Genetics of metabolic syndrome and related traits

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Chapter 1

General Introduction

METABOLIC SYNDROME

Metabolic syndrome (MetS) refers to a cluster of risk factors for type 2 diabetes (T2D), cardiovascular disease (CVD) and stroke (Figure 1) that are strongly associated with the Western life style[1,2,3,4]. This life style is a result of the overwhelming and readily available supply of high-energy food that can be consumed at relatively low cost in westernized societies and is characterized by excess food intake and limited physical exercise. The term syndrome implies a complex etiology and this is confirmed by the variety of definitions for MetS that have been formulated over the years (Table 1).

The concept of MetS has been recognized for at least 80 years and during this period the syndrome has been (re)defined several times. MetS was introduced for the first time in the 1920s by the Swedish clinician Eskil Kylin[5]. Kylin discovered that several individual risk factors for CVD, like hypertension, obesity, hyperglycemia and dyslipidemia, tend to cluster. Such individual risk factors are still considered part of MetS and involve an increased risk for T2D, CVD and stroke. However, other components such as urinary albumin content and different measures for obesity like body mass index (BMI) or waist to hip ratio (WHR) can be considered determinants for the diagnosis of MetS. Systemic inflammation is widely regarded as associated with MetS, but is not part of any MetS definition. Table 1 presents four different MetS definitions which were formulated over the last decade. Alternative definitions of MetS have been developed, such as the definition of the American Association of Clinical Endocrinologists (AACE), and these are more focused on diabetes and insulin resistance[6,7,8]. Applying the different criteria to a single data set will lead to different patients being classified as MetS

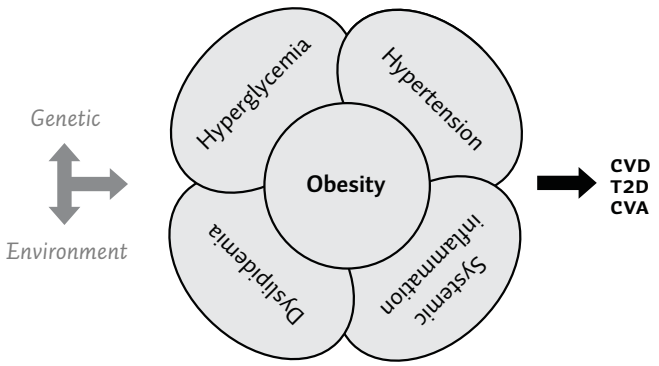

Fig. 1: Schematic representation of biologically relevant components contributing to the metabolic syndrome (MetS): Obesity, Hyperglycemia, Hypertension, Dyslipidemia and Systemic inflammation. The latter component is virtually absent in MetS definitions. MetS refers to a clustering of risk factors for cardio vascular disease (CVD), type II diabetes mellitus (T2D) and stroke.

The International Diabetes Federation MetS definition (2006) includes central obesity as an essential component for the manifestation of the syndrome. The obligatory presence of obesity in this definition is driven by the observed strong association between excess energy intake over expenditure and MetS. As such, obesity is a clear indication that excess energy intake has taken place for some period of time in an individual.

ENERGY HOMEOSTASIS

In the fasting state, the body relies on the production of glucose and lipids by the liver for the supply of energy to peripheral organs. The brain requires glucose while skeletal and heart muscle can also utilize fatty acids (FA) as substrates for oxidation. Glucose is secreted directly into the blood and FA's are packaged into VLDL particles after secretion by adipose tissue or liver in the form of triglycerides (TG).

Upon ingestion of a meal composed of lipids and carbohydrates, the body will respond by producing a variety of hormones and neuronal signals which cause the system to switch the system from a catabolic to an anabolic state. Carbohydrates are converted to glucose in the gut and are directly

Table 1 Four metabolic syndrome definitions

	WHO (1999)	EGSIR (1999)	ATPIII (2001)	IDF (2006)[a]
Components (N) [b]	2 + OGTT	2 + IR	3	2 + WC
_ WC (cm)	-	≥ 94	>102	≥ 94
_ WC (cm)	-	≥ 80	> 88	≥ 80
BMI (kg/m2)	> 30 [c]		-	-
_ WHR	> 0.9 c		-	-
_ WHR	> 0.85 c		-	-
FPG (mmol/L)	-	≥ 6.0	≥ 6.1	≥ 5.6 [d,f]
AE (_g/min)	≥ 20	-	-	-
OGTT	< glucose 25%	-	-	-
IR	-	> insulin 25% [c]	-	-
SBP (mm Hg)	> 140	≥ 140	≥ 135 e	≥ 130 e
DBP (mm Hg)	> 90	≥ 90	≥ 85 e	≥ 85 e
_ HDL-C (mmol/L)	< 0.9 [g]	< 1.0	< 1.0	< 1.03 [g]
_ HDL-C (mmol/L)	< 1.0 [g]	< 1.0	< 1.3	< 1.29 [g]
TG (mmol/L)	≥ 1.7 [g]	≥ 2.0	≥ 1.7	≥ 1.7 [g]

WHO; World Health Organization, EGSIR; European Group for the study of Insulin Resistance, IDF; International diabetes federation, ATPIII; National Education Control Panel Adult Treatment Panel III. a Europids. b Minimal composition of components required for diagnosis MetS. WC; waist circumference, BMI; body mass Index, WHR; waist to hip ratio; FPG; fasting plasma glucose, AE; albumin excretion, OGTT; oral glucose tolerance test, IR; insulin resistant, SBP; systolic blood pressure, DBP; diastolic blood pressure, HDL-C; HDL-cholesterol, TG; total plasma triglycerides., c BMI or WHR, d top 25% of fasting insulin values from non-diabetic population, e pharmacological treated hypertensive patients included, f pharmacological treated type 2 diabetes patients included, g pharmacological treated dyslipidemic patients included.

absorbed in the blood. Lipids are converted to chylomicrons and enter the blood via the lymph. The production of insulin by the pancreas is considered as the most metabolically important hormone signal. Insulin secretion is prompted by the relatively rapid increase in plasma glucose. Insulin represses the secretion of glucose by the liver and increases the uptake of glucose by adipose and muscle tissue. Insulin also represses the secretion of VLDL by the liver.

