMI	1,341 individuals (Framingham)	NR	Intergenic Intergenic Intergenic	1 x 10-7 2 x 10-6 8 x 10-6	NR NR NR
“	WC traits	1,341 individuals (Framingham)	NR	GDAP1 Intergenic	2 x 10-7 2 x 10-6	NR NR
Frayling et al., 2007	BMI	10,657 adults	19,424 adults, 10,172 children	FTO	2 x 10-20	0.36 [NR] kg/m2 per copy in adults
Kathiresan et al. 2008	HDL-C	2,758 individuals	18,544 individuals	GALNT2	2 x 10-13	0.07 [0.05-0.09] SD higher

Study	Disease/ Trait	Initial Sample Size	Replication Sample Size	associated Gene – important examples	P-value	OR per copy or B-coefficient for HetZ and [95% CI]
Kathiresan et al. 2008	LDL-C	2,758 individuals	18,544 individuals	CELSR2, PSRC1, SORT1	3 x 10-29	0.16 [0.14-0.18] SD lower
				CILP2, PBX4	3 x 10-8	0.10 [0.06-0.14] SD lower
"	TG	2,758 individuals	18,544 individuals	BCL7B, TBL2, MLXIPL	7 x 10-22	0.14 [0.25-0.53] SD lower
				TRIB1	4 x 10-17	0.08 [0.06-0.10] SD lower
				GALNT2	7 x 10-15	0.08 [0.06-0.10] SD higher
				CILP2, PBX4	4 x 10-9	0.10 [0.06-0.14] SD lower
				ANGPTL3, DOCK7, ATG4C	2 x 10-8	0.11 [0.07-0.15] SD lower
Kooner et al., 2008	TG	2,011 individuals	10,536 individuals	MLXIPL	1 x 10-10	10.50 [5.3-17.7]% higher
Levy et al., 2007	BP	644-1,327 individuals, depending on measure (Framingham)	NR	Intergenic	2 x 10-6 (SBP)	NR
				Intergenic	3 x 10-6 (DBP)	NR
				Intergenic	3 x 10-6 (DBP)	NR
Liu et al., 2008	Obesity	1,000 individuals	896 obese individuals 2,916 lean individuals	NA	NS	NA
Loos et al., 2008	BMI	16,876 individuals	60,352 individuals	MC4R	3 x 10-15	0.05 (0.04-0.06) unit increase in log (BMI)
Ridker et al., 2008	CRP	6,345 women	NR	LEPR	7 x 10-21	0.17 [NR] mg/dl decrease
				HNF1A	7 x 10-17	0.15 [NR] mg/dl decrease
				GCKR	7 x 10-15	0.14 [NR] mg/dl increase
				Unknown	1 x 10-10	0.12 [NR] mg/dl decrease

Study	Disease/ Trait	Initial Sample Size	Replication Sample Size	associated Gene – important examples	P-value	OR per copy or B-coefficient for HetZ and [95% CI]
Ridker et al., 2008				IL6R	2 x 10-8	0.10 [NR] mg/dl decrease
Sandhu et al. 2008	LDL-C	11,685 individuals from 5 GWAS	5,036 individuals	CELSR2	1 x 10-33	0.15 [.13-.19] SD decrease in LDL
Saxena et al., 2007	T2DM	1,464 cases, 1,467 controls	5,065 cases, 5,785 controls	IGF2BP2	9 x 10-16	1.14 [1.11-1.18]
				CDKN2A, CDKN2B	8 x 10-15	1.20 [1.14-1.25]
				CDKAL1	4 x 10-11	1.12 [1.08-1.16]
Scott et al., 2007	T2DM	1,161 cases, 1,174 controls	1,215 cases, 1,258 controls	IGF2BP2	9 x 10-16	1.14 [1.11-1.18]
				CDKN2A, CDKN2B	8 x 10-15	1.20 [1.14-1.25]
				CDKAL1	4 x 10-11	1.12 [1.08-1.16]
				Intergenic	4 x 10-7	1.25 [1.15-1.37]
Sladek et al., 2007	T2DM	1,380 cases, 1,323 controls	2,617 cases, 2,894 controls	SLC30A8	6 x 10-8	1.18 [0.69-1.67]
				HHEX	3 x 10-6	1.19 [0.82-1.56]
Steinthorsdottir et al., 2007	T2DM	1,399 EA cases, 5,275 EA controls	2,437 EA cases, 7,287 EA controls	CDKAL1	8 x 10-9	1.20 [1.13-1.27]
Willer et al., 2008	HDL-C	8,656 individuals	11,399 individuals	GALNT2	3 x 10-14	1.11 [NR] mg/dl higher
				MVK, MMAB	3 x 10-8	0.48 [NR] mg/dl higher
"	LDL-C	8,589 individuals	7,440-10,783 individuals	CELSR2, PSRC1, SORT1	6 x 10-33	5.48 [NR] mg/dl higher
				NCAN, CILP2	3 x 10-9	3.32 [NR] mg/dl higher
				B3GALT4	5 x 10-8	1.91 [NR] mg/dl higher
"	TG	8,684 individuals	5,312-9,707 individuals	GCKR	6 x 10-32	8.59 [NR] mg/dl higher
				TRIB1	7 x 10-13	6.42 [NR] mg/dl higher
				MLXIPL	2 x 10-12	8.21 [NR] mg/dl higher

Study	Disease/ Trait	Initial Sample Size	Replication Sample Size	associated Gene – important examples	P-value	OR per copy or B-coefficient for HetZ and [95% CI]
Willer et al., 2008				ANGPTL3	2 x 10-10	7.12 [NR] mg/dl higher
				NCAN, CILP2	3 x 10-9	6.10 [NR] mg/dl higher
WTCCC, 2007	HT	1,952 cases, 2,938 controls	NR	RYR2, CHRM3, ZP4	8 x 10-7	1.54 [1.03-2.31]
				NR	6 x 10-6	1.03 [0.80-1.32]
"	T2DM	1,924 cases, 2,938 controls	(see Zeggini 2007)	FTO	2 x 10-7	1.34 [1.17-1.52]
				CDKAL1	3 x 10-7	1.18 [1.04-1.34]
				NR	3 x 10-6	1.16 [1.03-1.33]
				NR	7 x 10-6	2.50 [1.53-4.09]
				NR	7 x 10-6	1.28 [1.11-1.49]
Zeggini et al. 2007	T2DM	1,924 cases, 2,938 controls	3,757 cases, 5,346 controls	IGFBP2	9 x 10-16	1.14 [1.11-1.18]
				CDKN2B	8 x 10-15	1.20 [1.14-1.25]
				FTO	1 x 10-12	1.17 [1.12-1.22]
				CDKAL1	4 x 10-11	1.12 [1.08-1.16]
				HHEX	6 x 10-10	1.13 [1.08-1.17]
Zeggini et al., 2008	T2DM	4,549 cases, 5,579 controls	24,194 cases, 55,598 controls	JAZF1	5 x 10-14	1.10 [1.07-1.13]
				CDC123, CAMK1D	1 x 10-10	1.11 [1.07-1.14]
				TSPAN8, LGR5	1 x 10-9	1.09 [1.06-1.12]
				THADA	1 x 10-9	1.15 [1.10-1.20]
				ADAMTS9	1 x 10-8	1.09 [1.06-1.12]

BMI body mass index**;** DBP diastolic blood pressure**;** DM incident diabetes mellitus; T2DM diabetes mellitus type 2; EA European-American; FI fasting insulin; HOMA-IR homeostasis model insulin resistance; HDL high density lipoprotein; HetZ heterozygous; HT hypertension; LDL low density lipoprotein; NA not applicable; NR none reported; NS not significant; OR odds ratio; PT phenotype; SBP systolic blood pressure; WC waist circumference

[NHGRI, 2008a] modified

2.7.1 T2DM, FPG, HOMA-IR%

The interplay of environmental factors and genetic factors is thought to be responsible for the T2DM development. Genetic factors may predispose somebody, for example, to higher fasting plasma glucose (FPG) or reduced insulin secretion.

Glucose and insulin metabolism regarding GWASs studied either genes that are associated with T2DM directly or associated with FPG. In addition, one GWAS, which was designed to investigate obesity related genes, showed an association with HOMA-IR%. For that reasons this part is split up into genes associated with T2DM, genes associated with FPG, and genes associated with HOMA-IR%.

Genes associated with type 2 diabetes mellitus

In case of T2DM, GWASs recently lead to the identification of several novel gene associations. In 2007, five GWASs were published with convincing evidence for six new gene regions involved in T2DM [SLADEK et al, 2007; STEINTHORSDOTTIR et al., 2007; SCOTT et al., 2007; ZEGGINI et al., 2007; SAXENA et al., 2007; THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007] in addition to the five gene regions that have been already identified by other approaches. Frayling reviewed above-mentioned GWASs and additional studies. He listed the best associations and showed that TCF7L2 has by far the best evidence and the biggest effect size regarding the risk of T2DM (Table 6) [FRAYLING, 2007b]. In 2008, another six loci with convincing evidence for T2DM involvement were published [ZEGGINI et al. 2008]. This GWAS boosted the number of gene regions involved in T2DM to the number of 17.

Table 6. 11 T2DM gene regions published 2007

These gene regions were identified by GWASs published 2007 [SLADEK et al, 2007; STEINTHORSDOTTIR et al., 2007; SCOTT et al., 2007; ZEGGINI et al., 2007; SAXENA et al., 2007; THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007] and additional studies [FRAYLING, 2007b]

Example variant	Closest gene	Current evidence (P-value)	Odds ratio (per allele)	RAF (UK)	N*
rs10946398	CDKAL1	2×10^{-18}	1.14 (1.11–1.17)	0.32	16,200

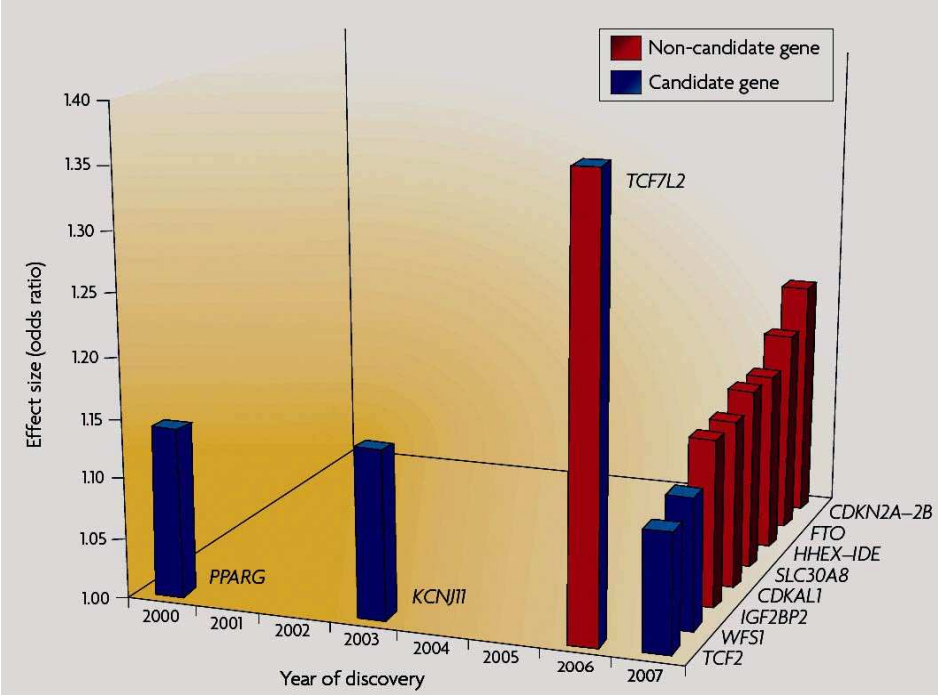
Example variant	Closest gene	Current evidence (P-value)	Odds ratio (per allele)	RAF (UK)	N*
rs10811661	CDKN2A–2B	8×10^{-15}	1.20 (1.14–1.25)	0.83	12,400
rs8050136	FTO	1×10^{-12}	1.17 (1.12–1.22)	0.40	10,400
rs1111875	HHEX–IDE	7×10^{-17}	1.15 (1.10–1.19)	0.65	12,800
rs4402960	IGF2BP2	9×10^{-16}	1.14 (1.11–1.18)	0.32	16,200
rs5215 (E23K)	KCNJ11	5×10^{-11}	1.14 (1.10–1.19)	0.35	15,600
rs1801282 (P12A)	PPARG	2×10^{-6}	1.14 (1.08–1.20)	0.87	>20,000
rs13266634	SLC30A8	1×10^{-19}	1.15 (1.12–1.19)	0.69	14,400
rs4430796	TCF2	8×10^{-10}	1.10 (1.07–1.14)	0.47	>20,000
rs7901695	TCF7L2	1×10^{-48}	1.37 (1.31–1.43)	0.31	2,760
rs10010131	WFS1	1×10^{-7}	1.11 (1.08–1.16)	0.60	>20,000

*Total number of cases and controls needed in a 1:1 ratio to provide 80% power to detect an effect at $P = 5 \times 10^{-7}$, on the basis of UK risk allele frequencies and assuming a 5% disease frequency.

[FRAYLING, 2007b] modified

Figure 9. Effect sizes of the 11 T2DM gene regions published 2007

These gene regions were identified by GWASs published 2007 [SLADEK et al, 2007; STEINTHORSDOTTIR et al., 2007; SCOTT et al., 2007; ZEGGINI et al., 2007; SAXENA et al., 2007; THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007] and additional studies [FRAYLING, 2007b]



[FRAYLING, 2007b] reproduced

CDKAL1

Steinthorsdottir et al. performed a 3-stage GWAS in Icelandic individuals with T2DM (1,399 individuals with T2DM; 5,275 controls). The top signals were pursued in a fast-tracking effort and so 47 SNPs were genotyped in 1,110 Danish T2DM cases and 2,272 controls. Two SNPs (rs7756992 and rs13266634) were then chosen for a further investigation in three other T2DM case-control groups of European ancestry. They found that SNP rs7756992 within the cyclin-dependent kinase (CDK5) regulatory subunit associated protein 1-like 1 (CDKAL1) gene is associated with T2DM. Meta-analysis of the five T2DM case-control studies showed that homozygous carriers of the risk allele G have a 1.50-fold (95% CI 1.31-1.72) increased risk and heterozygous carriers of the risk allele G have a 1.15-fold (95% CI 1.06-1.24) risk to develop T2DM. The allelic OR amounts to 1.20 (95% CI 1.13-1.27) [STEINTHORSDOTTIR et al., 2007].

