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3`UTR	Untranslated region
ACC	Acetyl-CoA Carboxylase
Acrp30	Adipocyte complement-related protein of 30 kDa
ALSPAC	Avon Longitudinal Study of Parents and Children
AMPK	Adenosine monophosphate kinase
apM1	Adipose most abundant gene transcript 1
APPL1	Adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding domain and leucin zipper motif
ARL15	ADP-ribosylation factor like 15
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BLSA	Baltimore Longitudinal Study of Aging
BMI	body mass index
BP	blood pressure
BRIGHT	The MRC British Genetics of Hypertension
CAD	Coronary artery disease
Cadherin	Calcium dependant adhhesion molecules
cAMP	Cyclic adenosine monophosphate
cDNA	Coloured DNA
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
CoLaus	Cohort Lausanne
CPT1	Carnitin palmitoyl transferase 1 (CPT 1)
CRP	C-reactive protein
CVD	Cardiovascular disease
DESIR	Data from an Epidemiological Study on the Insulin Resistance Syndrome
DNA	Desoxiribonucleic acid
EGIR	European Group for the Study of Insulin Resistance
EPIC norfolk	European Prospective Investigation of Cancer - Norfolk
ERF	Erasmus Rucphen Family Study
ERK 1/2	Extracellular signal-related kinase 1/2
FFA	Free fatty acids
FOS	Framingham Offspring Study
Framingham	Framingham Heart Study
GBP28	Gelatine binding protein of 28 kDa
GEMS	Genetic Etiology of Metabolic Syndrome
GIANT	General Practice Implementation in Asia of Normoglycaemic Targets)
GPCR	G-proteine coupled receptor
GRB2	Growth factor receptor bound protein 2
GWAS	Genome-wide association study
HDL	High-density lipoprotein
HMG	3-Hydroxy-3-Methylglutaryl
HMW	High molecular weight
HYPEST	Hypertension in Estonia
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
InChianti	Invecchiare in Chianty Study
IR	Insulin resistance
IRS 1/2	Insulin receptor substrate 1/2

JAK	Janus kinase
KORA	Kooperative Gesundheitsforschung in der Region Augsburg
LDL	Low-density lipoprotein
LMW	Low molecular weight
lod score	Logarithm of odds
LOLIPOP	London Life Science Population
MetS	Metabolic Syndrome
MMW	Middle molecular weight
mRNA	Messenger RNA
NCEP ATP III	National Cholesterol Education Program - Third Adult Treat Panel
NFBC	Northern Finland Birth Cohort Study
NHBPEP	National High Blood Pressure Education Program
NHGRI	National Human Genome Research Institute
OB	Obese gene
OR	Odds ratio
P38 MAPK	p38 mitogen-activated protein kinase
PI3K	Phosphatidlyinositol 3 kinase
PPAR	Peroxisome proliferator activated receptor
PTP1B	Phosphotyrosine phosphatase 1B
PTPN1	Protein tyrosine phosphatase non-receptor type 1
QTL	Quantitative trait loci
SH2	Src homology 2 domain
SH2B	SH2 domain containing phosphatase 2
SH2P	SH2 domain containing phosphatase 2
SNP	Single nucleotide polymorphism
SOCS3	Suppressor of cytokine signalling 3
STAT	Signal transducer and activator of transcription
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TRL	Triglyceride-rich lipoproteins
TWINS UK	Twins Research and Genetic Epidemiology in the UK
US	United States
VLDL	Very low-denisity lipoproteins
WAT	White adipose tissue
WC	Waist Circumference
WHO	World Health Orgnisation
WHR	Waist-to-Hip ratio
WTCCC	Wellcome Trust Case Control Consortium

1. Introduction

The attention to metabolic syndrome (MetS) has risen constantly over the years. The increasing number of people affected by obesity, cardiovascular diseases and/or diabetes is alarming as it is not only a problem of premature death but also of disability, illness and economic costs. It is out of question that the change of the environment in the last decades, which comes with sedentary life style and poor nutrition habits, is blameable for the substantial part of this situation. But how significant is the genetic part? Are there really genes that are accountable for this development, too? If yes, which ones are to blame?