From an evolutionary perspective, the system is exquisitely suited to ensure survival of prolonged periods of chronic food deprivation, but seems much less suited to deal with chronic over consumption. A chronic excess intake of energy leads to obesity and is associated with low grade inflammation, disturbances in both glucose and lipid metabolism and high blood pressure. These aspects of MetS are discussed in detail below. In addition to quantity, the quality of the food plays a role in the development of pathology. For example, the Mediterranean diet has been associated with less pathology presumably due to the increased dietary levels of unsaturated FA inherent to a high intake of olive oil and fish[9,10]. The mechanism of excess of food intake leading to MetS is under debate. Although most of the processes that are involved are not disputed to play a role in MetS, their relative contribution and the sequence of events leading to MetS are the source of the debate. These processes and their overlaps are shown in Figure 1.

PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME

OBESITY

A misbalance between energy intake and expenditure is thought to be the cause of the current increase in prevalence of MetS[11,12]. Although obesity may not be the first pathological metabolic consequence of excess food intake, its presence does prove that, for a prolonged period of time, there has been higher

energy intake than is required for expenditure has taken place. Expansion of adipose tissue requires an increased influx of FA, but also extensive tissue proliferation and remodeling including precursor cell differentiation, extracellular matrix breakdown and neovascularization. Excess adipose tissue is also associated with increased effluxes of FA and altered function of the adipose tissue itself.

In the definitions of MetS in Table 1, obesity is defined by a threshold BMI or WC. The major difference between these two obesity measures is the focus on body composition (BMI) versus central obesity (WC) and the corresponding risk for MetS. BMI is the most widely used general measure for obesity and is defined by the ratio of weight and squared height (kg/m^2). Because BMI involves the total sum of bone, fat and muscle mass, it does not make a distinction between specific fat depots. WC specifically measures central obesity. Although WC is more region specific than BMI, WC measures the total sum of visceral and subcutaneous fat depots and does not distinguish between these two functionally different fat depots. Alternatively, body composition can be expressed as waist to hip ratio (WHR) and is defined by the ratio between specific fat depots in hip and waist. This measure is generally used to distinguish between the benign pear-shaped overweight individuals and the more pathogenic apple-shaped overweight individuals. As an isolated measure, WHR does not actually represent the level of obesity.

ADIPOSE TISSUE

It is generally accepted that adipose tissue functions as an endocrine organ and can respond to neuronal and hormonal input by secreting biologically active substances in addition to FA, namely adipocytokines or adipokines. Chronic disturbances in the endocrine function of adipose tissue clearly play a role in the pathology associated with MetS. In this respect, the different adipose tissue depots, such as visceral and subcutaneous fat, are not each other's equivalent in terms of the production and secretion of adipokines[13]. Visceral fat directly drains on the portal vein, and is thus likely to have a much more direct signaling and metabolic relation with the liver in comparison to subcutaneous fat. The adipokine family can be divided in two types of signaling molecules, namely those with a metabolic/immunological function, which include interleukins 1β, 6, 8, 10 or 18, tumor necrosis factor alpha (TNF-α) and transcription growth factor beta (TGF-β), and those with an endocrine function, which include leptin, retinol binding protein-4 (RBP-4), adiponectin and resistin. All adipokines are thought to affect many different tissues throughout the body, including liver, gall bladder, skeletal muscle, brain and pancreas.

The metabolic/immunological adipokines are, in general, pro-inflammatory and positively associated with obesity and trigger a response which results in infiltration of immune cells, such as macrophages. Macrophages are involved in the clearance of all forms of debris, including dead cells, but are themselves also a source of pro-inflammatory cytokines. Once macrophages are abundant in adipose tissue, they maintain the inflammatory state of the adipose tissue in a vicious circle, which results in chronic systemic low-grade inflammation. This inflammatory state is far less severe than pathogen-induced inflammation[14,15].

The adipokines with endocrine function do not only affect the liver, skeletal muscle and pancreas, but also the central nervous system (CNS)[16]. The adipokine leptin affects the arcuate nucleus in the brain, which is involved in the regulation of appetite and energy expenditure. Increased plasma leptin functions as a signal of increased adipose tissue mass. As such leptin is, potentially, an ideal weight loss hormone. However, obesity is associated with leptin resistance limiting the use in

weight reduction[17,18]. The adipokines resistin and adiponectin are negatively associated with adipose tissue mass and insulin sensitivity, whereas RBP-4 is positively associated with adipose tissue mass and insulin sensitivity[19,20,21,22,23,24]. The precise role of the adipokines resistin and adiponectin in the development of obesity and insulin resistance remains to be fully characterized. The role of RBP-4 in the manifestation of T2D seems to involve a secondary mechanism[25]. Still, in animal intervention studies it has been shown that modulation of adipokine levels can determine the development of insulin resistance[24,16]. Although pharmacological interventions in patients that improve insulin sensitivity clearly ameliorate plasma adipokine levels, interventions that are directly aimed at adipokines have yet to be performed[26].