Scott et al. showed that another SNP within CDKAL1 is associated with T2DM, namely rs7754840 (OR 1.12; 95% CI 1.08-1.16; $P = 4.1 \times 10^{-11}$). To note is that the results presented from Scott at al. are actually results from a meta-analysis, because most of the associations reached only significance when they pooled the data. Additional data came from the Diabetes Genetics Initiative (DGI) and the Wellcome Trust Case Control Consortium UKT2D United Kingdom T2D Genetics Consortium/United Kingdom T2D Genetics Consortium (WTCCC/UKT2D) [SCOTT et al., 2007].

The role of CDKAL1 is currently unknown, but it is speculated that the gene product has a role in the inhibition of the CDK5/p35 complex in pancreatic beta cells. The complex regulates the insulin gene expression, which is decreased when too much glucose is present (glucotoxicity). By inhibition of the complex, the insulin gene expression is not declined; the response to glucotoxicity is not impaired [STEINTHORSDOTTIR et al., 2007]. Expression of CDKAL1 mRNA can be found in human pancreatic islet and skeletal muscle [ZEGGINI et al., 2007]. Steinthorsdottir et al. showed, by the way, that rs7756992 is associated with a lower insulin response. Homozygous (GG) carriers of the risk allele showed significant 22 % lower corrected insulin response than noncarriers. In contrast, only 2 % of the heterozygous (AG)

carriers showed a nonsignificant lower corrected insulin response [STEINTHORSDOTTIR et al., 2007].

CDKN2A/B

SNP rs10811661 within the cyclin-dependent kinase inhibitor 2A (CDKN2A/B) gene is associated with T2DM (OR 1.20; 95% CI 1.14-1.25; P = 7.8 x 10^{-15}) as shown by Scott et al. all data (FUSION, DGI, and WTCCC/UKT2D) [SCOTT et al., 2007].

Grarup et al. confirmed the association with SNP rs10811661 by investigating 10,705 Danish subjects. The candidate gene study was designed to validate and extent GWASs. The T-allele was associated with a 1.30-fold increased risk in an additive genetic model (OR 1.30 per risk allele; 95% CI 1.16–1.47; P_{add} = 1 x 10^{-5}). Moreover, they showed that variations within CDKN2A/B loci are responsible for an impairment of glucose- and totbutamide-induced insulin release in middle-aged and young healthy individuals leading to the assumption that there is a dysfunction of the beta cells [GRARUP et al., 2007].

FTO

Pooled data from FUSION, DGI, and WTCCC/UKT2D showed that the fatso/Fat mass and obesity associated (FTO) gene is associated with T2DM. Risk allele A of SNP rs8050136 increased the T2DM risk 1.17-fold (95% CI 1.12-1.22; P = 1.3 x 10^{-12}) [SCOTT et al., 2007].

As mentioned before data from the WTCCC was included in that meta-analysis. Results from this study alone provided evidence for an additional SNP within FTO. They showed that variant rs9939609 increases the T2DM risk 1.27-fold (95% CI 1.16-1.37; P = 5 x 10^{-8}) [THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007].

This gene contributes to the T2DM risk through the mediation of obesity, because the association disappears when adjusted for BMI [ZEGGINI et al. 2007].

IDE-HHEX-KIF11

Two SNPs (rs1111875 and rs7923837) within chromosome 10 were identified by Sladek et al. to be associated with T2DM. Homozygous carriers of the risk allele A had an increased risk for T2DM in the range of 1.44-1.45; heterozygous carriers of the risk allele A had an increased risk for T2DM in the range of 1.19-1.22. Those SNPs are located in a LD block (Figure 8) that contains two genes potentially involved in beta cell development or function, namely the insulin-degrading enzyme (IDE) and the homeodomain protein HHEX – as well as kinesin-interacting factor 11 (KIF11) [SLADEK et al, 2007].

The association of one of those SNPs (rs1111875) was also confirmed by a meta-analysis of three GWASs (FUSION, WTCCC/UKT2D, DGI) (OR 1.13; 95% CI 1.09-1.17; $P = 5.7 \times 10^{-10}$) [SCOTT et al., 2007] and in a case-control study of 10,705 Danish subjects (OR 1.13 per allele; 95% CI 1.03–1.23; $P_{add} = 0.008$). This case-control study showed, moreover, that variations within the HHEX/KIF11/IDE are related to pancreatic beta cell dysfunctions by conferring an impairment of glucose- and totbutamide -induced insulin release in middle-aged and young healthy subjects [GRARUP et al., 2007].

It is to notice that likewise TCF7L2, HHEX is a target of the Wingless-type (Wnt) signalling pathway [SLADEK et al, 2007].

IGF2BP2

Scott et al. contributed to the identification of a T2DM-associated variant near the insulinlike growth factor 2 mRNA binding protein 2 (IGF2BP2) gene. All data (FUSION, DGI, and WTCCC/UKT2D) showed an association of SNP rs4402960 near IGF2BP2 with T2DM (OR 1.12; 95% CI 1.08-1.16; $P = 4.1 \times 10^{-11}$) [SCOTT et al., 2007].

The association of IGF2BP2 (rs4402960) with T2DM was replicated in a candidate gene study; the variant showed an association in a dominant genetic model (OR 1.17; 95% CI 1.04–1.32; $p_{dom} = 0.01$) [GRARUP et al., 2007].

Additionally, SNP rs1470579 within IGF2BP2 showed association with T2DM (OR 1.17; 95% CI 1.11-1.23, P = 1.3 x 10^{-9}). This was found by analysing DGI data [SAXENA et al. 2007].

IGF2BP2 variants that predispose to diabetes may function by regulation of the IGF2 translation. IGF2 is known as a member of the insulin family of polypeptide growth factors. It stimulates insulin secretion and is involved in the development and growth [SCOTT et al., 2007].

KCNJ11

Pooled data from FUSION, DGI, and WTCCC/UKT2D showed that the potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) gene is associated with T2DM. Risk allele T of SNP rs5219 increased the T2DM risk 1.14-fold (95% CI 1.10-1.19; P = 6.7 x 10^{-11}). The identified SNP rs5219 within KCNJ11 is a non-synonymous one (Glu23→Lys23). Another SNP (rs5215) is nearly in a perfect association (r2 = 0.995) [SCOTT et al., 2008].

KCNJ is a reasonable candidate gene for T2DM, because it encodes a component of a potassium channel with a key role in beta cell physiology; therefore, it is a target for anti-diabetic therapies [FRAYLING, 2007b].

PPARG

SNP Pro12Ala is the most investigated SNP within the peroxisome proliferator-activated receptor gamma (PPARG) gene. It is a non-synonymous SNP, which is the result of a CCA-to-GGA missense mutation [SOOKOIAN et al., 2005]. Although it is the most investigated, all data (FUSION, DGI, WTCCC/UKT2D) showed a strong, but not a genome-wide significant association, between SNP rs1801282 (Pro12Ala) and T2DM (OR 1.14; 95% CI 1.08-1.12; P = 1.7 x 10^{-6}) [SCOTT et al., 2007].

Similar was shown by the GWAS of Zeggini et al. Indistinguishable from that signal at rs1801282 (Pro12Ala) was the signal at SNP rs17036101. Latter increased the risk of T2DM 1.15-fold (P = 2.0 x 10^{-7}), but the prespecified threshold of P = 5.0 x 10^{-8} was

not met. Interestingly, the SNP was not convincingly associated with BMI or other T2DM related traits [ZEGGINI et al., 2008].

In addition, a meta-analysis of candidate gene studies, which aimed to clear the question whether SNP Pro12Ala is truly associated with T2DM, showed no conclusive evidence. They analysed 41 published and 2 unpublished studies (all together 42,910 individuals from Asia, Europe, and North America) and showed that Ala12 carriers have a 19 % T2DM risk reduction. However, the observed association was highly heterogeneous (P = 0.005). This was partly explained by the BMI of the control group; the risk reduction was greater when the BMI was lower. Heterogeneity remained after stratifying according to the three different geographic regions (Asia, Europe, and North America) in the European subgroup (P = 0.02). Furthermore, they stratified the data from Europe according to a North-South gradient. This analysis showed that the risk reduction is bigger in North Europe (26 % reduction, P < 0.0001) than in Central Europe (10 %, P = 0.04) and non-existing in South Europe (Table 7). The authors suggested that this result might be due to publication bias, because it is unlikely that negative results from Northern Europe have been published, but they included negative results from Italian studies [LUDOVICO et al., 2007].

Table 7. Risk of T2DM according to PPARG Ala12 variant stratified by region

Study	**OR (95 % CI)**
All Asians	0.65 (0.54-0.79)
All Europeans	0.86 (0.79-0.95)
All North-Europe (Scandinavians, British)	0.74 (0.66-0.83)
All Central-Europe (Poles, Germans, French, Czechs)	0.90 (0.82-1.00)
All South-Europe (Italians, Spaniards)	1.01 (0.80-1.28)
All North Americans	0.82 (0.67-1.01)

[LUDOVICO et al., 2007] modified

Ludovico et al. assumed that the heterogeneous risk in Europe might be through different genetic and/or environmental background [LUDOVICO et al., 2007].

That the observed heterogeneity may partly be explained by physical activity was reported by a Finnish study. Increased physical activity decreased the effect of the risk alleles of rs17036314 and rs1801282 (Pro12Ala) on the conversion from IGT to T2DM.

After adjustment for baseline fasting glucose only SNP rs17036314 remained significantly associated [KILPELÄINEN et al., 2008].

PPARG belongs to the peroxisome proliferator-activated receptors (PPARs) family. These endogenous receptors for fatty acids and lipid metabolites modulate our rates of macronutrient oxidation, regulate the systemic inflammatory response, and improve systemic insulin sensitivity when a ligand binds. Furthermore, their activation inhibits the hypertrophy of adipocytes, which leads to insulin resistance via a decreased plasma adiponectin level [GURI et al., 2008].

SLC30A8

The non-synonymous SNP rs13266634 (Arg325→Trp325) [FRAYLING, 2007b] was repeatedly associated with T2DM [SLADEK et al., 2007; STEINTHORSOTTIR et al., 2007; SCOTT et al., 2007]. This SNP is located within the solute carrier family 30 (zinc transporter), member 8 (SLC30A8) gene and was identified by Sladek et al. as the most significant SNP compared to the other newly associated SNPs. They found that homozygous (CC) individuals have a 1.53-fold increased risk and heterozygous (CT) individuals have a 1.18-fold increased risk to develop T2DM [SLADEK et al, 2007].

The GWAS conducted by Steinthorsdottir et al. showed smaller effect sizes of that SNP when analysing data from five T2DM case-control studies (replication sample). The CC type of SNP rs13266634 was associated with a 1.26-fold (95% CI 1.10-1.43) increased risk and the CT type was associated with a 1.05-fold (95% CI 0.93-1.19) increased risk. Every risk allele accounted for a 1.15-fold increased risk (allelic OR 1.15; 95% CI 1.08-1.22; $P = 7.7 \times 10^{-9}$) [STEINTHORSDOTTIR et al., 2007].

Pooled data from FUSION, DGI and WTCCC/UKT2D showed also that rs13266634 is associated with T2DM. Risk allele C increased T2DM risk 1.12-fold (OR 1.12; 95% CI 1.07-1.16; $P = 5.3 \times 10^{-8}$). The association was even stronger when they adjusted for BMI (or waist circumferences). An adjustment for blood pressure variables showed no effect [SCOTT et al., 2007].

The zinc transporter, which is encoded by SLC30A8, is solely found in pancreatic beta cells and is involved in the final stages of insulin biosynthesis. This is because of the

insulin co-crystallization with zinc [SLADEK et al, 2007]. A dysfunction of the transporter, through variation of SLC30A8, may affect zinc accumulation and subsequently alter insulin stability, storage, or secretion [SCOTT et al., 2007]. To mention is that Steinthorsdottir et al. observed a significant lower insulin response in individuals carrying the risk allele C [STEINTHORSDOTTIR et al., 2007].

TCF7L2

The transcription factor 7-like 2 (TCF7L2) gene is the most important susceptibility gene for T2DM, because variants showed the best evidence and the biggest effect sizes in comparison to the other T2DM susceptibility genes (Table 6).

Pooled data (FUSION, DGI and WTCCC/UKT2D) showed that the risk allele T of SNP rs7901695 increase the T2DM risk 1.37-fold (95% CI 1.31-1.43; $P = 1 \times 10^{-48}$) with an association even stronger after adjustment for BMI (or waist circumferences). Adjustment for blood pressure variables showed no effect [SCOTT et al., 2007].

Sladek et al. related another SNP (rs7903146) within TCF7L2 to T2DM with homozygous carriers (TT) having a 2.77-increased risk and heterozygous carriers (CT) having a 1.65-increased risk to develop T2DM [SLADEK et al., 2007].

Steinthorsdottir et al. confirmed the association with SNP rs7903146 within TCF7L2 (allelic OR 1.38; $P = 1.82 \times 10^{-10}$) [STEINTHORSDOTTIR et al., 2007].

However, the transcription factor 7-like 2 (TCF7L2) gene was already linked to T2DM by other gene association approaches. In 2006, researchers followed up a previously reported linkage of T2DM to chromosome 10q by using a high density of genotyped microsatellite markers. They identified an association of the composite allele X (microsatellite marker DG10S478X, nonzero alleles), within intron 3 of the TCF7L2 gene, with T2DM in an Icelandic, in a Danish and in a US cohort. The combined RR from all cohorts (in each case individuals with T2DM and controls) was 1.56 (95% CI 1.41-1.73) with a 4.7×10^{-18} P-value. To reach significance with the Bonferroni correction a P-value of 7.8×10^{-15} was needed. From the five investigated SNPs allele G of SNP rs12255372 showed the best, nearly perfect, association with DG10S478

(r^2=0.95; P = 5.53 x 10^{-38}). The SNP with the second best association was rs7903146 (r^2=0.78). Both were recommended for further research [GRANT et al. 2006].

Zhang et al. confirmed the association of the TCF7L2 gene (rs12255372) with T2DM risk among Caucasians. Moreover, they pooled their data with data from Grant et al. All in all, they investigated 3,347 case and 3,947 control subjects. This meta-analysis showed that each copy of the T-allele was associated with a 1.48-fold (95% CI 1.37-1.60; P < 10^{-16}) increased risk for T2DM. The calculated OR for heterozygous carries (GT) was 1.42 (P = 5 x 10^{-10}) and 2.11 for homozygous carriers (TT) (P < 10^{-16}). The authors stated that they could not ascertain whether the rs12255372 SNP is a causative variant or a proxy for an underlying causal variant [ZHANG et al, 2006].