This thesis is focusing on leptin and adiponectin as well as their receptors. These two adipocytokines have brought some furore into research as they have changed the knowledge about adipose tissue completely. Both have been associated with obesity and type II diabetes as well as MetS. Researchers have also shown that they play a crucial role in hunger and satiety, which might have a substantial effect on obesity, a feature of MetS. But what about their influences on insulin resistance, dyslipidemia or hypertension?

Returning to the question from above, this thesis is assuming that there are genes that have an influence on the development of MetS. Furthermore it wants to show if there are substantial causal effects primarily due to polymorphisms in the leptin and adiponectin coding genes, their receptor coding genes or the genes that encode their signalling pathway. Genome-wide association studies (GWAS) are the method of choice in finding these polymorphisms in the DNA associated with changes in leptin and adiponectin efficiency. If researchers are able to find significant results, they might be able to understand to which part genetic variance plays a role in pathogenesis. This might either lead to new and individual therapies or to the conclusion that the incorporation of better health habits is the only way of treatment of MetS.

However, it remains interesting to find out what these adipocytokines really are, how they function in the human body and what effects they have when they do and do not function properly. This thesis has tried to consider all these aspects. At first the MetS and its features of pathophysiology are described, then the adipocytokines and their signalling pathway in the human body. The following explanation of genome-wide association studies is an essential background to understand and interpret the study results. Those have been divided into real GWAS and further studies as there are not many of the first ones and the latter have shown promising outcomes. A discussion at the end of every subchapter points out the up-to-date knowledge and shows the related difficulties. General results are then summarized in the conclusion.

Various opinions, numerous definitions of MetS, indefinite cut-off points and new detection methods are only a few difficulties that researchers and this thesis have to deal with. As already shortly mentioned, those are pointed out in the thesis, as they might be crucial when interpreting studies.

It starts with the question: “What is actually the MetS?”

2. Metabolic Syndrome

2.1. Definitions

To understand how leptin and adiponectin can play a role in development of MetS it is important to know what MetS actually is. In general it describes a "cluster of risk factors for type II diabetes (T2D), cardiovascular disease (CVD) and stroke" [HENNEMAN 2010], which are all diseases that are associated with the modern, sedentary, `high energy intake` lifestyle.

Although KYLIN has introduced the concept already in 1923, a standardized definition of MetS has not yet been formulated. At the moment there are four definitions widely used for classification for MetS, formulated by the World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III) and the International Diabetes Federation (IDF). All four definitions agree upon the core components of the MetS: obesity, insulin resistance (IR), dyslipidaemia and hypertension. However, they offer different clinical criteria to identify it. Each of them has slightly different levels for each factor and they use different combinations of components to diagnose the cluster of risk factors. Therefore direct comparisons between studies remain to be difficult or impossible. In the chapter "Prevalence" this problem is pointed out and explained in detail as it also has an effect on the interpretation of study results later on.

The four definitions named above are explained subsequently. They are also put into a table with the exact, corresponding components for easier comparison of the cut-off points.

2.1.1. WHO

The definition of the WHO is based on the assumption that insulin resistance is the major contributor to the MetS. Their clinical criteria are: glucose intolerance, impaired glucose tolerance (IGT) or diabetes mellitus and/or insulin resistance together with two or more of the other components like hypertension, elevated plasma triglycerides, central obesity and microalbuminuria [WHO 1999; ALBERTI et al. 2006]. Within this definition central obesity is measured as waist-to-hip ratio (WHR), which is an index of the relative accumulation of abdominal fat. Eye-catching is the fact that WHO also uses microalbuminuria as a factor, which is not common within other definitions.