HYPERGLYCEMIA

Hyperglycemia is defined by elevated levels of fasting plasma glucose. The threshold values of these levels vary between the different MetS definitions (Table 1). Hyperglycemia is thought to be characterized by insulin resistance (IR). IR is defined as an impaired response of a specific process or organ to insulin. In a normal physiological state, insulin suppresses glucose production by the liver and increases glucose uptake by peripheral organs such as muscle and fat. As a consequence of IR, plasma insulin levels increase to achieve normal plasma glucose levels[27].

Many physiological and pathological processes have been identified that can modulate the response of cells to insulin and thus affect insulin sensitivity. These processes include nutritional status, circadian rhythms, inflammation, ER stress and intracellular lipid levels[28,29,30,31]. A current important challenge lies in the understanding of the integration of these (patho) physiological processes in the development of insulin resistance.

Hyperglycemia is generally defined as T2D when fasting plasma glucose levels consistently rise up to 7 mmol/L. T2D is a specific risk factor for retinopathy, neuropathy and nephropathy[32]. The progression of insulin resistance to diabetes is thought to result from the chronic nature of the triggers that induce insulin resistance. It seems more than likely that as time progresses, some of these triggers exacerbate as a consequence of aging-induced changes, i.e. hormonal status and physical activity. At some point, the insulin resistance may fail to be compensated by increased insulin secretion, resulting in failure of the glucose homeostasis and thus in hyperglycemia. Since high levels of glucose are cytotoxic, the hyperglycemia will contribute further to the overall deterioration of the system. Eventually, this vicious cycle may progress to pancreatic beta-cell deterioration, at which point the diabetic state has become irreversible.

It should be noted that the threshold levels for fasting hyperglycemia in the various definitions of MetS (5.6-6.1 mM/L, Table 1), are all below the accepted level that is considered to be minimal for treatment (>7.0 mM/L).

DYSLIPIDEMIA

The dyslipidemia associated with MetS is defined by hypertriglyceridemia and low HDL-cholesterol levels. Both have been identified as risk factors for cardiovascular disease[33] and especially hypertriglyceridemia is a target for drugs aimed at CVD prevention. These drugs include fibrates, which act mainly via the nuclear receptor PPAR-alpha. Similar to hyperglycemia, the level of hypertriglyceridemia required for MetS diagnosis is well below the level required for pharmacological intervention.

The lipids TG and cholesterol are transported via lipoproteins in the blood. After a meal, food derived TG and cholesterol are packaged into chylomicrons in the intestine and secreted via the lymph into the circulation. The main fate of the TG in chylomicrons is lipolysis in peripheral muscle and storage in fat. In the fasting state, TG and cholesterol are transported from the liver to the periphery via very-low-density-lipoprotein particles (VLDL). Once chylomicrons and VLDL are depleted of TG, their remnants are cleared by the liver. A fraction of VLDL remnants progresses to be metabolized in cholesterol-rich LDL particles. LDL particles are specific transporters of cholesterol to the periphery and LDL cholesterol is a well defined risk factor for CVD. A separate lipoprotein, HDL, is responsible for cholesterol transport from the periphery to the liver, which is referred to as reverse cholesterol transport. High levels of HDL cholesterol are associated with low levels of CVD[34,35].

From epidemiological studies it is well known that plasma TG and HDL-cholesterol are highly correlated inversely. The enzyme Cholesterol-Ester Transfer Protein (CETP) balances the levels of TG and HDL-cholesterol and is thus responsible for the mutual exchange of TG and cholesterol ester between apoB-containing lipoproteins (chylomicrons, VLDL and LDL) and HDL. It has been suggested that CETP activity explains some of the high TG levels and low HDL levels, observed in persons with MetS[36].

It has recently been hypothesized that hepatic insulin resistance of glucose and lipid metabolism may be differentially affected in persons with MetS. In individuals with MetS, insulin-mediated suppression of hepatic glucose output may be decreased, but insulin-mediated stimulation of *de novo* lipogenesis may be increased. This will result in increased hepatic lipid accumulation, increased VLDL production and thus ensuing hypertriglyceridemia[37,38].

HYPERTENSION

Hypertension is characterized by chronically elevated systolic (SBP) and/or diastolic (DBP) blood pressure. The diagnosis hypertension can be subdivided in two types: primary hypertension, which involves an unknown cause or origin and secondary hypertension, which involves a known cause or origin. Threshold values for hypertension within the different MetS definitions differ slightly (Table 1). For diastolic blood pressure this threshold range between 85-90 mm Hg and for systolic blood pressure the threshold ranges between 135-140 mm Hg. The increased blood pressure found in persons with MetS is in general of unknown cause (primary hypertension). Similar to the previously discussed MetS traits, the threshold for hypertension in the definition of MetS is well below the level that requires pharmacological treatment.

The exact cause for the increased blood pressure associated with MetS is not known but there is evidence suggesting that genetic predisposition plays an important role. However, it has also been suggested that MetS components hyperglycemia, dyslipidemia and low grade systemic inflammation affect the functioning of the vascular endothelium. A dysfunctional endothelium will not properly respond to physiological stimuli that increase NO production, an important signaling molecule involved in vascular contraction-relaxation and subsequent hypertension[39]. Such mechanism may occur independently of a joint genetic etiology.