Interesting results came from the Finnish Diabetes Prevention Study (DPS), which investigated the association of SNPs rs12255372 and rs7903146 with incident T2DM. For one thing, they confirmed the association of rs12255372 again and for the other thing, they showed that lifestyle-interventions can reduce the risk conferred by genetic factors. 507 individuals with IGT, divided into an intensive diet and lifestyle intervention group (individually customized advice to reduce weight as well as total and saturated fat intake and individually customized advice to increase fibre intake and physical activity) or control group (general information on the benefits of weight reduction, physical activity and healthy diet), were included in that study. Remarkably, only the control group showed association between the TT genotype of rs12255372 and the risk of incident diabetes (HR 2.85; 95% CI 1.17-6.95, P = 0.021). No association was seen in the intervention group. SNP rs7903146 was not significantly associated with the risk of diabetes [WANG et al., 2007].

The exact mechanism by which the TCF7L2 gene is related to T2DM is not known yet. Data indicates that the regulating of glucagon-like peptide 1 (GLP-1) through the Wnt signalling pathway, which is one of the key developmental and growth regulatory mechanisms of the cell, is to consider. GLP-1 is an insulinotropic hormone that can lower blood glucose levels through several pathways e.g. stimulation of insulin secretion and biosynthesis [ZHANG et al, 2006]. Interestingly, the above-mentioned Finnish study showed that the T allele of rs12255372 within TCF7L2 is significantly associated with decreased insulin secretion [WANG et al., 2007].

Genes associated with type 2 diabetes mellitus published in 2008

Zeggini et al. carried out a meta-analysis of the DGI, FUSION, and WTCCC (all in all 10,128 samples; 4,549 cases and 5,579 controls) data and enlarged SNP coverage by including untyped SNPs (based on HapMap) to identify additional, to the already published, associated SNPs. This meta-analysis was followed by a 2-stage study including 53,975 individuals. On the basis of the combined stage 1-3 analyses six additional SNPs showed evidence for association with T2DM ($p_{add} = 5.0 \times 10^{-8}$). SNPs near the genes JAZF1, THADA, ADAMTS, NOTCH2 and between genes CDC123 and CAMK1D as well as between TSPAN 8 and LGR5 belonged to the significant SNPs. ORs ranged from 1.09 to 1.15. None of the SNPs was convincingly associated with BMI or other T2DM-related traits [ZEGGINI et al., 2008].

Table 8. Six T2DM gene regions published 2008

These gene regions were identified by one GWAS published 2008. All data (Stage 1-Stage 3) presented [ZEGGINI et al., 2008]

SNP	Nearest gene	Chr	Odds ratio (95%CI)	P-value
rs4607103	ADAMTS9	3	1.09 (1.06-1.12)	1.2×10^{-08}
rs12779790	CDC123, CAMK1D	10	1.11 (1.07-1.14)	1.2×10^{-10}
rs864745	JAZF1	7	1.10 (1.07-1.13)	5.0×10^{-14}
rs10923931	NOTCH2	1	1.13 (1.08-1.17)	4.1×10^{-08}
rs7578597	THADA	2	1.15 (1.10-1.20)	1.1×10^{-09}
rs7961581	TSPAN8, LGR5	12	1.09 (1.06-1.12)	1.1×10^{-09}

ADAMTS9 a disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 9; CDC123 cell division cycle protein 123 homolog; CAMK1D calcium/calmodulin-dependent protein kinase i-delta; JAZF1 juxtaposed with another zinc finger gene 1; NOTCH2 notch, drosophila, homolog of, 2; THADA thyroid adenoma-associated gene; TSPAN8 tetraspanin 8; LGR5 leucine-rich repeat-containing G protein-coupled receptor 5

[ZEGGINI et al., 2008] modified

Genes associated with fasting plasma glucose

Two GWASs were published in 2008 that found a new gene association for fasting plasma glucose (FPG) [CHEN et al., 2008; BOUATIA-NAJI et al., 2008].

G6PC2

Variants located between the genes glucose-6 phosphatase catalytic subunit 2 (G6PC2) and ATP-binding cassette, sub-family B (MDR/TAP), member 11 (ABCB11) showed significant association with fasting glucose concentration in two genome wide scans including a total of 5,088 nondiabetic individuals from Finland and Sardinia. The significance remained after adjustment for BMI, leading to the assumption that the main contribution to the observed association is not mediated by obesity. Anyhow, the found associations were then partly replicated in additional 18,436 nondiabetic individuals of mixed European decent. Pooled data from all studies (n = 24,046) showed that rs563694 (SNP with the strongest association within gene G6PC2) is associated with FPG by a p-value of 6.4×10^{-33}. Across these studies, each copy of the SNP rs563694 major allele increased the fasting glucose concentration by 0.01-0.16mM. This amounts to an approximately 1% contribution to the total variation observed in fasting glucose. Interestingly, no association was observed between fasting glucose and SNP rs563694 and rs560887 in individuals with T2DM [CHEN et al., 2008].

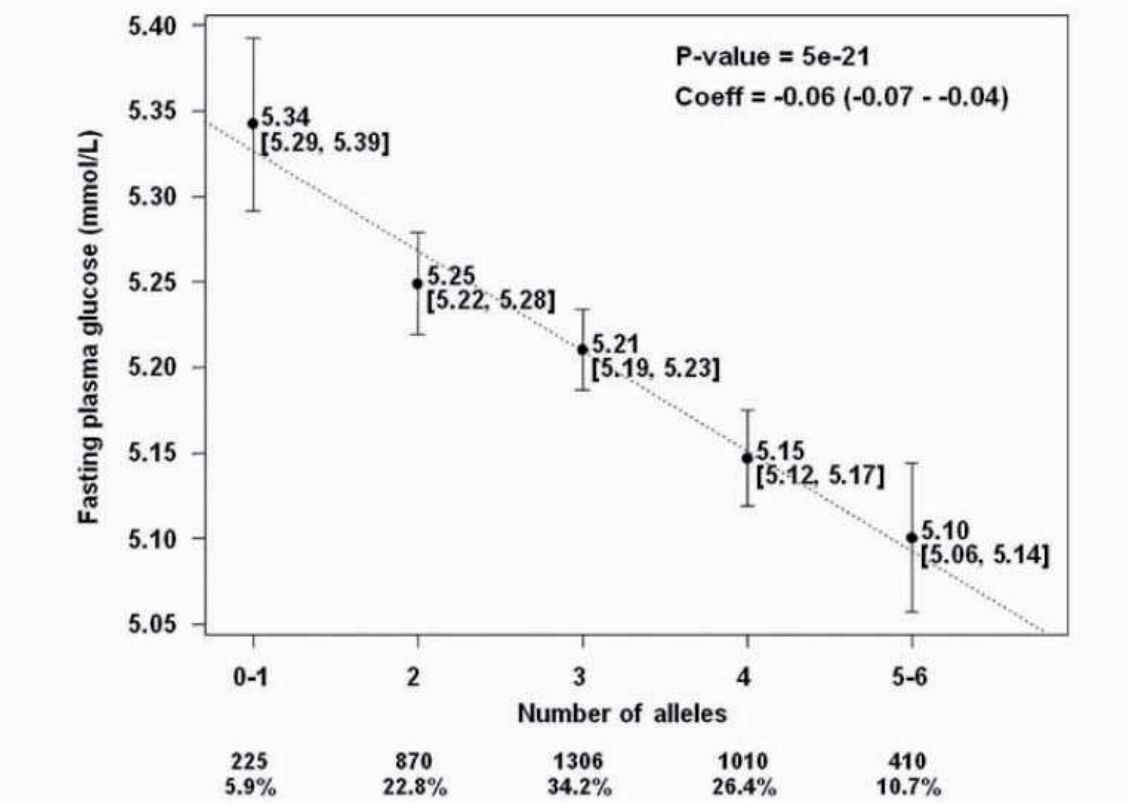
In contrast to Chen et al., Bouatia-Naji et al. observed the strongest association between rs560887 and fasting plasma glucose. Anyhow, SNP rs560887 is in high LD with SNP rs563694 ($D' = 0.99$, $r^2 = 0.84$), but opposite to rs563694, rs560887 is not located in between two genes, it is located directly in intron 3 of G6PC2. Interestingly, the seen association was, like the association in the former study, independent of BMI. 654 normoglycemic participants were investigated in that GWAS followed by an investigation of three independent populations (n = 9,353). The overall meta-analysis of the replication samples showed that each copy of the A allele results in a 0.06 mmol/l FPG decrease (combined $P = 4 \times 10^{-23}$) and is associated with pancreatic beta cell function (HOMA-B model, combined $P = 3 \times 10^{-13}$). In addition, the interaction of rs560887 with SNPs formally associated with FPG, namely GCK (rs1799884) and GCKR (rs1260326), was investigated. They observed that these three SNPs have an

additive effect on FPG ($P = 5 \times 10^{-21}$). Carriers of more than four alleles related to low FPG showed a mean 0.24 mmol/l (4.5%) decrease of FPG compared with those individuals carrying only one allele (Figure 10). Remarkably, no association of a SNP within G6PC2 and T2DM risk was observed. The authors suggested that the genetic determinants that regulate FPG might be different from those that predispose somebody to T2DM [BOUATIA-NAJI et al., 2008].

The protein encoded by G6PC2, also known as islet-specific glucose-6 phosphatase related protein (IGRP), is involved in the glucose metabolism, more precisely, the protein is involved in the gluconeogenic and glycogenolytic pathways. The mainly in the liver expressed gene ABCB11 is involved in the secretion of bile salts [CHEN et al., 2008].

Figure 10. Combined effects of gene variants on FPG levels

Combined effects of rs560887 (G6PC2), rs1260326 (GCKR), and rs1799884 (GCK) variants on FPG levels. Data are presented as mean [95% CI], and the P-value is for the beta coefficient in the linear regression model (adjusted for age, sex, and BMI) of FPG levels on the number of alleles associated with low FPG



[BOUATIA-NAJI et al., 2008] reproduced

Genes associated with the homeostasis model assessment of insulin resistance

MC4R

In 2008, a GWAS was published, which investigated IR and related phenotypes in individuals from the UK of Indian Asian or European ancestry. They showed that the SNPs rs12970134, rs477181, rs502933 and rs4450508 within the melanocortin 4 receptor (MC4R) gene are significantly associated with WC, HOMA-IR, WHR, weight, BMI and MetSyn (Table 9) in the population of Indian Asian ancestry and in the combined analysis. SNP rs12970134 was, for example, associated with HOMA-IR % by an effect size of 5.17 (95% CI 2.96-7.42; P = 3.2 x 10^{-6}). Analysis of the European ancestry population was not significant. Multiple rare mutations of the MC4R gene cause hyperphagia and subsequently severe childhood obesity. Furthermore, they are associated with increased insulin levels. MC4R is, therefore, an interesting candidate gene when investigating the genetic determinants of IR [CHAMBERS et al., 2008].

Table 9. Association of a variant near MC4R with IR and related phenotypes

Data is shown for SNP rs12970134 allele A in stage 2 participants

	Indian Asian ancestry (n = 7,394)		**European ancestry (n = 4,561)**		**All stage 2 participants (n = 1,955)**		**HT p**
	Effect size or OR (95% CI)	P	Effect size or OR (95% CI)	p	Effect size or OR (95% CI)	p	
HOMA-IR (%)	5.39 (2.78-8.07)	4.2 x 10^{-5}	5.16 (1.18-9.29)	0.01	5.17 (2.96-7.42)	3.2 x 10^{-6}	0.95
Waist-hip ratio	0.004 (0.002-0.006)	9.8 x 10^{-4}	0.004 (0.000-0.007)	0.02	0.004 (0.002-0.006)	7.7 x 10^{-5}	0.87
Weight (kg)	0.82 (0.40-1.24)	4.2 x 10^{-5}	1.21 (0.48-1.94)	0.001	0.93 (0.56-1.31)	1.0 x 10^{-6}	0.37
BMI (kg/m2)	0.25 (0.10-0.39)	1.3 x 10^{-4}	0.28 (0.05-0.51)	0.02	0.25 (0.13-0.38)	6.4 x 10^{-5}	0.86
MetSyn	1.13 (1.05-1.21)	0.002	1.13 (1.00-1.27)	0.01	1.12 (1.06-1.20)	2.3 x 10^{-4}	0.93

HT Heterogeneity

Effect sizes or OR under an additive genetic model, with adjustment for age, gender and ethnic group in stage 2 and in the combined analysis. Statistical significance was given with a P-value of P < 0.006 after Bonferroni correction for nine phenotypes tested.

[CHAMBERS et al., 2008] modified

2.7.2 Obesity

As shown before FTO is related to T2DM through the altering of BMI, but this gene is not the only gene that was found to be associated with obesity traits. GWASs reported a vast number of – not always genome-wide significant – associated genes [FRAYLING et al., 2007a; LOOS et al., 2008; CHAMBERS et al., 2008; FOX et al., 2007]. The most important of them are listed and described (Table 10). The basic paradigm for a genetic predisposition to obesity is shown in Figure 12, located at the end of that chapter.

Table 10. Eight gene regions associated with overweight/obesity or related traits identified by GWAS

Gene regions associated with overweight/obesity or related traits identified by GWASs [FRAYLING et al., 2007a; LOOS et al., 2008; CHAMBERS et al., 2008; FOX et al., 2007]. Because additional evidence from candidate gene studies or a CRP-GWAS [RIDKER et al., 2007] exists, even genes that didn´t reach the genome-wide significance are presented.

Closest gene	BMI	Weight	WHR	WC	FM	OV	OB
ADIPOQ*				x			
CTNNBL1	x				x		x
FTO	x	x	x	x	x	x	x
IL6R*				x			
INSIG2*	x						
LEPR*				x			
MC4R	x	x			x	x	x
PPARG*	x			x			

*reached not the genome-wide significance
BMI Body mass index; WHR waist to hip ratio; WC waist circumference; FM fat mass; OV overweight; OB obesity

ADIPOQ

The Adiponectin, C1Q and Collagen Domain Containing (ADIPOQ) gene is one of the genes with former evidence for association, but no genome-wide significant association has yet been observed.

In the Framingham Heart Study-GWAS (100 k project; n = 1,341) it was reported that SNP rs1042464 is related to mean WC. Association was given for the family based

association test with a P-value of 0.024. To notice is that this finding reached not the required genome-wide significance [FOX et al., 2007].

In humans, the level of plasma adiponectin is inversely associated with BMI and body fat mass; loosing weight increases plasma adiponectin levels [SONG et al., 2006].