2.1.2. EGIR

The members of the EGIR have criticised the syndrome definition by the WHO Expert Committee and proposed an altered definition, which has to be used only in non-diabetic cases [BALKAU and CHARLES 1999]. They proposed to use fasting insulin sensitivity to estimate insulin resistance and impaired fasting glucose (IFG) instead of IGT and also slightly altered cut-points for hypertension, triglycerides and high-density lipoprotein (HDL) cholesterol. For central obesity measurement they recommend to use waist circumference (WC) instead of the WHR [ALBERTI et al. 2006].

2.1.3. NCEP ATP III

The definition made by NCEP ATP III was developed to facilitate the diagnosis of MetS in clinical practice. It differs substantially to the other two definitions because it does not include a measurement of insulin resistance and it treats glucose abnormalities with equal importance as the other components. For them three or more of the five suggested factors (obesity, HDL-cholesterol, triglycerides, blood pressure (BP) and fasting plasma glucose) have to be present to diagnose MetS [GRUNDY et al. 2004; ALBERTI et al. 2006].

2.1.4. IDF

As already mentioned numerous definitions make comparisons between individual studies very difficult. Consequently, the IDF decided to create a new worldwide definition for MetS as well as to establish new clinical criteria that should replace other definitions. The NCEP ATP III definition from 2001 was used as a fundament and then modified and updated (e.g ethnicity specific cut-points for WC). For IDF a person, to be identified as having the MetS, must have central obesity (defined as waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women) plus any two of four additional factors [ALBERTI et al. 2006]. Despite all attempts it did not replace the other definitions but added new criteria instead. Therefore the problem of comparability remains.

Factors	**WHO (1999)**	**EGIR (1999)**	**NCEP ATP III (2004)**	**IDF**
	Glucose intolerance, IGT or diabetes and/or insulin resistance together with two or more of the following:	Insulin resistance Plus two of the following:	Three or more of the following five risk factors:	Central obesity plus two of four additional risk factors.
Fasting plasma glucose		≥ 6.1 mmol/l (110 mg/dl) but non-diabetic	≥ 5.6 mmol/l (100 mg/dl)	≥ 5.6 mmol/l (100 mg/dl) or previously diagnosed Type 2 diabetes.
Blood pressure	≥140/90 mmHg	≥ 140/90 mmHg or treatment	≥ 130/ ≥ 85 mmHg	≥ 130/ ≥ 85 mmHg or treatment
Triglycerides	Raised plasma triglycerides: ≥ 1.7 mmol/l (150 mg/dl) and/or	> 2.0 mmol/l (178 mg/dl) or treatment and/or	≥ 1.7 mmol/l (150 mg/dl)	≥ 1.7 mmol/l (150mg/dl) or specific treatment
HDL- cholesterol	Men: < 0.9 mmol/l (35 mg/dl) Women: < 1.0 mmol/l (39 mg/dl)	< 1.0 mmol/l (39 mg/dl) or treatment	Men: < 1.03 mmol/l (40 mg/dl) Women: < 1.29 mmol/l (50 mg/dl)	Men: < 1.03 mmol/l (40 mg/dl) Women: < 1.29 mmol/l (50 mg/dl) or specific treatment
Obesity	Men: WHR > 0.90 and/or BMI > 30 kg/m^2 Women: WHR > 0.85 and/or BMI > 30 kg/m^2	Men: WC ≥ 94 cm Women: WC ≥ 80 cm	Men: WC > 102 cm Women: WC > 88 cm	Ethnicity specific cut-points of WC
Microalbuminuria	Urinary albumin excretion rate ≥20 mg/min or albumin: creatinine ratio ≥30mg/g			

Table 1: Comparison of four different MetS definitions [ALBERTI et al. 2006].

2.2. Pathophysiology of the Metabolic Syndrome

The pathogenesis of each of the MetS components is complex and until now not fully elucidated. Therefore the syndrome as a whole, which means the interplay of all the single components, remains to be an interesting subject for research. Various factors, e.g IR, genetic profile, physical inactivity, hormonal dysregulation, etc., have been proposed to be causal factors for development of MetS. In the following subchapters the most important factors that have been associated with the cluster of diseases are explained.