Alternatively, mild hypertension may be involved in the etiology of MetS in that hypertension can contribute to the presence of MetS as an independent risk factor. Especially when hypertension is the consequence of endothelial dysfunction not directly associated with MetS (i.e. genetic factors or smoking), this endothelial dysfunction may, in turn, affect blood supply to organs and thus affect

tissue function. Insulin sensitivity of the blood supply to adipose tissue, muscle and liver is an important regulator of glucose and fat fluxes.

INFLAMMATION

Low grade systemic inflammation and/or hepatic inflammation are generally not included in MetS definitions. However, in addition to the inflammation associated with adipose tissue as discussed above, hepatic inflammation is also strongly associated with MetS[14,15]. Markers such as C-reactive protein, several hepatic enzymes like alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) and cytokines like IL-6 can serve as determinants (biomarkers) of the inflammatory state[40]. Hepatic inflammation associated with MetS but not due to excessive alcohol intake is termed non-alcoholic steatohepatitis (NASH). NASH is thought to be a consequence of hepatic lipid accumulation. NASH is also characterized by hepatic insulin resistance. As discussed above, the differential insulin resistance of hepatic glucose output and hepatic *de novo* lipogenesis may explain the development of NASH in individuals with MetS. This explanation is attractive in its simplicity, but remains to be thoroughly investigated and confirmed. For example, the specific triggers that lead from lipid accumulation to inflammation remain to be fully characterized. Moreover, the role of immune cells, such as macrophages, in relation to the inflammatory state of the hepatocyte remains to be characterized.

As discussed above, the low grade systemic inflammation has been hypothesized to present a continuous insult to the endothelium, leading to endothelial cell dysfunction and abnormal blood pressure regulation. The loss of cell function can also extend to the endothelial barrier. When the endothelial barrier function is compromised, LDL and other potentially harmful substances may enter the sub-endothelial space more easily and trigger an inflammatory response. The chronic accumulation of LDL, subsequent oxidation of LDL and uptake by invading macrophages leads to foam cell formation, which is considered the first step in the development of an atherosclerotic plaque. As such, chronic low grade inflammation, hypertension and dyslipidemia all represent chronic triggers for the development of atherosclerosis[34,41,42].

EPIDEMIOLOGY OF THE METABOLIC SYNDROME AND ITS COMPONENTS

PREVALENCE OF METS

The prevalence of MetS has increased dramatically over the last decades in societies with a Western lifestyle. In the US, studies have shown that the prevalence of MetS in adults and, in particular, in adolescents and female adults was growing constantly over the years 1984 to 2000. In this time frame, the prevalence of MetS in adults ranged from approximately 25% to 30%, and in it adolescents ranged from approximately 4% to 9%, with a higher prevalence in males than in females[43,44,45]. In Europe, studies in the general Caucasian population of the prevalence of the metabolic syndrome are scarcer and quite diverse due to different study methods. For example, the prevalence of MetS among healthy French families ranged from approximately 7% to 9%[46], whereas the prevalence in an urban and rural German population ranged from 20% to 40%[47]. Although consistent and comparable information about the increase of the prevalence of the metabolic syndrome in European is scarce, there is no doubt that the prevalence of metabolic components such as obesity and hyperglycemia

is increasing in Europe[48,49,50]. The macro-economic consequences but also individual morbidity and mortality associated with an increase in the prevalence of MetS, are substantial[51,52].

PREVALENCE OF OVERWEIGHT AND OBESITY

In westernized countries the prevalence of overweight and obesity has increased dramatically over the last few decades[53,54,55,56]. A particularly worrying development is the rise in the manifestation of overweight and obesity in young children and adolescents[57]. In 2002, the prevalence of overweight (BMI ≥ 25 kg/m²) or obesity (BMI ≥ 30 kg/m²) in young US children, adolescents and adults, exceeded the astonishing level of 60%. In a time span of approximately 25 years, a 25% increase of overweight was seen in children from age 6 to 11 [58]. In the period 1997-2001, the prevalence of European obese children between 13 and 17 years old ranged, in general, from 10 to 15%. However, in Greek children the prevalence of obesity was much higher, at 22 to 30%[59,57].

The prevalence of overweight and obesity in the Dutch population is carefully monitored by the "Centaal Bureau voor Statistiek" (CBS; http://www.cbs.nl) and by the "Rijksinstituut voor Volksgezondheid en Milieu" (RIVM; http://www.rivm.nl). These studies are cross sectional and prospective and include males and females, children and adolescents. Figure 2 illustrates the cross sectional increase of the prevalence of overweight in the Dutch population between 1987 and 2007. The mean prevalence of overweight in Dutch adults (>20 years old) increased from 33% in 1983 to 45% in 2007.

The prevalence of overweight (BMI>25 kg/m²) and/or obesity (BMI>30 kg/m²) in Dutch men and women (age> 20 years old) rose with 10% in the timeframe of 1981 to 2007. In general, the prevalence in men is approximately 10% higher than in women. The prevalence of obesity alone in this timeframe rose from 4% to 10% in women and from 6% to 12% in men. In Dutch adolescents, a similar increase of the prevalence of overweight is seen. Especially in children / adolescents (age > 8 years old) the prevalence of overweight increased by approximately 10% between the years 1997 and 2004 in both genders.