CTNNBL1

Opposite to the following studies a genome-wide association scan for obesity (examining approximately 500,000 SNPs) in a sample of 1,000 unrelated U.S. Caucasians provided no evidence for the FTO association, in contrast, they identified a novel gene, the catenin, beta like 1 (CTNNBL1) gene, which showed association with BMI and fat mass. The T allele of the most significant SNP rs6013029 was associated with a fat mass increase of 5.96 kg (P-value of 2.7×10^{-7}) and with a BMI increase of 2.67 kg/m^2 (P-value of 5×10^{-8}). In a second stage they genotyped the most significant variants within CTNNBL1 in a case-control sample (n = 3,812). They repeated the association and showed that homozygous TT carriers have an increased risk of obesity (OR = 1.42) compared to homozygous GG carriers. The exact implication mechanism of CTNNBL1 is not known yet; however, as the protein structure of CTNNBLI is homolog to the beta-catenin structure, the function may be similar. Beta-Catenin is involved in the regulation of the adipogenic gene expression [LIU et al., 2008].

FTO

Frayling et al. investigated the T2DM susceptibility gene FTO in a meta-analysis of 13 cohorts with 38,759 participants (population-based studies and T2DM case and control studies including WTCCC data) further. They found an additive association of the variant rs9939609 with BMI. The analysis of the pooled data (population-based studies only) showed that each additional copy of the rs9939609 A allele was associated with a BMI increase of a mean of 0.10 Z-score units (95% CI 0.08-0.12; $P = 2 \times 10^{-20}$, equivalent to ~ 0.4 kg/m^2). Moreover, rs9939609 A allele was associated with higher weight (overall per-A allele increase = 0.09 Z-sore units; 95% CI = 0.07–0.11; $P = 4 \times 10^{-17}$; equivalent to ~ 1.2 kg) and higher WC (overall per-A allele = 0.08 Z-score units;

95% CI = 0.05-0.11; P = 4 x 10^{-9}; ~ 1 cm). In a meta-analysis of all studies, homozygous risk carriers (AA; 16% of the population) were at increased risk of being overweight (OR = 1.38; 95% CI =1.26-1.52; P = 4 x 10^{-4}) or obese (OR =1.67; 95% CI 1.47-1.89; P = 1 x 10^{-14}) compared with those individuals homozygous for the low-risk T allele (37% of the population). The SNP rs9939609 explained ~1% of BMI variance. In addition, they found that the variant contributes to childhood obesity [FRAYLING et al., 2007a].

Another variant (SNP rs1121980) was reported to be associated with obesity traits in the GWAS conducted by Loos et al. They showed by meta-analysis of four European population-based studies and three disease-case studies that the per allele effect for rs1121980 is equivalent to 0.27 kg m^{-2} (beta = 0.060; 95% CI 0.039-0.082 Z-score units; P = 3.6 x 10^{-8}). Z-score units have been defined as "log_{10}- transformed BMI, standardized by gender and age" [LOOS et al., 2008].

SNP rs1121980 was also associated with childhood obesity and severe adult obesity (2,900 cases and 5,100 controls). The T allele of SNP rs1121980 was strongly associated with severe adult obesity (OR = 1.55; 95% CI 1.39-1.73; P = 5.3 x 10^{-16}). Furthermore, rs1421085 and rs17817449, potentially functional SNPs, showed associations [DINA et al., 2007].

Do et al. aimed to investigate the mechanism by which the FTO variants (rs17817449, rs1421085) influence obesity traits; therefore, they genotyped 908 participants of the Quebec Family Study. SNP rs17817449 was associated either under an recessive model or an additive model with anthropometric parameters of obesity (weight, BMI, FM, waist and hip circumferences, WHR, percentage body fat and skinfold thickness), but also with fasting insulin, HOMA-IR, an insulin sensitivity index derived from an oral glucose tolerance test, resting metabolic rate and plasma leptin levels. The association with insulin sensitivity, resting metabolic rate, and plasma leptin levels disappeared when they adjusted for BMI. To note is that SNPs rs9939609, rs17817449, rs3751812, rs1421085 and rs8050136 are all in strong LD (r^2 from 0.92 to 1) [DO et al., 2008].

The contribution of the FTO gene to the development of obesity might be mediated by the encoded 2-oxoglutarate-dependent nucleic acid demethylase. This enzyme is most frequently present in the hypothalamus [AL-ATTAR et al., 2008].

IL6R

Although no genome-wide significance was shown for an association of the interleukin 6 receptor (IL6R) gene with obesity, this gene is mentioned, because another GWAS, which investigated CRP, showed genome-wide significant association [RIDKER et al., 2008]. Anyhow, Fox et al. showed by the family based association test that SNP rs4129267 within IL6R is related to mean WC (P-value of 0.003) [FOX et al., 2007].

This gene encodes for a protein that is a subunit of the IL6 receptor complex. Variations in IL6R may lead to dysfunction of IL6, a cytokine that regulates cell growth and differentiation; furthermore, it plays an important role in immune response [NCBI, 2008b].

INSIG

Hinney et al. reviewed the latest findings regarding gene-obesity associations. They stated that the insulin-induced gene 2 (INSIG2) showed positive data, but this was not reliably replicable by now [HINNEY and HEBEBRAND, 2008]. Interestingly Fox et al. showed that five SNPs within INSIG2 are related to mean BMI. Significance, but not genome-wide significance, was given by the additive generalized estimating equations with P-values from 0.001 to 0.035 [FOX et al., 2007].

The protein that is encoded by INSIG2 inhibits the synthesis of cholesterol and fatty acid [SOOKOIAN and PIROLA, 2007].

LEPR

The same situation that is true for the IL6R gene is true for the leptin receptor (LEPR) gene. Only Fox et al. showed, by the additive generalized estimation equations, that the SNP rs2025804 within LEPR is related to mean WC, but the association is not a genome-wide significant one [FOX et al., 2007].

LEPR is a single-transmembrane-domain receptor of the cytokine receptor family. Through that receptor operates leptin, an adipocyte-specific hormone that regulates

adipose-tissue mass through hypothalamic effects on energy expenditure and satiety [NCBI, 2008b].

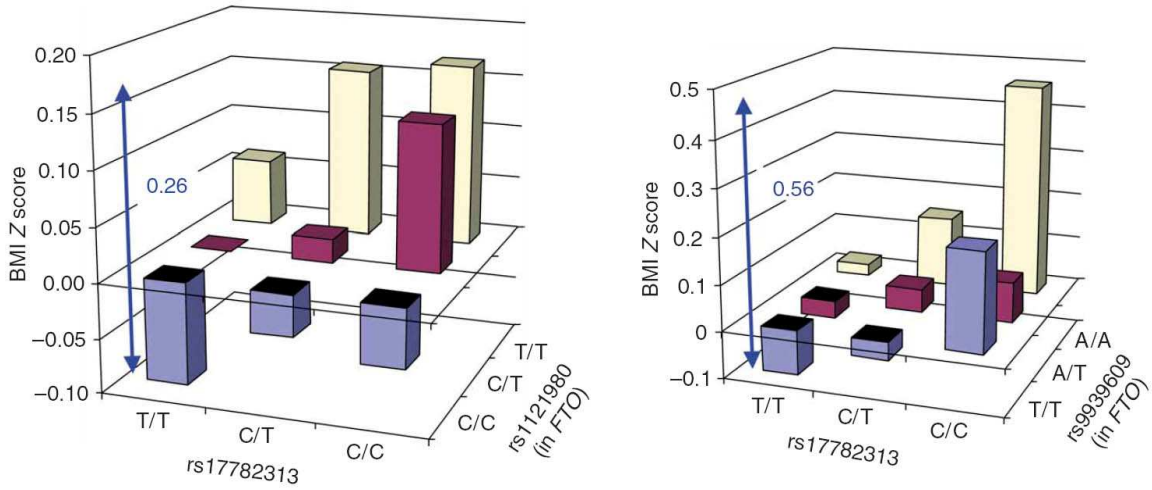
MC4R

Additional to the SNP within the FTO gene there is substantial evidence that variants within the melanocortin-4 receptor (MC4R) gene have an effect on body weight. Two recently published GWASs showed associations between MC4R variants and obesity [LOOS et al., 2008; CHAMBERS et al., 2008].

Loos et al. reported that SNP 17782313 near MC4R is associated with fat mass, weight, and risk of obesity. The large-scale GWAS (n = 16,876) in individuals phenotyped for adult BMI was followed by replication studies (n = 66,340). Meta-analysis of all studies showed a BMI association in adults (per-allele effect for rs17782313C = 0.049; 95% CI 0.037-0.061 Z-score units; $P = 2.8 \times 10^{-15}$; equivalent to 0.22 kg m^{-2}) and a BMI association in children aged 7-11 (per-allele effect for rs17782313C = 0.13, Z-score units; $P = 1.5 \times 10^{-8}$). The association with BMI was mediated through weight (per-allele effect for rs17782313C = 0.059; 95% CI 0.047-0.071 Z-score units; $P = 2.8 \times 10^{-15}$; equivalent to ~760g) and height (per-allele effect = 0.030; 95% CI 0.018-0.042 Z-score units; $P = 8.7 \times 10^{-7}$; equivalent to ~0.21 cm) in adults, but only through weight in children. Adults had an increased risk of overweight (OR = 1.08; $P = 1.6 \times 10^{-9}$) as well as an increased risk of obesity (OR = 1.12; $P = 5.2 \times 10^{-9}$) when carrying an rs17782313C allele. Anyhow, it is to notice that the contribution to the observed variance is rather small. The identified SNPs near MC4R account for ~0.14% variance in adult BMI and ~0.26% variance in fat mass at age 9. In addition to the already stated results, they found an additive effect on BMI that was attributed to FTO variants as well as MC4R variants (Figure 11) and reported that genetic determinants of monogenic and multifactorial forms of the same condition can overlap. Latter was found, because the same SNP, which was associated with obesity in the population-based studies, was also associated in populations with severe early onset obesity. SNP rs17782313 increased the risk of extreme obesity 1.30-fold (95% CI 1.20-1.42; $P = 8.0 \times 10^{-10}$) [LOOS et al., 2008].

Figure 11. Additive effect of FTO variants and MC4R variants

Association between the combined SNPs (rs17782313, rs1121980) and BMI in adults and association between the combined SNPs (rs17782313, rs9939609) and BMI in children. BMI difference amounts to 0.26 Z-score units (or ~ 1.17 kg m^{-2}) in adults and 0.56 Z-score units in children when comparing individuals with no risk alleles at either locus (19% of the population) with those homozygous at both (1% of the population).



[LOOS et al., 2008] reproduced

Chambers et al., showed in their 2-stage GWAS of IR and related phenotypes in a population cohort of individuals from the UK of Indian Asian or European ancestry that the strongest association with WC was near the MC4R gene with rs12970134, followed by rs471181, rs502933, rs4450508. All of those SNPs are in high LD (r^2 = > 0.5) on chromosome 18. Three of the four SNPs reached genome-wide significance when a combined analysis of stage one and two data (Indian Asians and European ancestry) was done. The effect size for the association of rs12970134 risk allele A with WC under an additive genetic model adjusted for age, sex, and ethnicity was 0.88 cm (95% CI 0.59-1.17; P = 1.7 x 10^{-9}). Moreover, SNP rs12970134 was significantly associated with WHR, weight, and BMI but only in the population of Indian Asian ancestry and in the combined analysis of all stage 2 participants (Indian Asian ancestry and European ancestry) (Table 9). Variant V103I was uncommon in that study (MAF 0.6%) and not in a LD with any of the mentioned SNPs [CHAMBERS et al. 2008].

The above-mentioned variant V103I was the first confirmed polygenetic variant that was associated with BMI with a higher frequency of Val103 in obese individuals. The effect size of the 103Ile allele on mean BMI was stated with -0.5 kg/m^{-2} [HINNEY and HEBEBRAND, 2008].

The association between V103I and risk of obesity was investigated by three UK population based cohort studies (n = 8,304 in total) that were followed by a meta-analysis of 29,563 individuals. The non-synonymous Val103Ile (rs2229616) polymorphism showed a protective function against human obesity. Carriers of the 103I allele had an 18% (95% CI 4–30%; P = 0.015) lower risk of obesity compared with non-carriers [YOUNG et al., 2007].

MC4R is a plausible biological candidate for the association with obesity, because rare MC4R mutations are associated with hyperphagia and severe childhood obesity [CHAMBERS et al., 2008]. However, evidence for the mechanism relating the found variants to MC4R expression is missing [LOOS et al., 2008].

PPARG

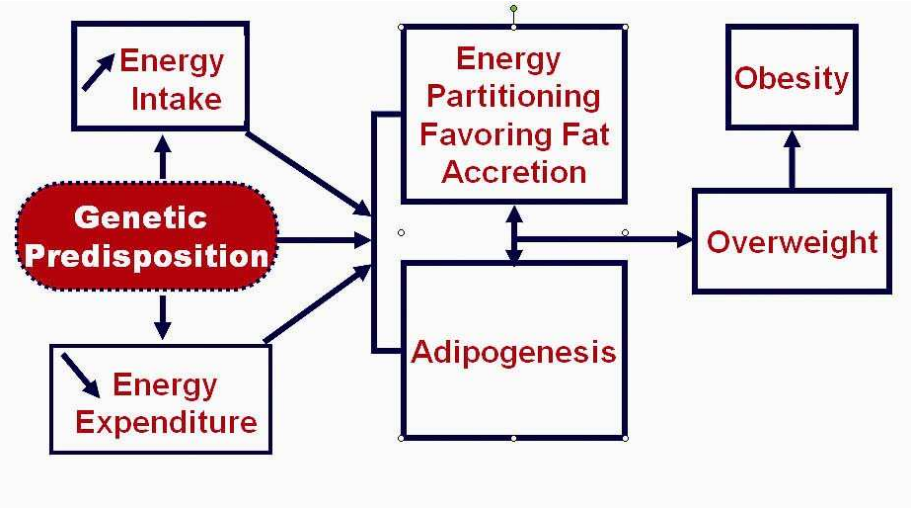
In a GWAS (Framingham Heart Study 100 k project; n = 1,341), six SNPs (rs2938392, rs709157, rs10510422, rs10510423, rs2454431, rs963163) within the PPARG gene were found to be related to mean BMI and mean WC. Significance, but not genome-wide significance, was either given for the family based association test or the additive generalized estimation equations. The P-values ranged from 0.003 to 0.047. An association of the SNP rs1801282 (Pro12Ala) with mean BMI or mean WC was not found [FOX et al., 2007].

As mentioned before, the Ala allele of the variant Pro12Ala (rs1801282) within the PPARG gene is seen as protective against T2DM [SCOTT et al., 2007]. Anyhow, a population-based study of 934 high-school students of Caucasian origin showed that there is no association between IR, BMI and leptin levels and PPARG genotypes. They found instead that the 12Ala variant is significantly associated with an increased WHR in normotensive subjects (OR = 2.37; 95% CI 1.05-5.33, P < 0.03), independently of age, sex, hypertension, plasma leptin, or IR [SOOKOIAN et al., 2005].

Additional susceptibility genes found by GWAS

The above-described genes are associated with obesity; however, there are many more susceptibility genes identified by at least Fox et al. This GWAS reported a vast number of associated genes. Some of them previously associated with obesity, some of them newly associated. The SSTR2 (somatostatin receptor 2) gene, the LRP1B (low density lipoprotein receptor-related protein 1b) gene, the VIP (vasoactive intestinal peptide) gene and the ESR1 Xba (estrogen receptor 1) gene are for instance under the identified associated genes. However, they found the best association with mean BMI and mean WC by generalized estimating equations with the intergenic SNP rs110683 ($P = 1.22 \times 10^{-7}$) and with the SNP rs4471028 ($P = 1.96 \times 10^{-7}$) near to GDAP1 (ganglioside-induced differentiation-associated protein 1) gene. To notice is that none of the associated SNPs reached genome-wide significance [FOX et al., 2007]. For that reason only the genes with additional evidence are discussed. For further details, cf. Fox et al.

Figure 12. Basic paradigm for a genetic predisposition to obesity



Source: http://www.pbrc.edu/huec7005/slides/bouchard.ppt

2.7.3 Dyslipidemia

In the last two years, GWASs confirmed gene associations and identified newly associated genes or new gene variants within confirmed associated genes, which are implicated in the individual variation of lipid concentrations. The number of associated genes and gene variants was, therefore, growing. To illustrate the point, Willer et al. identified more than 25 independent common variants, which are significantly associated with lipid concentrations ($P < 5 \times 10^{-8}$) [WILLER et al., 2008].

The authors of the reviewed GWASs mostly distinguished between a newly associated gene and an already known associated gene. For that reason this part of this review is also split up, in already known associated genes and newly associated genes.

Already known associated genes

Table 11. Ten previously in lipid concentrations implicated gene regions

Previously in lipid concentration implicated genes successfully replicated by GWAS [KOONER et al., 2008; KATHIRESAN et al, 2008; WILLER et al., 2008; SAXENA et al. 2007] and associated traits

Closest gene(s)	Associated trait			
	LDL-C	HDL-C	TG	additional
ABCA1		x		
APOA1-APOC3-APOA4-APOA5		x	x	
APOB	x		x	apoB
APOE cluster	x			
CETP		x		apoA-I
LDLR	x			
LIPC		x	x	
LIPG		x		
LPL		x	x	
PCSK9	x			

ABCA1

Kathiresan et al. found that SNP rs3890182 within the ATB-Binding cassette, subfamily A, member 1 (ABCA1) gene is associated with HDL-C ($P = 3 \times 10^{-10}$) when carrying

out a meta-analysis of one GWA and three replication studies [KATHIRESAN et al., 2008].

Willer et al. also showed association between a variant within ABCA1 and HDL-C. Rs4149268 was associated by a two-sided P-value of 1.2×10^{-10} in the combined stage 1 and stage 2 analysis. Allele C at rs4149268 increased TG concentration by 0.82 mg/dl. ABCA1 encodes a protein that is a known transporter of cholesterol [WILLER et al., 2008].

APOA1-APOC3-APOA4-APOA5

As shown in the GWAS conducted by Saxena et al., apolipoprotein A5 (APOA5) is associated with the TG level (SNP rs481843; $P = 3.3 \times 10^{-5}$) in Europeans [SAXENA et al., 2007].

Kooner et al. carried out two genome-wide associations scans. One GWAS was in 1,005 European men and one in 1,006 Indian Asian men aged 35-65 years ascertained on NCEP-ATPIII criteria for MetSyn. The genomes scan in the Indian Asian population showed that SNP rs1558861 and rs17120139 are significantly related to TG levels ($P = 3.8 \times 10^{-5}$ and $P = 0.011$, respectively, after Bonferroni correction). Those SNPs are located in the vicinity of the APOA1-APOC3-APOA4-APOA5 associated cluster. Stage 2 and stage 3 results also confirmed associations with variants near the cluster and TG concentration. To notice is that stage 2 investigated partly individuals of European ancestry and stage 3 investigated only individuals of European ancestry [KOONER et al., 2008].

Another SNP near the cluster (rs28927680) was found to be associated with HDL-C ($P = 2 \times 10^{-5}$) and TG-levels ($P = 2 \times 10^{-17}$) when carrying out a meta-analysis of one GWA and three replication studies. To notice is that the prespecified significance level was 5×10^{-8}, the HDL-C association is, therefore, not genome-wide significant [KATHIRESAN et al., 2008].

Willer et al. also showed association between variants within APOA1-APOC3-APOA4-APOA5 gene cluster and TG level. Rs12286037 was associated by a two-sided P-value of 1.0×10^{-26} and rs662799 was associated by a two-sided P-value of 2.4×10^{-15} in the

combined stage 1 and stage 2 analysis. To note is that SNP rs12286037 accounted for the biggest effect size (25.82 mg/dl) compared to the other SNPs associated with TG concentration [WILLER et al., 2008].

APOB

A GWAS of five study populations consisting of up to 11,685 participants showed a significant association ($P < 1.0 \times 10^{-7}$) between apolipoprotein B (APOB) SNPs and concentrations of LDL-C. Within the APO B variants, SNP rs562338 accounted for the biggest effect size (change in LDL-cholesterol concentration per additional minor allele beta = -0.04, SE 0.01; $P = 1.4 \times 10^{-9}$). However, there was substantial evidence for heterogeneity between studies after adjustment for multiple testing. The same researchers conducted another GWAS in three UK study populations consisting of up to 4,337 participants. This GWAS showed significant association of a different APOB variant (rs1713222) with LDL-C concentrations (change in LDL-cholesterol concentration per additional minor allele beta = -0.17, SE 0.03, $P = 1.0 \times 10^{-8}$). This time there was no heterogeneity between the study populations [SANDHU et al., 2008].

Kathiresan et al. confirmed that variants within APOB influence LDL-levels and TG-levels when analysing pooled data form the DGI-GWAS and three replication studies. Variant rs693 was significantly associated with LDL-C ($P = 1 \times 10^{-60}$) and TG ($P = 2 \times 10^{-7}$) [KATHIRESAN et al. 2008].

Variant rs693 was also associated with LDL-C in the GWAS conducted by Saxena et al. ($P = 7.1 \times 10^{-7}$). Moreover, they found an association between that SNP and apoB ($P = 9.4 \times 10^{-5}$) [SAXENA et al., 2007].

Willer et al. reported additionally evidence for the association between the variant rs693 and LDL-C. The SNP was associated by a two-sided P-value of 3.1×10^{-9} in the combined stage 1 and stage 2 analysis. Furthermore, SNPs rs562338 (two-sided P-value 5.6×10^{-22}) and rs754523 (two-sided P-value 8.3×10^{-12}) showed associations. Apolipoproteins are encoded by APOB [WILLER et al., 2008].

APOE and APOE-APOC cluster

Kathiresan et al. confirmed the association of the apolipoprotein E (APOE) gene and blood lipids. They showed that variant rs4420638 within APOE-C1-C4-C2 is significantly related to LDL-C ($P = 1 \times 10^{-60}$) when they analysed pooled data from the DGI-GWAS and three replication studies [KATHIRESAN et al., 2008].

SNP rs4420638 was also reported to be significantly associated with LDL-C in the GWAS conducted by Saxena et al. ($P = 3.4 \times 10^{-13}$) [SAXENA et al., 2007] and in the GWAS conducted by Willer et al. (two-sided $P = 3.0 \times 10^{-43}$). Additionally, SNP rs10402271 near APOE/C1/C4 was found to be associated with LDL-C (two-sided $P = 1.2 \times 10^{-9}$) in the combined stage 1 and stage 2 analysis [WILLER et al., 2008].

CETP

Kooner et al. reported that five SNPs (rs711752, rs5882, rs1800777, rs5880, rs7205804) in or around the cholesteryl ester transfer protein, plasma (CETP) gene are significantly associated with HDL-C ($P < 7 \times 10^{-5}$ after Bonferroni correction) in the combined analysis of stage 2 and stage 3. Interestingly, SNP rs711752 within CETP was also associated in the initial scan conducted in 1,005 Northern Europeans men who were diagnosed with the MetSyn defined by the NCEP-ATPIII criteria ($P = 7.2 \times 10^{-5}$ after correction for 216,774 tests) [KOONER et al., 2008].

Kathiresan et al. replicated the association with SNP rs1800775. They found that this SNP is associated with HDL-C ($P = 1 \times 10^{-73}$) by carrying out a meta-analysis of one GWAS and three replication studies [KATHIRESAN et al., 2008].

Saxena et al. also identified an association of SNP rs1800775 with HDL-C ($P = 2.5 \times 10^{-13}$). Moreover, an association of that variant with another lipoprotein trait, namely apoA-I ($P = 2.7 \times 10^{-6}$) was shown [SAXENA et al., 2007].

Willer et al. identified five SNPs (rs3764261, rs1864163, rs9989419, rs12596776, rs1566439), all of them not mentioned before, to be associated with HDL-C when analysing the combined stage 1 and stage 2 data. SNP rs3764261 was the most significant of them ($P = 2.3 \times 10^{-57}$). Interestingly, SNP rs1864163 was the variant with

the biggest effect size (4.12 mg/dl) in comparison to the other SNPs associated with HDL-C. A transporter for cholesterol ester is encoded by CETP [WILLER et al., 2008].

LDLR

A GWAS of three UK study populations consisting of up to 4,337 participants showed significant association of LDL-C receptor (LDLR) variants (rs2228671, rs11668477) with LDL-C concentrations (change in LDL-cholesterol concentration per additional minor allele beta = -0.18, SE 0.03, P = 1.1 x 10^{-8} and beta = -0.15, SE 0.03, P = 1.5 x 10^{-8}, respectively) [SANDHU et al., 2008].

In the GWAS conducted by Kathiresan et al., another SNP within LDLR was found to be associated with LDL-C. The combined analysis of the DGI-GWAS and three replication studies showed that rs6511720 is strongly related to LDL-C (P = 2 x 10^{-51}). In one of the replication studies, the Malmö Diet and Cancer Study, LDL-C values varied by ~ 7 mg/dl per copy of the minor allele at rs6511720 [KATHIRESAN et al., 2008].

Interestingly, Willer et al also reported an association of SNP rs6511720. The variant accounted for the best effect size (9.17 mg/dl) within the SNPs associated with LDL-C in the combined stage 1 and stage 2 analysis (two-sided P-value of 4.2 x 10^{-26}) [WILLER et al., 2008].

LIPC

SNP rs1800588 within the lipase, hepatic (LIPC) gene was found to be associated with HDL-C (P = 2 x 10^{-32}) when carrying out a meta-analysis of one GWAS and three replication studies [KATHIRESAN et al., 2008].

Saxena et al. showed that another variant within LIPC, namely rs261332, is associated with HDL-C (P = 3.4 x 10^{-5}) [SAXENA et al.2007].

That variant (rs261332) was also found to be strongly associated with HDL-C (two-sided P-value 3.2 x 10^{-20}) in the combined stage 1 and stage 2 analysis of the GWASs conducted by Willer et al. Furthermore, they reported rs4775041 to be associated with

HDL-C (two-sided P-value 2.3 x 10^{-15}) and to be associated with TG (two-sided P-value 1.6 x 10^{-8}) [WILLER et al., 2008].

LIPG

Willer et al. reported an association between a variant within the lipase, endothelial (LIPG) gene and HDL-C. Rs2156552 was associated by a two-sided P-value of 6.4 x 10^{-12} in the combined stage 1 and stage 2 analysis. Allele C at rs2156552 increased HDL-C concentration by 3.62 mg/dl [WILLER et al., 2008].

LPL

Kooner et al. found five SNPs (rs325, rs326, rs328, rs174109, rs4406409) around the lipoprotein lipase (LPL) gene to be related to TG-levels. All SNPs, except for rs4406409, reached the genome-wide significance. To mention is that SNP rs326 was also significantly associated with HDL-C [KOONER et al., 2008].

The above-mentioned SNP rs328 was also found to be associated with HDL-C (P = 9 x 10^{-23}) and TG-level (P = 2 x 10^{-28}) when carrying out a meta-analysis of one GWA and three replication studies [KATHIRESAN et al., 2008].

Another SNP, namely rs17482753, was shown to be associated with HDL-C (P = 3.6 x 10^{-5}) and TG (P = 4.9 x 10^{-7}) by Saxena et al. [SAXENA et al., 2007].

Willer et al. showed that two variants (rs10503669, rs2197089) are associated with HDL-C and TG (for HDL-C: two-sided P-value of 4.1 x 10^{-19} and of 1.0 x 10^{-11}; for TG two-sided P-value of 3.9 x 10^{-22} and of 1.1 x 10^{-12}, respectively) in the combined stage 1 and stage 2 analysis. Moreover, they showed SNP rs6586891 to be associated with HDL-C (two-sided P-value of 2.9 x 10^{-9}) [WILLER et al., 2008].

PCSK9

Kathiresan et al. confirmed that variants within the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene influence LDL-C concentration when analysing

pooled data form the DGI GWAS and three replication studies. Variant rs11591147 was significantly associated with LDL-C ($P = 2 \times 10^{-44}$) [KATHIRESAN et al. 2008].

Willer et al. also showed association between a variant within PCSK9 and LDL-C. Rs11206510 was associated by a two-sided P-value of 3.5×10^{-11} in the combined stage 1 and stage 2 analysis. Allele T increased LDL-C concentration by 3.04 mg/dl [WILLER et al., 2008].

Newly associated genes

In February 2008, three GWASs were published that investigated whether common variants are associated with HDL-C, LDL-C and TG [KOONER et al., 2008; KATHIRESAN et al, 2008; WILLER et al., 2008]. All in all, they identified eight newly associated loci (MLXIPL, ANGPTL3, MVK and MMAB, GALNT2, CELSR2-PSRC1-MYBPHL-SORT1, TRIB1, NCAN, PBX4 and CILP2) and more than a dozen previously implicated genes were confirmed (Figure 13) [LUSIS and PAJUKANTA, 2008]. The newly found association of GCKR and TG-level was already reported in 2007 [SAXENA et al., 2007]. This finding increased the number of newly associated genes respectively gene regions to nine (Table 12).