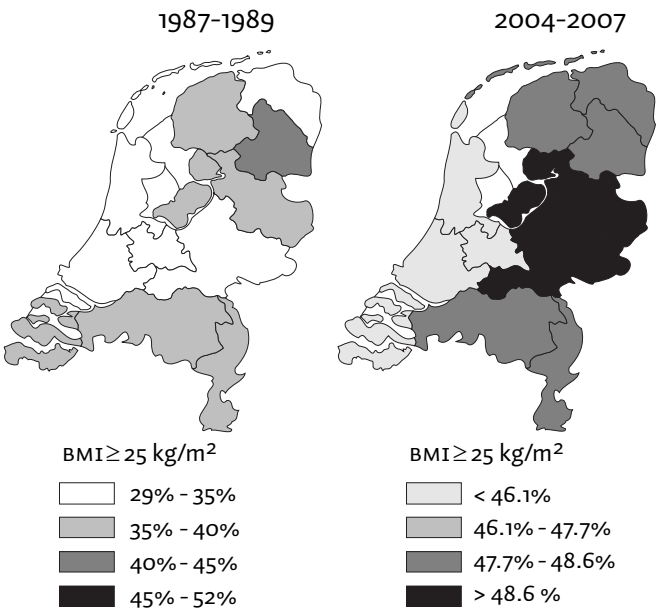

Fig. 2: Prevalence of overweight in Dutch population in the periods between 1987-1989 and 2004-2007. Prevalence adjusted for gender and age. source: CBS

PREVALENCE OF HYPERGLYCEMIA

The prevalence of hyperglycemia as entity in general populations is mainly monitored by the prevalence of diabetes mellitus (fasting plasma glucose > 7mM/L). However, within MetS an impaired glucose metabolism is diagnosed using a variety of measurements (see Table 1). There are large differences in the definition of impaired glucose metabolism between the four MetS definitions. The WHO uses the oral glucose tolerance test (OGTT), whereas the EGSIR includes fasting plasma glucose or the top 25% of fasting insulin values from the (non-diabetic) population. Both the NCEP ATPIII and IDF use fasting plasma glucose levels, though with different thresholds. The choice of such a parameter is a matter of availability (within clinics), cost-efficiency and personal preference. The outcome of epidemiological studies of the presence of impaired glucose metabolism and MetS are affected by these methodological differences.

The increase of the prevalence of hyperglycemia or T2D is as dramatic as the increase of the prevalence of obesity[58,60]. In 2007 the incidence of diagnosed T2D in the general US population was 5.9%. It should be noted that about 2% of the population was estimated to be suffering from undiagnosed diabetes (source: National Diabetes Information Clearinghouse: NCDIC; http://diabtes.niddk.nih.gov/DM/PUBS/statistics).

The prevalence of hyperglycemia (fasting plasma glucose > 6.1, T2D included) was monitored in two cross-sectional Dutch cohorts, the MORGEN and PREVEND studies[61]. The prevalence of hyperglycemia ranged between 5%-20% in men and 3%-9% in women. The age of the individuals in these studies ranged from 28 to 59 years old. The overall incidence of T2D in 2007, as monitored by the RIVM (http://www.rivm.nl), was approximately 4.5%. The incidence of T2D in the Dutch population has increased in the last decade. Between 1990 and 2007, the incidence of T2D increased by approximately 50% in men and 40% in women. However, it should be noted that in addition to the increase of obesity, the increase in average age of the Dutch population also contributes to this increase of the prevalence of T2D.

Although T2D is typically a late onset disease, the increasing incidence of T2D is also seen in young children and adolescents[57]. The prevalence of T2D or other rare forms of diabetes among US children ranged between 1-2%. In the last decade however, several reports have indicated an increase of incidence of up to 50% of newly identified non immune-mediated diabetes in US young children[62]. In Europe the increase in the prevalence of T2D in children is limited[63]. Recently a prevalence of T2D or impaired glucose tolerance of 2.5% was reported in German children with a low socioeconomic status[64].

PREVALENCE OF DYSLIPIDEMIA

Two of the 5 traits defining the metabolic syndrome are dyslipidemias, namely high TG and low HDL-cholesterol. These traits are not independent, since high TG and low HDL are strongly correlated. This correlation may be caused by the activity of the enzyme Cholesteryl Ester Transfer Protein (CETP), as discussed above[65,36]. The prevalence of HTG (> 1.7 mM/L) in the MORGEN and PREVEND studies, ranged between 13% in women and 24% to 29% in men (age ranging from 28 to 59 years old)[61]. The prevalence of low HDL-cholesterol (men <1.0 and women <1.3 mM/L) ranged between 28% and 36% in the MORGEN and PREVEND studies[61].

Both high TG and low HDL are classical risk factors for CVD and stroke[66]. However, the other classical lipid risk factor for CVD and stroke, LDL-cholesterol, is not part of the definition of the

metabolic syndrome. This is due to the independent association of LDL cholesterol with CVD/stroke risk. High levels of plasma LDL-cholesterol do not consistently cluster with other components of MetS, such as obesity and insulin sensitivity. In some cases, a specific genetic cause (Familial Hyper cholesterolemia; FH) lies at the basis of this particular impairment[67].