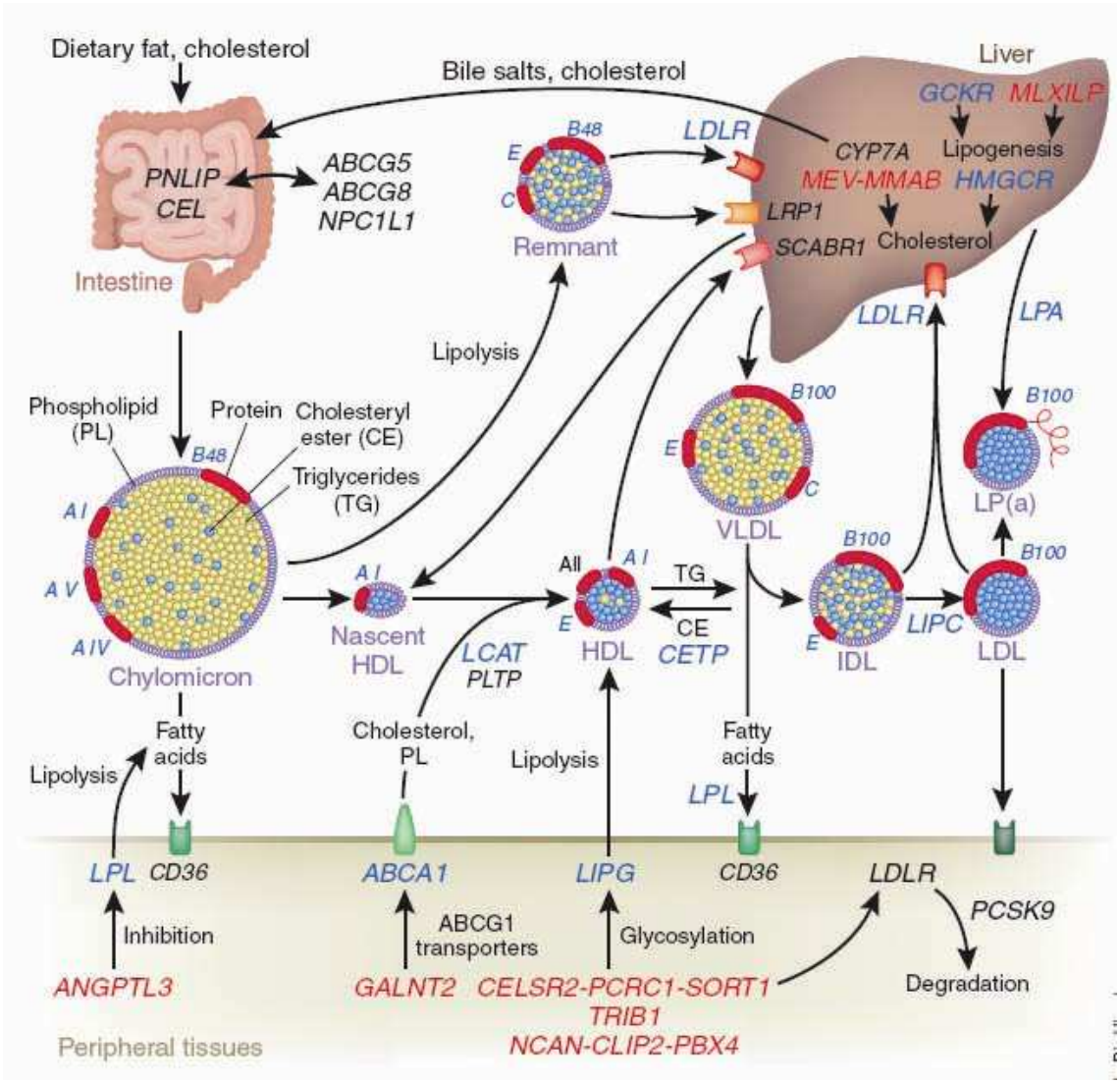
Table 12. Nine newly associated gene regions implicated in lipid concentrations

Newly associated gene regions implicated in lipid concentrations identified by GWASs [KOONER et al., 2008; KATHIRESAN et al, 2008; WILLER et al., 2008; SAXENA et al. 2007] and associated traits

Closest gene(s)	Associated trait		
	LDL-C	HDL-C	TG
ANGPTL3			x
CELSR2-PSRC1-MYBPHL-SORT1	x		
GALNT2		x	
GCKR			x
MLXIPL		x	x
MVK and MMAB		x	
NCAN	x		
PBX4 and CILP2	x		
TRIB1			x

Figure 13. Primary pathways for the metabolism of human plasma lipoproteins

The intestine secrets triglyceride (TG)-rich lipoproteins as chylomicrons and the liver as VLDL. Lipolysis in the circulation subsequently delivers fatty acids to tissues. Chylomicron remnants and about half of the VLDL remnants are taken up by the liver, while the remainder of the VLDL remnants is further metabolised to cholesterol-rich LDL, the main cholesterol carrying particles in humans. Lipid-poor apolipoproteins secreted by liver and intestine and surface components sloughed during lipolysis of TG-rich lipoproteins lead to the formation of HDL in circulation. Blue symbols indicate previously identified genes confirmed in these studies to contribute to common variations in the concentrations of the major lipoproteins. Loci or genes newly identified in these genome-wide association studies are shown in red.



[LUSIS and PAJUKANTA, 2008] reproduced

GCKR

In the GWAS of the Diabetes Genetics Initiative (DGI) of Broad Institute of Harvard and MIT and Novartis Institutes for BioMedical Research it was reported that the SNP rs780094 within the glucokinase regulatory protein (GCKR) gene is associated with triglyceride levels ($P = 3.7 \times 10^{-8}$). That variation was responsible for 1% of the residual variance in TG levels. This association was confirmed by testing another 5,217 individuals from the Malmö Diet and Cancer Study, Cardiovascular Arm ($P = 8.7 \times 10^{-8}$) [SAXENA et al., 2007].

Willer et al. replicated the association between the variant rs780094 and TG (two-sided P-value of 6.1×10^{-32}) in the combined stage 1 and 2 two analysis. Allele T at rs780094 increased TG concentration by 8.59 mg/dl [WILLER et al., 2008].

GCKR encodes for a protein that inhibits the pancreatic and hepatic glucokinase [Ridker et al., 2008]. Glucokinase is the first glycolytic enzyme and overexpression of glucokinase has been shown to lower fasting blood glucose and increase triglyceride levels [SAXENA et al., 2007].

MLXIPL

Kooner et al. showed a significant association of TG and HDL-C with the nonsynonymous SNP rs3812316 (G771C, Gln241His) within the MLX interacting protein-like (MLXIPL) gene in the combined analysis of stage 2 and stage 3 data ($P = 9.9 \times 10^{-8}$). The nonsynonymous SNP conferred an odds ratio of 1.29 per copy of the major C allele for TG levels > 1.7 mmol/l [KOONER et al., 2008].

In the GWAS conducted by Kathiresan et al., SNP rs17145738 within MLXIPL was associated with blood TG ($P = 7 \times 10^{-22}$) [KATHIRESAN et al., 2008].

Willer et al. found the same SNP (rs17145732) as Kathiresan et al. to be associated with TG. Allele C increased TG concentration by 8.21 mg/dl [WILLER et al., 2008].

The product of the gene MLXIPL is involved in the glucose and triglyceride metabolism as well as in the regulation of the energy storage [LUSIS and PAJUKANTA, 2008].

Additional susceptibility genes

Despite no association with blood lipids was found by the reviewed GWASs, the following genes are worth to mention in this chapter. Both are discussed as MetSyn candidate genes [SONG et al., 2006] and were found by GWAS that investigated other MetSyn traits [SCOTT et al., 2007; FOX et al., 2007].

Table 13. 2 additional susceptibility genes associated with lipid concentrations

Genes associated with lipid concentrations identified by GWASs that investigated T2DM [SCOTT et al., 2007] or obesity related features [FOX et al., 2007]

Closest gene	Associated trait		
	LDL-C	HDL-C	TG
PPARG	x		
ADIPOQ	x		

PPARG

The Ala12 variant (rs1801282) within PPARG was associated with T2DM in a protective manner [SCOTT et al., 2007]; however, that SNP is suggested to be associated with blood lipid levels too. This was recently confirmed in a study conducted in 285 obese children and adolescents. The SNP was not associated with BMI, HDL-C, TG or IR, but wild type carriers of PPARG Pro12Ala ($P < 0.05$) had higher total and LDL-C levels adjusted for age, gender, BMI and insulin sensitivity [JOHANSSON et al., 2008].

ADIPOQ

The candidate gene study conducted by Johansson et al. genotyped also the G276T SNP within ADIPOQ. They found that homozygous (TT) carriers of the G276T have higher total and LDL-C levels adjusted for age, gender, BMI, and insulin sensitivity ($P < 0.001$). Like the PPARG variation, the ADIPOQ variation was not associated with BMI, HDL-C, TG, or IR. Interestingly, they calculated a combined risk resulting from a PPARG Pro/Pro and an ADIPOQ 276 T/T genotype. The carriers of that genotype had

higher total (5.0; CI 4.1-5.8 vs. 4.1; CI 3.6-4.6 mmol/l) and LDL-C levels (3.7; CI 95% 2.9-4.5 vs. 3.0; CI 95% 2.5-3.5 mmol/l) compared with carriers of other combinations ($P < 0.001$) [JOHANSSON et al., 2008].

The hormone adiponectin is implicated in the regulation of the lipid metabolism, the glucose metabolism, and the energy homeostasis. It is secreted by adipocytes [SONG et al. 2006].

2.7.4 Hypertension

The aforementioned Wellcome Trust Case Control Consortium (WTCCC) investigated the association of SNPs with T2DM in a GWAS of 14,000 cases and 3,000 shared controls, but they also investigated six other common diseases. One of those diseases was hypertension. However, they only found moderate evidence for association (range of the P-value: 10^{-4} to 10^{-7}). Misclassification bias due to the presence of hypertensive individuals within the control samples was seen as one explanation for that findings [THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007].

Another GWAS that aimed to investigate blood pressure and arterial stiffness (Framingham Heart Study 100 K Project; included up to 1,327 individuals) neither found associations that reached genome-wide significance. The best association was seen between diastolic blood pressure and the intergenic variant rs1963982 ($P = 3.31 \times 10^{-6}$) and between systolic blood pressure and the intergenic rs10493340 ($P = 1.7 \times 10^{-6}$). The authors call for further GWASs with larger sample sizes and more dense genome-wide coverage of common variants [LEVY et al., 2007].

Additional susceptibility genes

PPARG

PPARG is a discussed MetSyn candidate gene [SONG et al., 2006], in spite of that no association with blood pressure or blood lipids was found by the reviewed GWASs for that gene and only a not genome-wide significant association was reported for T2DM [SCOTT et al., 2007]. However, it is worth to mention in this part, as candidate gene studies also support a role of that gene in hypertension.

The Ala allele of the variant Pro12Ala (rs1801282) within the PPARG gene has been associated with protection against T2DM [SCOTT et al., 2007]. In contrast, a population-based study of 934 high-school students of Caucasian origin showed that carriers of the 12Ala variant have higher diastolic blood pressure ($P = 0.002$) and lower puls pressure than non-carriers, particularly in hypertensive and overweight adolescents with features of the MetSyn [SOOKOIAN et al., 2005].

2.7.5 Inflammation – CRP

Although the assessment of the C-reactive protein (CRP) level is not required to diagnose somebody with the MetSyn, evidence exists that elevated levels of CRP independently predict incident MetSyn, T2DM, myocardial infarction and stroke in healthy men and women. Because of the seen importance of that protein and the known heritability, a GWAS aimed to investigate the influence of the genetic variation on CRP level. This was done among 6,345 apparently healthy women in the Women's Genome Health Study. They identified seven loci (46 SNPs) to be in an association with plasma CRP at levels achieving genome-wide statistical significance (P = from 1.9×10^{-8} to 6.2×10^{-28} for lead SNPs within the seven loci). Those common variants explained 10.1% of the remaining variation in CRP after adjustment for the clinical and environmental factors (Table 14). The polymorphism in the CRP gene explained the most remaining variation (3.4%) compared to the others [RIDKER et al. 2008].

Table 14. Seven gene regions associated with plasma CRP levels
Women's Genome Health Study data

Number of SNPs clustered in chromosome locus	Closest gene	Chromosome locus	Locus-wide variance explained (%)
2*	APOE	19q13.32	1.5
20*	CRP	1q23.2	3.4
3*	GCKR	12q23.31	1.1
8*	HNF1A	12q24.31	1.1
2	IL6R	1q21.3	0.6
9*	LEPR	1q31.3	1.6
2	Unknown	12q23.2	0.8

*Remained significant after the analyses was restricted to those study participants with CRP levels < 10 mg/l; Results were adjustment for age, smoking, BMI, hormone therapy, and menopausal status

[RIDKER et al., 2008] modified

2.7.6 Metabolic syndrome itself

Each MetSyn component, except hypertension, was previously associated genome wide significantly with gene variants; nevertheless, it is currently unknown if the interaction of such variants leads to the development of the MetSyn or if there is a MetSyn locus, which is alone responsible for the onset of the disease. Latter theory was and is investigated by linkage studies.

Linkage studies

In 2006, a genome-wide linkage scan using 250 German families (affected sib pairs only) was published that supported the existence of a MetSyn locus on chromosome 1p36.13 (37.05cM, D1S3669). The scan aimed originally to identify T2DM risk loci only, but when the researchers compared their results with results from former linkage scans, evidence for a MetSyn locus appeared; other studies had already reported linkages with parameters of lipid metabolism, hypertension and obesity close to their identified T2DM locus [HOFFMANN et al., 2007]. Interestingly, that linkage study reported no linkage to the previously mentioned region around DG10D478, which was found to be associated with T2DM in Icelandic, Danish, and US cohorts [GRANT et al. 2006].

Another linkage analysis (n = 4,549) showed that the composite factor (BMI, WHR, subscapular skinfold, TG, HDL-C, HOMA, plasminogen activator inhibitor-1 antigen, and serum uric acid) is significantly associated (LOD 3.34) with a region between D2S427 and DS21279 on chromosome 2q36. Chromosomes 7, 12, 14, and 15 showed suggestive associations (LOD between 2.42 and 2.84) [TANG et al., 2003].

Edwards et al. recently confirmed a linkage for MetSyn phenotypes on chromosome 2, but at a different position (2q12.1-2q13) [EDWARDS et al., 2008].

Obviously, various chromosomal regions have emerged that are linked to the MetSyn and even more chromosomal regions spanning several thousand genes are linked to MetSyn components. In a recently published review a clue was given, how many chromosomal regions or how many genes are suggested to be associated. The researchers used a bioinformatics tool (ENDEAVOUR) to prioritise the selection of

candidate genes in MetSyn and listed the first 20 genes out of 1,518 (located in chromosomal regions previously associated with MetSyn components). CYP3A43 (7q21.1), INSRR (1q21-q23), ADIPOQ (3q27), and SERPINC1 (4q21) are several of the first listed genes [SOOKOIAN and PIROLA, 2007].