PREVALENCE OF HYPERTENSION

Similar to T2D, hypertension is a late onset and common disease in the general population. Hypertension in the general adult US population shows a prevalence of approximately 25 up to 36% (SBP ≥ 140 mm Hg and DBP ≥ 90mm Hg or use of medication, reported from 1988 to 1998)[68,44]. This prevalence of hypertension in general USA adults increased up to 41% in the period between 1999-2000[44]. In general, other countries show lower percentages of hypertension[68,69]. The prevalence of hypertension (according to NCEP ATPIII, see Table1) in the MORGEN and PREVEND studies, ranged between 42% and 44% in men and from 21% to 26% in women (age ranging from 28 to 59 years old)[61].

Analogous to hyperglycemia, dyslipidemia and hyperglycemia, many hypertensive patients are not aware that they are suffering from elevated blood pressure. 45% of US adults in the period between 1988 and 1993 were not aware of their elevated blood pressure. Hypertension develops gradually and eventually does result in overt problems in the patient. Since anti-hypertension medication is generally prescribed life long and may have unwanted side-effects, a large proportion of patients does not adhere to therapy; 29% of US adults suffering from hypertension in the period between 1988 and 1993 did not adhere properly to therapy[68,70].

APPROACHES IN GENETIC EPIDEMIOLOGY

INTRODUCTION TO GENETIC EPIDEMIOLOGY

Genetic diseases can be divided in disorders with a monogenic inheritance pattern and disorder with a complex inheritance pattern. Monogenic disorders show autosomal or X-linked dominant or recessive inheritance patterns. Complex disorders are characterized by inheritance patterns where only some of the mutation carriers are affected. This is referred to as reduced penetrance. In complex disorders, environmental variables or additional genetic factors contribute to the manifestation of the disease. For the genetic part, this means that multiple genes and interactions may contribute to the disease according to a threshold model.

An example of a monogenetic x-linked recessive disorder is Duchenne muscle dystrophy (DMD). DMD is characterized by a progressive dystrophy of skeletal muscles and eventual respiratory or heart failure. The genetic basis of this neuralmuscular disorder lies, in general, in a disrupted reading frame in the dystrophin gene (Xp21) caused by nucleotide insertions or deletions of variable length. Such disruption of the reading frame results in a truncated (dysfunctional) or absent dystophin protein[71]. An example of a complex disorder is the disease hyperlipidemia (HLP) type III, which is characterized by elevated plasma levels of VLDL triglyceride and cholesterol. In the last 3 decades, researchers have found that patients suffering from HLP type III were predominantly homozygote carriers of the apoE2 protein variant. Since homozygosity of this variant is mainly present in healthy controls, this metabolic disorder is characterized by a reduced penetrance. Functional analyses indicated that apoE2 has a defect in binding to the hepatic LDL receptor and is thus poorly cleared

from the circulation[72,73,74]. In addition, a number of rare variants of apoE have been identified that contribute to the expression of type III HLP or HTG[75,76]. Family analyses of these variants revealed clear co-segregation of disease and variant and these variants were completely absent from healthy controls. This provided convincing evidence for causation of disease, which was confirmed by *in vivo* analyses in transgenic mouse models[77,78]. Thus, monogenic disorders are completely or predominantly caused by variations in one single gene. In contrast, complex disorders result from joint effects of multiple genetic -and environmental causes with each factor having only a minor contribution to the expression of the disease[79].

In general, two methods are available for the identification of loci involving monogenetic or complex diseases and these are illustrated in Figure 3. Linkage analysis is a family based method and was the first robust method in genetic epidemiology. The second method is association analysis which is based on cases and controls or quantitative trait analysis. Both methods are described below with regard to their design and statistical power in the following sections.

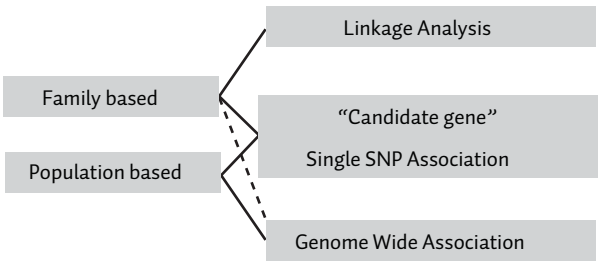

Fig. 3: Two main types of cohorts with regards ro feasible typs of analyses in genetic research

LINKAGE ANALYSIS

Early techniques which made large scale genotyping possible enabled a novel strategy for the identification of disease loci, namely large scale linkage analysis. This type of analysis is based on the characterization of a large number of short tandem repeats (STRs) or micro-satellites distributed over the genome. At present, large genotyping platforms involving SNPs are also used in linkage analysis, as described in the section below.

Linkage analysis focuses on chromosomal regions that are transmitted to diseased offspring more often than expected. Linkage analysis is based on the fact that particular loci do not show independent inheritance patterns. This means that between such loci the probability of recombination approaches o within a family of closely related subjects. This phenomenon of linked loci is also called linkage[80]. Linkage analysis is thus a family-based approach where the segregation of the disease within the families can be linked to a specific chromosome region (locus). Linkage analysis was and still is a robust method to identify novel disease loci. After determining the chromosomal location of the causal gene, these loci often contain multiple interesting genes with regard to the disease of interest. The most interesting genes overlap or involve a certain pathway which is impaired in the disease. These are called "candidate genes". The method for validation or replication of the involvement of such candidate genes in the disease is described in the section "validation and replication". In addition, new pathways not implicated earlier in the disease may also be discovered. Linkage analysis can be performed with a relatively small number of samples and genotypes. Nevertheless, information about the pedigree structure is essential.