Candidate gene studies

As already mentioned, no GWAS was published so far – to my knowledge – that directly investigated the association of gene variants with the MetSyn. For that reason, some results from candidate gene studies are presented that showed direct gene associations with the MetSyn. Only genes with former evidence from GWASs are discussed.

Table 15. Five genes associated with MetSyn components and with the MetSyn itself

Genes associated with MetSyn components identified by GWASs [RIDKER et al., 2008; CHAMBERS et al., 2008; SCOTT et al., 2007] and with the MetSyn itself as identified by candidate gene studies [SIMA et al., 2007; HEID et al., 2008; AL-ATTAR et al., 2008]

Closest gene	Associations				
	T2DM, HOMA-IR%	obesity	blood lipids	hypertension	CRP
APOA5			x		
APOE					x
FTO	x	x			
MC4R	x	x			
TCF7L2	x				

APOA5

A recently published candidate gene study showed that the coding SNP rs3135506 (c.56C > G) within APOA5 is directly associated with the MetSyn. The study was conducted in 1,354 Caucasian subjects of the population-based Cooperative Health Research in the Region of Augsburg (KORA) survey S4 and in 1,770 subjects of the

Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) Study. The association between APOA5 and MetSyn itself as well as the association with features of the MetSyn (lipid parameters, WC, glucose-related parameters, blood pressure, uric acid) was investigated. It was reported that the minor allele of rs3135506 is significantly associated with higher TG levels and lower HDL-C; latter was only significant in the SAPHIR population. None of the other MetSyn related features was associated, nevertheless, MetSyn (as defined by NCEP-ATPIII criteria) was directly associated. In the additive model, SNP rs662799 increased the MetSyn risk in the KORA population 1.43-fold (95% CI 1.04-1.99; P = 0.03) and 1.48-fold in the SAPHIR population (95% CI 1.10-1,99; P = 0.009). Only the association in the SAPHIR population remained significant after correction for multiple testing. The authors reported, furthermore, that an additional SNP (rs662799, 1131T>C) might have an additive effect to the effect seen with rs3135506. The estimated MetSyn risk was increased (but not significantly) in subjects heterozygous for minor alleles of both variants [GRALLERT et al., 2007].

APOE

This gene was directly associated with the MetSyn in a small candidate gene study (n = 279). The subjects were divided into a control group (1), a MetSyn group (2) and a group of obese patients with coronary heart diseases (3). They found a higher frequency of the apo E4/3 genotype in the second and the third group as well as higher levels of TG and lower levels of HDL-C compared to group 1. Sima et al. concluded, therefore, that the epsilon 4 allele is an independent risk factor for MetSyn [SIMA et al., 2007].

FTO

FTO was associated with the MetSyn directly in a candidate gene study conducted in a non-Caucasian multi-ethnic sample (n = 2,121; Canadians of South Asian and Chinese descent, Oji-Cree, Inuit). The researchers reported an increased risk of MetSyn when carrying an rs9939609 A allele (Table 16). This was especially true for men. Individuals with ≥ 1 copy of the rs9939609 A allele were significantly more likely to have IDF-defined MetSyn (35.8%) than those individuals carrying no copy (31.2%). The results

for NCEP-ATP III-defined MetSyn were very similar to the results for IDF-defined MetSyn. This association was induced by a higher proportion of subjects with lowered HDL-C and a trend towards increased WC [AL-ATTAR et al., 2008]. Although this study was conducted in non-Caucasians, it might be that the variant shows a similar association in Caucasians, because SNP rs9939609 was repeatedly associated with increased BMI and T2DM in Caucasians [THE WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007; FRAYLING et al., 2007a].

Table 16. MetSyn prevalence in subjects when classified in accordance to their FTO genotype

Data is shown for SNP rs9939609

	TT carriers in %	AA&AT carriers in %	OR 95% CI	P-value
IDF	31.2	35.8	1.12 (1.01-1.50)	0.036
NCEP-ATPIII	21.1	25.2	1.26 (1.02-1.57)	0.036

[AL-ATTAR et al., 2008] modified

MC4R

That MC4R SNP V103I (rs2229616) is associated with features of the MetSyn was shown in a population-based cross-sectional study (n = 7,888), with allele 103I to be protective. Furthermore, those individuals carrying a 103I polymorphism had a reduced risk of having three or more components of the MetSyn (OR 0.43, P = 0.003). The exact implication mechanism is not known yet, but it is thought that the variant influences appetite regulation, physical activity, and dietary intake. Results from that study support that theory, because MC4R102I showed a borderline association with carbohydrate intake (OR = 1.26, P = 0.059) [HEID et al., 2008].

SNP V103I is not the only variation within MC4R that is associated with the MetSyn. Variants (rs12970136, rs477181, rs502933, rs4450508) were also shown to be associated with the MetSyn and with features of the MetSyn (Table 9). For example, SNP rs12970134 increased the risk of MetSyn 1.12-fold (95% CI 1.06-1.20; P = 2.3 x 10^{-4}) in all stage 2 participants (n = 11,955). To note is that stage 2 included a

population of Indian Asian ancestry and a population of European ancestry [CHAMBERS et al., 2008].

TCF7L2

Two candidate gene studies published opposite findings regarding the association of TCF7L2 variants with the MetSyn.

Marzi et al. confirmed that rs12255372 and rs7903146, both within the TCF7L2 gene, are strongly associated with T2DM, but they also showed in that population-based study comprising 1,404 male and female KORA participants aged 55-74 years that those SNPs are not related to the MetSyn as defined by the IDF or by NCEP [MARZI et al., 2007].

Sarzani et al. studied 204 obese and hypertensive patients (<65 years old) without T2DM and 222 unselected adults. They found that obese and hypertensive patients with the MetSyn (defined by NCEP-ATPIII criteria) have higher frequencies of TCF7L2 DG10S478 allele X (66.2%) than those without MetSyn (40%). Individuals with DG10S478X had a 2.94-increased risk (95% CI 1.37-6.28, P = 0.0006) to develop the MetSyn in an unadjusted model. The effect size was even higher when they adjusted for age, gender, BMI, and rs7903146 (OR 3.25; 95% CI 1.43-7.4; P = 0.005) [SARZANI et al., 2008].

3 Discussion

The number of different MetSyn-definitions makes a statement about the quality of each definition to a rather daunting task. The most important definitions (in numbers 6) are described in that paper. A number of six definitions does not sound that much and those definitions seem – at the first glance – not too different, but on closer examination, one can see momentous differences [DAY, 2007]. This was pointed out by showing different prevalence rates. For example, the assessed MetSyn prevalence in men ranged from 27% to 35.9% according to definitions of WHO, NCEP-ATPIII and IDF [THE DECODE STUDY GROUP, 2005]. To determine the quality of a definition or to give a recommendation, which definition to prefer with regard to the prediction of CVD and T2DM – one has to investigate studies that compared different definitions in the same population and under the same conditions at best. There are indeed some studies, even meta-analyses, that compared the definitions and those also came up with some suggestions; nonetheless, the results are to question, because they did not compare all definitions among each other and meta-analyses only exists, to my knowledge, for the prediction of CVD.

What can be stated is that every definition has its advantages and disadvantages. To illustrate the point, the WHO definition is due to the presence of IR more difficult to implement in clinical practice. In addition, the criteria are often modified when used in studies, because microalbuminuria data is lacking, and finally, a rather low sensitivity to identify persons at risk on MetSyn was attributed to that definition [LORENZO et al., 2007]. Nonetheless, the WHO definition seems to be on forefront to predict CVD [GAMI et al., 2007] and T2DM [LORENZO et al., 2007] when comparing the RRs (or ORs). In that context it is to mention that individuals with the MetSyn have a ~ 2-fold increased risk for CVD and a ~ 6-fold increased risk for T2DM [LORENZO et al., 2007]. That sounds a lot, but this does not imply a practical effect, because the MetSyn was shown to be inferior to the established disease predicting models (Diabetes Risk Score, Framingham Risk Score) [GRUNDY et al.; 2005; STERN et al.; 2004, LORENZO et al. 2007]. Even an addition to the risk, assessed by the Diabetes Risk Score and the Framingham Risk Score is unclear [STERN et al., 2004]; if it adds, the

question arises whether the MetSyn adds to the risk as a syndrome or whether the simple summation of the risks conferred by the single components is pivotal [GAMI et al., 2007; THE DECODE STUDY GROUP, 2006; LAWLOR et al., 2006]. Further investigations are needed to clear the essential question whether the MetSyn is more than the sum of its components.

In face of a questioned existence of the MetSyn and inferiority to the established predicting models, there are still some positive aspects about the MetSyn.

First, it is assumed that the MetSyn is useful to assess people who are on long-term risk (> 10 years) for CVD; whereas Framingham Risk Score should be performed to estimate 10-year risk [GRUNDY et al. 2005]. Second, it was shown that the MetSyn may complement Framingham scoring for men aged ≥ 45 years and women aged ≥ 55 years and that the prediction of T2DM is beyond glucose intolerance alone [LORENZO et al., 2007]. Third, the DECODE Study Group justified the use of the MetSyn in clinical practice, because the MetSyn detect further men at risk of cardiovascular death beyond those identified by a risk score [THE DECODE STUDY GROUP, 2007] and finally the syndrome can serve as a reminder in clinical practise. Physicians might be encouraged to search for additional metabolic abnormalities when one component of the MetSyn occurs.

In spite of all the disadvantages shown in this paper, the positive aspects of the MetSyn overweigh to my opinion. The evidence leads to the assumption that the MetSyn is at least useful to identify additional individuals at long-term risk, which could benefit from early prevention measures like lifestyle modifications (weight loss, increased physical activity, healthy diet) [LEVESQUE and LAMARCHE 2008]. With the population getting older, it is especially important to fight against an increasing prevalence, as the MetSyn is age-dependent [FORD et al., 2008]. The older the individuals, the higher the MetSyn prevalence, and the more individuals would benefit from an early prevention.

To assess individuals who are at-risk to develop the MetSyn and subsequently CVD and T2DM, one has to consider that the interplay of environmental factors and genetic variations is thought to be causative for the MetSyn [SONG et al., 2006].

A genetic contribution to the development of the MetSyn is not to deny, because the MetSyn and its components are heritable, with components having a higher heritability and with components having a lower heritability. An example for latter one is systolic blood pressure, with heritability estimates between 14% [FREEMAN et al. 2002] and 28% [MILLS et al., 2004], but also the heritability for the MetSyn itself is not so high. The highest estimate was 24% [LIN et al., 2005], but that study was conducted in a Caribbean Hispanic high-risk population leading to the assumption that the heritability in a European not at-risk population might be lower. The heritability for HDL-C is, however, rather high. The estimates range from 44% [FREEMAN et al., 2002] to 76% [SOUREN et al., 2007]. The range of variety may surprise, but can be partly explained by the heterogeneity of the studies (different study population with different genetic background, different statistical methodologies).

As the evidence for the genetic contribution to the development of the MetSyn is conclusive it makes subsequently sense to search for susceptibility genes, but that is less easy than one had expected. As discussed above, there are a number of MetSyn definitions; that is a problem for the search of susceptibility genes, because studies apply different definitions, thus the results are hardly to compare. However, this is not the only problem. None of those definitions is precise and that hinders the identification of genes involved in the disease on a basic principle. How can a genotype be related to a not defined phenotype? It is remarkable how many phenotypes are feasible, when somebody is diagnosed with the MetSyn [DESPRÉS and PÉRUSSE, 2008]. This phenotype variety occurs due to the reason that the discussed definitions require either obesity or IR or no special criteria for their definition and not all of the additional components have to be fulfilled. An example: A person with an elevated waist circumference, hypertension and reduced HDL-C and a lean person with elevated TG, elevated fasting glucose and hypertension are both diagnosed with the MetSyn by the AHA/NHLBI definition. In consequence of this phenotype variety, a precise definition has to be created, before any approach of finding susceptibility genes is applied.

Anyhow, several genes were identified by GWASs to be associated with MetSyn components or with CRP, which elevated levels are assumed to independently predict

incident MetSyn [RIDKER et al. 2008]. Some of those genes were linked to the MetSyn itself or/and with MetSyn components in candidate gene studies too (Table 17).

Table 17. Ten gene regions implicated in MetSyn components identified by GWASs and partly linked to MetSyn in CGSs

Gene or gene cluster	Associations					
	T2DM, HOMA-IR%, FPG	obesity	dys-lipidemia	hyper-tesion	CRP	MetSyn
ADIPOQ		GWAS[$]	CGS			
APOA1-APOC3-APOA4-APOA5			GWAS			CGS
APOE			GWAS		GWAS	CGS
CETP			GWAS[#]			
FTO	GWAS	GWAS				CGS*
GCKR	GWAS		GWAS		GWAS	
IL6R		GWAS[$]			GWAS	
LEPR		GWAS[$]			GWAS	
MC4R	GWAS	GWAS				CGS
PPARG	GWAS[$]	GWAS[$]	CGS	CGS		
TCF7L2	GWAS					CGS

* Not in Caucasians; [#] Stage 1 conduced in a MetSyn population; GWAS gene wide association study; CGS candidate gene study; [$] not genome-wide significant

As one can notice, no GWAS was published so far that investigated the MetSyn itself. Anyhow, it is currently unknown if there is a MetSyn locus, which is alone responsible for the onset of the disease or if the interaction of polymorphisms located within or near the genes associated with the MetSyn components leads to the development of the MetSyn. Assuming the latter theory, several disease susceptibility genes with more or less evidence exist. Each of them will be discussed briefly to summarize the evidence.

ADIPOQ

Song et al. stated in their review that "biochemical, cellular, animal, and human epidemiological data support the role of adiponectin in MetSyn" [SONG et al., 2006]. This assumed implication of ADIPOQ variations in the development of the MetSyn is underpinned by another review [SOOKOIAN and PIROLA, 2007]. Moreover, a SNP within ADIPOQ (G276T) was associated with higher total and higher LDL-C in a small candidate gene study [JOHANSSON et al., 2008]. Anyhow, ADIPOQ was only found in one GWAS to be associated with obesity and that finding did not reach genome-wide significance [FOX et al. 2007].

APOA1-APOC3-APOA4-APOA5

GWASs showed that SNPs near or within the APOA1-APOC3-ACOA4-APOA5 cluster are strongly associated with the TG-level and account for effect sizes up to 25.82 mg/dl [WILLER et al., 2008]. Noteworthy, one of these GWASs carried out their initial genome scan in individuals affected by the MetSyn [KOONER et al., 2008]. An association with HDL-C was also shown by one GWAS, but that association was not genome-wide significant [KATHIRESAN et al., 2008]. Additionally to the found blood lipid associations, it was shown by a candidate gene study that the variant rs3135506 within APOA5 increases the risk of MetSyn in the range from 1.43-1.48. Moreover, evidence exists that the variant rs662799 within the cluster also increases the risk of MetSyn [GRALLERT et al., 2007]. Most interestingly, this variant belongs to the before mentioned SNPs that are associated with the TG-level [WILLER et al., 2008].