GENOME WIDE ASSOCIATION

Family based genome wide linkage analysis is especially powerful for the detection of association of rare genetic variants with rare diseases, since large chromosomal regions are linked to the disease. Traditionally, genetic association has been used for fine mapping of the linked region. Moreover, the last decade (genome wide) genetic association was also used. The basic underlying assumption is the "common disease – common variant" (CD-CV) hypothesis. This hypothesis involves the idea that a prevalent disease in the general population (common) is caused by many common genetic variants. Thus, according to the CDCV hypothesis, proposed in the last decade of the 20th century, common prevalent diseases like hypertension, CVD or T2D, might be caused by (multiple) common variants in genes throughout the general population[81]. Discovery of novel loci using linkage analysis is not suitable in CDCV because different genes may be involved in the same family. Thus, for the search for common variants, causing common disease, preferably large cohorts with extensive genotype data are used. Within such large cohorts, cases and controls can be selected for binary association or alternatively, quantitative trait analyses can be performed. The statistical power (see section *statistical power*) of the two study designs, linkage analysis and genome wide association, are illustrated in Figure 4.

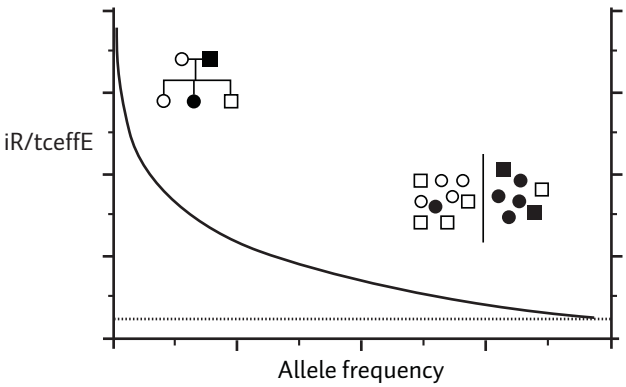

Fig. 4: Schematic overview of the most powerful approach for discovering genes based on minor allele frequency.

To find novel loci according the CDCV hypothesis, extensive genotyping is necessary and the available techniques have evolved rapidly in recent years. Extensive genotyping techniques are based on micro-array technology. Two major companies, Affymetrix and Illumina, have developed micro-arrays, among which SNP-arrays. At present, SNP micro-arrays involving 6K to 1000K SNPs are available. The SNPs present on the Illumina SNP arrays are based on their tag property. This implies that these SNPs were selected because each SNP covers a relatively large region in linkage disequilibrium (LD). Such tag property of the SNPs is of less importance in the Affymetrix design. By contrast, Affymetrix SNP arrays also contain known "coding" mutations.

Since it is likely that common variants are associated with small effects, large cohorts are needed to achieve sufficient statistical power. Therefore, in Genome Wide Association studies (GWAS), the aim is to accumulate the highest achievable number of genotypes of as many subjects as possible. When GWAS results in novel loci, the "candidate gene" approach is generally chosen to search for and validate the causal variant[82].

VALIDATION AND REPLICATION

A classical method in genetic research of disease is the "candidate gene" approach. Candidate genes can be selected after indication obtained through several different methods, namely by means of: (1) linkage analysis, (2) GWAs or by (3) selecting a gene / protein according to its biochemical characteristic in the disease process or pathway. In general, fine mapping of the gene of interest is performed using sequencing analysis. Replication of a candidate gene is, mostly, performed using association analysis. This association analysis can be performed using SNPs and is based on the fact that most genetic variants causally related to the trait are expected to be more or less prevalent in patients than in controls, depending on whether they increase or decrease the risk of disease. However, also the SNPs close to the causal ones are expected to be increased or decreased in patients, when SNPs are close to each other. This phenomenon is called linkage disequilibrium (LD).

Thus causal mutations can be identified using SNP analysis or sequencing analysis. Mutations that cause overt changes in protein function (i.e. reading frame shifts leading to stop codons) provide strong proof for being the cause of a genetic disease. Less overt changes that nevertheless lead to protein dysfunction (i.e. missense mutations) can be identified by comparing the prevalence of the variants in patients versus controls. Putative dysfunction of the proteins encoding these genes can subsequently be characterized *in vitro* in material (i.e. blood cells) derived from patients versus healthy controls. Finally, a SNP that is located in a gene desert may very well influence the expression of a gene in the region (*cis* regulation) or elsewhere (*trans* regulation).

ALTERNATIVE STUDY COHORT

Most association studies are conducted in the general population. Alternatively, the design of a study can be based on a genetically isolated population. In short, this design is a mix between a family based cohort and a general cohort. It requires an extensive and more expensive collection procedure of study subjects, as in most cases a pedigree confirmation is required to rule out possible admixture with the general population. The statistical power of association analysis in such genetically isolated populations or founder populations is thought to be much stronger due to the fact that it is based on a limited gene pool[83,84,82]. This limited gene pool is a result of a limited number of founders in combination with a fast expansion of the population. Furthermore, a genetically isolated population is characterized by minimal immigration, due to social, geographical or religious reasons. Genetically isolated populations are liable to genetic drift. Genetic drift is defined by the phenomenon that rare genotypic variants disappear or, vice-versa, that rare variants become overrepresented with regard to the general (out bred) population. Common genetic variants are, however, generally not affected in genetically isolated populations and their frequencies are expected to be similar to those in the general population[83].

STATISTICAL POWER IN ASSOCIATION STUDIES

To perform linkage analysis or GWAs with sufficient statistical power, the study cohort must be of sufficient size and this requires significant effort and finances. Statistical power represents the measure of confidence to detect an (genetic) effect in a particular number of samples. However, the

problem with both linkage analysis and GWAs is the enormous number of tests which might result in false positive signals; type I errors. Statistical methods generally use a 95% confidence interval, which implies that five percent of the associations that are found are actually type I errors. When performing a single test, the 5% probability of finding a false positive result is acceptable. However, when performing half a million tests such as in GWA, the amount of false positive results will be large.

To address the multiple test correction issue in GWAs, methods like the method of Bonferroni are used to overcome the problem of accumulating type I errors[85,86]. In brief, the method of Bonferoni decreases the probability of a true finding by dividing the confidence (represented by the P-value) by the number of independent tests performed. This method is, however, a very stringent multiple test correction which might result in a high probability of type II errors; or false negatives. Therefore, other multiple test correction methods, like the method of Benjaminii – Hochberg, have been developed. This correction method also reduces the number of false positive associations, but also takes into account the possibility of false negative findings[85,86].

META-ANALYSIS OF ASSOCIATION STUDIES

To validate GWAs results and tackle the remaining probability of both false positive and false negative associations, meta-analyses are performed[87]. Meta-analysis is a statistical method to compare and strengthen similar observed candidate loci for disease associations in different studies/cohorts. Meta-analysis is capable of detecting in several different study cohorts consistent, yet small significant associations, but is also capable of excluding single significant false positive associations[88]. Meta-analysis is now generally accepted as a powerful tool in genetic epidemiology and the application of meta-analysis in the field of genetic epidemiology is widely used. Large international consortia have been formed over the last years, resulting in studies exceeding 30.000 samples. Meta-analysis on such large number of samples resulted for example in the discovery of several new loci associated with T2D and obesity and several quantitative traits such as plasma lipids[89,90,91,92].

Three common problems in meta-analysis are (1) the use of different types of cohorts, (2) inconsistency in phenotyping and (3) inconsistency in genotyping. The use of different types of cohorts in GWAs meta-analysis, at least with regard to ethnicity, should be avoided or at least carefully monitored since population specific genetic associations might unjustly be disregarded. Differences in (the accuracy of) phenotyping might also result in false negative findings. For example, in a meta-analysis of plasma glucose GWAs, consistent use of information about the fasting state of the samples and the use of glucose lowering medication should be included in each individual GWA. Genotypic inconsistencies between cohorts are for example caused by the use of different genotyping platforms. In this respect, the design of two major suppliers of genome wide genotyping platforms, Illumina and Affymetrix, are totally different.

To overcome the problem of missing SNPs, the statistical tool of imputation was developed[93,94]. Imputation is based on the fact that most genetic variants are more or less in LD with nearby genetic variants. Based on the genetic data of Hapmap, which involves about 2.500.000 genetic variants, differences in genetic variants between platforms can be filled in using estimation of the missing genetic variants[95]. This way, different platforms (cohorts) can be forced towards similarity in genetic variation and thus be used in meta-analysis.

OUTLINE THESIS

In this thesis several aspects of metabolic syndrome are addressed. The focus involves questions concerning the genetics of obesity, TG and cholesterol and hyperglycemia. Since we hypothesized that obesity is the most important trigger of metabolic impairment, the MetS definition in this thesis was chosen to include the obesity measure waist circumference as an essential component. In the study described in **chapter 2**, the heritability of the metabolic syndrome was addressed and compared to the heritability of its individual components. Since the individual components of MetS were shown to be more heritable than MetS itself, the studies described in **chapter 3 and 4** focused on the genetics of the individual MetS component plasma TG. For this purpose, a candidate gene approach was employed using HTG patients and healthy controls. The involvement of a series of candidate genes was confirmed. The study described in **chapter 5** followed a similar approach to that used in the studies described in **chapter 3 and 4**. Several candidate genes were studied in patients suffering from hyperlipoproteinemia (HLP) type III, which is characterized by elevated levels of total plasma cholesterol and plasma TG. HLP type III is characterized by APOE2 homozygosity. Contributing genetic factors in the (metabolically stressed) APOE2/2 environment were confirmed. Plasma adiponectin, an adipose tissue secreted hormone (adipokine), has been suggested to be a biomarker for MetS. In **chapter 6** we describe a study which particularly aimed to determine the effect of menopause on the discriminating accuracy of adiponectin to predict MetS. Especially low levels of plasma adiponectin in postmenopausal women were found to be a risk for MetS. However, the discriminating accuracy of adiponectin for the presence of MetS was exceeded by BMI in men and pre –and post menopausal women. Since plasma adiponectin levels are very well correlated with MetS components or related traits, the study described in **chapter 7** addressed the question whether these correlations are caused by a genetic overlap (genetic correlation). The genetic correlation was mono-laterally validated with regard to the adiponectin gene (*ADIPOQ*). **Chapter 8** describes a study towards finding novel loci associated with adiponectin or loci that are possibly involved in the genetic overlap between adiponectin and MetS components or related traits. This study followed a genome-wide association (GWA) approach. The results of this GWA were used in a joined analysis with two other cohorts in a meta-analysis. In addition, a selected proportion of SNPs was submitted for replication in several cohorts. **Chapter 9** provides a general discussion by reviewing all previous chapters in the thesis. Furthermore, chapter 9 includes suggestions and proposals for future analyses towards unraveling genetic and environmental factors involved in the expression and manifestation of metabolic risk factors.

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