APOE cluster

The APOE polymorphisms C112R (rs429358) and R158C (rs7412), responsible for the APOE isoforms, are known to influence the lipid levels of children and adults. [JOHANSSON et al., 2008]. Both were directly associated with the MetSyn by a candidate gene study [SIMA et al., 2007]. However, those SNPs were not identified by GWASs. Instead of, SNP rs4420638 showed overwhelming evidence ($P = 1 \times 10^{-60}$) for the association with LDL-C [KATHIRESAN et al., 2008]. In addition, SNPs rs769449

and rs2075650 were identified to be associated with CRP-levels with genome-wide significance [RIDKER et al., 2008].

CETP

Although this gene was only associated with HDL-C and apoA-I in GWASs [KATHIRESAN et al., 2008; SAXENA et al., 2007; KOONER et al., 2008], a further investigation would be interesting. This is motivated by the fact that one of the GWASs carried out their initial genome wide scan in European men affected by the MetSyn and in that initial scan, SNP rs711752 within CETP was significantly associated with HDL-C [KOONER et al., 2008].

FTO

Hinney et al. reviewed the latest findings regarding gene-obesity associations. They stated that the variants within FTO belong to those gene variants that are truly associated with obesity [HINNEY and HEBEBRAND, 2008]. The findings of this thesis confirm this statement. GWASs showed that FTO-variants are involved in the development of T2DM via modulation of BMI [SCOTT et al., 2007]. Furthermore, FTO-variants modulate weight, WHR, WC, and FM [DO et al., 2008]. Risk of overweight and obesity are also influenced by those polymorphisms. Although each additional risk allele copy of the most promising SNP (rs9939609) increase BMI of a mean of 0.10 Z-score units (95% CI 0.08-0.12; $P = 2 \times 10^{-20}$, equivalent to ~ 0.4 kg/m^{-2}) it accounts only for 1% of BMI variance [FRAYLING et al., 2007a].

GCKR

Variant rs780094 showed associations with the TG-level in GWASs [SAXENA et al., 2007; WILLER et al., 2008] and was associated by a P-value of 6.73×10^{-15} with CRP-levels [RIDKER et al. 2008], whereas another SNP (rs1260326) within GCKR showed an additive effect on FPG [BOUATIA-NAJI et al., 2008]. Noteworthy, the evidence that GCKR influence TG-levels was newly ascertained by GWASs. Considering all evidence, this gene becomes a new interesting candidate gene.

IL6R

This gene was related to mean WC in a GWAS, but not with a genome-wide significance [FOX et al., 2007]. Interestingly, the same SNP that was associated with mean WC was also associated with plasma CRP levels and this time the association reached genome-wide significance. SNP rs4129267 was related to CRP level with a P-value of 1.97×10^{-8} [RIDKER et al., 2008].

LEPR

This gene is an attractive candidate gene, because of its known function. Despite of that, it was only identified by one GWAS as obesity associated gene and not even with genome-wide significance [FOX et al., 2007]. However, a GWAS found nine SNPs within that gene to be associated with plasma CRP levels ($P < 3.28 \times 10^{-9}$) [RIDKER et al., 2008].

MC4R

Variants of MC4R have been associated with BMI, weight, height, fat mass, risk of overweight/obesity [LOOS et al., 2008], and waist circumference [CHAMBERS et al., 2008]. The previously by candidate gene studies identified association between SNP Val103Ile and BMI (effect size of the 103Ile allele -0.5 kg/m^{-2}) [HINNEY and HEBEBRAND, 2008] was not identified by the reviewed GWASs. Instead of, SNP rs17782313 within MC4R showed the best evidence. That SNP showed a per-allele effect of 0.049 (95% CI 0.037-0.061 Z-score units; equivalent to 0.22 kg m^{-2}) with a P-value of 2.8×10^{-15} in adults. Although common variants near MC4R influence obesity traits, they make only a modest contribution to overall variance (~0.14 % for adult BMI) [LOOS et al., 2008]. Furthermore, variants of that gene have been associated with HOMA-IR in one GWAS, which was conducted in a population of Indian Asian ancestry [CHAMBERS et al. 2008]. To what extent an association with HOMA-IR in a population of European ancestry exist, remain to be investigated.

PPARG

PPARG is assumed to be a MetSyn candidate gene [SONG et al., 2006], however, the evidence therefore is not convincing. Even if a meta-analysis of three GWASs and one separate GWAS found associations between variant rs1801282 (Pro12Ala) within PPARG and T2DM that are close to genome-wide significance [SCOTT et al., 2007; ZEGGINI et al., 2008], inconclusive results were shown by a meta-analysis of 43 CGS [LUDOVICO et al., 2007]. In addition, a GWAS found no association of SNP rs1801282 with mean BMI or mean WC, but six other SNPs within PPARG were related to; notably the prespecified genome-wide significance level was not reached [FOX et al., 2007]. Moreover, associations with LDL-level [JOHANSSON et al., 2008] and hypertension [SOOKOIAN et al., 2005] were shown by candidate gene studies.

TCF7L2

The association between SNPs within TCF7L2 and T2DM was first detected by linkage studies [GRANT et al., 2006] followed by candidate gene studies [WANG et al., 2007; ZHANG et al., 2006] and then confirmed by GWASs. Those GWASs showed that SNPs within that gene have good, if not the best associations in comparison to the other T2DM susceptibility genes [STEINTHORSDOTTIR et al, 2007; SCOTT et al., 2007]. Hence, variations within TCF7L2 are currently the most important ones that are associated with T2DM risk. The variant with the best evidence (rs7901695; $P = 1 \times 10^{-48}$) leads, by risk allele, to a 1.37-fold increased T2DM risk [FRAYLING, 2007b]. Although there truly is an association with T2DM the association with MetSyn is to question. Two CGSs have published conflicting results, with variants (DG10S478X) to be associated [SARZANI et al., 2008] and variants (rs12255372, rs7903146) to be not associated [MARZI et al., 2007].

In my opinion, all of those genes qualify for a follow-up, but no recommendation can be given which SNP or SNPs within those genes should be investigated further. Anyhow, conspicuous is that those SNPs found formally in candidate gene studies to be associated, mostly were not identified by GWASs and when they were identified, the evidence was not with genome-wide significant. That leads to the assumption that we

were possibly stiffed on the wrong SNPs till now. It follows that we should focus more on the newly identified SNPs.

A numerousness of gene variants has been identified, but as they all account for only modest effect sizes, they cannot be the answer to the seen trait-variations. Not either combining the effect sizes fills the gap as shown by various studies.

Loos et al. provided data about the combined effect of a variant within FTO and a variant within MC4R. The BMI difference between adults with no risk alleles at either locus and those homozygous at both was ~1.17 kg m^{-2} [LOOS et al., 2008].

Saxena et al. reported that even though each of the identified T2DM SNPs (within CDKN2B, IGF2BP2, CDKAL1, HHEX, SLC30A8, TCF7L2, KCNJ11, PPARG) account for a substantial population attributable risk, each contributes only little to the variation of diabetes risk (0.04 to 0.5%, ~2.3% combined across the eight SNPs) [SAXENA et al., 2007].

In the review of Lusis and Pajukanta it is stated that "despite the number of found associations, the SNPs that meet the genome-wide level of significance, including the previously identified genes, explain only 5-8% of the variation in HDL-C, LDL-C and TG, after accounting for sex, age and diabetes status" [LUSIS and PAJUKANTA, 2008].

In hypertension, however, GWASs have not provided any gene wide significant data so far [LEVY et al., 2007] and therefore no data for combined effect sizes is available.

To what extent the hitherto identified genes, which are not included in those calculations, account for the variance is to question. Especially the contribution of CTNNBL1 to obesity and the contribution of the newly associated six T2DM gene regions is interesting, although those gene regions showed only modest ORs (1.09 to 1.15) [SAXENA et al., 2007]. On the contrary, CTNNBL increased the odd ratios of obesity 1.42 fold and showed multiple BMI and fat mass effect sizes compared to FTO and MC4R, but only in one GWAS [LIU et al., 2008].

Obviously, many more loci remain to be found that account as risk factors for T2DM, obesity, hypertension, and dyslipidemia. Those could explain the remaining variance

seen in affected individuals. Alternatively, they have already found the right loci, but not the causative variants that explain bigger parts of the variance. Extensive resequencing and fine mapping is required to answer that question. Another possibility is, that variants with rarer frequencies (< 1%) compared to the so far investigated (> 1%), but with bigger effect sizes, exist.

Lots of speculations about the genetic contribution to the variance can be stated; however, one should not underestimate the chance that the remaining variance could arise from environmental factors too. Freeman et. al showed that unexplained trait variations ranged, still, from 32% to 59% after adjustment for genetic and household effects [FREEMAN et al., 2002]. In addition, it was shown that lifestyle-interventions reduce the risk conferred by genetic factors. That is because the association between the risk genotype and the risk of incident diabetes was only seen in the group without intervention [WANG et al., 2007].

As above pointed there are still several disease susceptibility genes and gene variants that have to be identified; but to identify is only one step. The further investigation, which should lead to the finding of causative variants and subsequently their pathways that contribute to pathogenesis, is crucial. To determine the pathways shows new opportunities to prevent and to treat a disease. Sladek et al. speculated for example that the knowledge of the association between the SNP within SLC30A8 and T2DM might lead to dietary interventions (zinc supplementation) or to the development of new medications [SLADEK et al, 2007].

In conclusion, well-designed GWASs offer great opportunities to identify genes and gene variants implicated in common diseases such as MetSyn. Anyhow, for a robust identification of variants with such small effect sizes as seen in common diseases large sample sizes or the exchange and pooling of data are needed, and with regard to the MetSyn – of course – a precise definition.

One may be curious about the future GWASs findings.

4 Summary

This thesis mainly intended to investigate the genetic determinants of the MetSyn and its components by reviewing gene-wide association studies (GWASs). Furthermore the most important definitions, the prevalence rates, the clinical relevance (a brief comparison of the MetSyn with established risk predicting models for CVD and T2DM is given), the underlying factors and the heritability estimates were discussed as well as the basic principles of a GWAS described.

To determine which genes or gene variants are involved in the development of the MetSyn GWASs were reviewed that investigated gene associations with components of the MetSyn, C-reactive protein or with known outcomes, like T2DM. This approach was due to the reason that no GWAS was published so far that investigated the MetSyn directly and the assumption that the interaction of gene variants associated with MetSyn components leads to the development of the MetSyn. The results of the reviewed GWASs were then compared and the ten genes or gene regions with overlapping or particular evidence (genome scan was conducted in individuals affected by the MetSyn) were discussed briefly to summarize the evidence.

The most important genes implicated in the development of the MetSyn seem to be APOE, FTO, GCKR, MC4R, and PPARG. SNPs of these genes are associated with at least two MetSyn components with genome-wide significance except of PPARG, which significance was borderline. Interestingly, those SNPs found formally in candidate gene studies to be associated, mostly were not identified by GWASs; for instance the variant of MC4R Val103Ile. All of the so far associated SNPs account only for modest effect sizes; therefore, they cannot explain the observed trait variations. Even the summation of effect sizes could not explain, for instance, more then 5-8% of the variation seen in blood lipids. It follows that many more implicated gene variants or other contributors to the observed trait variation, as environmental factors, have to be identified.

In conclusion, genetic variations influence the development of MetSyn components and the MetSyn itself, but to what extent remains unanswered. However, the GWAS is a powerful method to further investigate the genetic contribution and the genetic determinants of the MetSyn and its components.

5 Zusammenfassung

Das Ziel dieser Diplomarbeit war es, die genetischen Determinanten des MetSyn durch ein Literaturreview der betreffenden genomweiten Assoziationsstudien (GWASs) zu bestimmen. Weiters wurden die wichtigsten Definitionen des MetSyn, die Prävalenzraten, die klinische Relevanz (dazu wurde das MetSyn mit bestehenden Prognosemodellen für kardiovaskulare Erkrankungen oder Diabetes verglichen), die zu Grunde liegenden Faktoren und die Heretabilität diskutiert sowie die Grundlagen einer GWAS beschrieben.

Zur Bestimmung der involvierten Gene bzw. Genvarianten wurden GWAS herangezogen, die Genassoziationen mit Komponenten des MetSyn sowie mit C-reaktiven Protein und Folgeerkrankungen, wie z.B. Diabetes mellitus untersuchten. Dieser Ansatz begründet sich dadurch, dass noch keine GWAS publiziert wurde, die das MetSyn als solches untersucht hat sowie durch die Annahme, dass die Interaktion verschiedener Genvarianten assoziiert mit den Komponenten des MetSyn zur Entwicklung des MetSyn beitragen kann.

Der Vergleich der durch die GWASs als assoziiert identifizierten Gene zeigte, dass neun Gene sich auf verschiedene MetSyn Komponenten gleichzeitig auswirken. Ein zehntes Gen wird dadurch interessant, da dessen Identifikation anhand einer an MetSyn erkrankten Gruppe erfolgte. Die Evidenz aller zehn Gene wird zusammengefasst dargestellt und diskutiert.

Zu den Genen mit der besten Evidenz zählen APOE, FTO, GCKR, MC4R und PPARG. Varianten dieser Gene wurden mindestes mit zwei Komponenten des MetSyn assoziiert, mit einer den genomweiten Assoziationsstudien entsprechenden Signifikanz. Eine Ausnahme bildet PPARG. Bei diesem Gen wurde nur eine grenzwertige Signifikanz festgestellt. Interessanterweise wurden vorwiegend SNPs als assoziiert identifiziert, die keine Evidenz aus Kandidatengenstudien mitbrachten. Ein Beispiel ist hierfür die Genvariante Val103Ile des MC4R Genes. Alle der bisherigen assoziierten Genvarianten zeigen relativ moderate Effektwerte und können somit die beobachtbaren Variationen nicht erklären. Ebenso kann die Summation der Effektwerte die Variationen nicht vollständig, bei den Plasmalipiden sind es z.B. 5-8%, erklären. Daraus lässt sich

schließen, dass es noch eine Vielzahl an assoziierten Genvarianten zu identifizieren gibt bzw. Umweltfaktoren einen großen Teil zur Variation beitragen.

Zusammenfassend kann man sagen, dass Genvariationen die Entwicklung der MetSyn Komponenten sowie des MetSyn beeinflussen. Es ist allerdings noch unklar zu welchem Anteil dies geschieht. GWAS sind jedenfalls eine erfolgreiche Methode, um die Beteiligung der Gene bzw. die genetischen Determinanten des MetSyn und seiner Komponenten weiter zu untersuchen.

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