2.2.1. Obesity

Obesity is a condition of excess body fat and is currently defined by using body mass index (BMI), WC and/or WHR [HILL et al. 2006].

BMI is calculated as weight (kg)/ height squared (m^2) and is independent of sex and also age in adults. Subjects with a BMI in the range of 25 to 30 are considered as overweight and those with a BMI of 30+ as obese [GALLAGHER et al. 2000]. This parameter is a suitable but not perfect surrogate for body fatness as there are individuals who are overweight but have a normal amount of body fat. This appears e.g. in bodybuilders that have a large muscle mass, which is accountable for the high weight. On the other hand there are also people with a normal BMI that have high body fat percentage but reduced muscle mass. Measuring the WC or WHR could be an additional check [HILL et al. 2006] for the correct identification of individuals. WC is highly correlated with the amount of visceral fat, which, in many studies, has been shown to increase risk for diabetes, hypertension, dyslipidaemia and ischemic heart disease [KISSEBAH et al. 1982]. The WHR is used to make a difference between the benign pear shaped and the malign apple shaped overweight individuals but, on its own, it does not represent obesity [HENNEMAN 2010].

Next to sedentary lifestyle and unhealthy nutrition it is suggested that genetic factors may play an important role in determining the response of body mass

and body fat stores to chronic alterations in energy balance [PERUSSE and BOUCHARD 2000]. Family studies show strong genetic influences on body weight. MAES et al. (1997) estimated in their review that up to 80% of variation in BMI is explained by genetic factors. But these results have to be looked at with great caution as it is often not clear where genetics end and environmental influence, in this case e.g. sharing the same food or the same exercise habits within a family, starts.

2.2.2. Insulin resistance

In a normal physiological state insulin increases glucose uptake by peripheral organs such as muscle and fat and suppresses glucose production by the liver [HENNEMAN 2010]. If IR, which is defined as “a decreased response of the peripheral tissues to insulin action” [XU H. et al. 2003], is present, insulin is not able to act normally in the regulation of nutrition and metabolism and therefore plasma levels have to be increased to achieve normal plasma glucose levels. If levels of fasting plasma glucose consistently rise up to 7 mmol/L it can be said that the person has T2D [AMERICAN DIABETES ASSOCIATION 2007]. This appears when the increased insulin secretion cannot compensate for the decreased response of the tissues anymore. The glucose homeostasis cannot be maintained – resulting in cytotoxic hyperglycaemia [HENNEMAN 2010].

Free fatty acids (FFA) have been shown to be a major link between obesity and IR [BODEN 2004]. FFA inhibit insulin-stimulated glucose uptake at the level of glucose transport and/or phosphorylation through mechanisms that involve accumulation of diacylglycerol and long-chain acyl-CoA activation of proteinkinase C and decreased tyrosine phosphorylation of insulin receptor substrate 1/2 (IRS-1/2) [BODEN et al. 2005]. FFA concentrations can be reduced by a small increase in insulin levels. That means that high plasma FFA concentrations can be prevented if large amounts of insulin can be secreted. If this does not happen high FFA concentrations increase hepatic glucose production, which is likely to lead to significant fasting hyperglycemia [REAVEN 1988].

2.2.3. Dyslipidemia

Dyslipidemia is a condition that shows no symptoms by itself but leads to symptomatic vascular disease. It is a disorder of lipoprotein metabolism, which is characterized by three major components: increased fasting and postprandial triglyceride-rich lipoproteins, decreased HDL and increased low-density lipoprotein (LDL) particles [RUOTOLO and HOWARD 2002].

Reasons for dyslipidemia can be diverse. Genetic, but especially lifestyle factors are proposed to have a big influence. Living a sedentary lifestyle with excessive food intake leads to hyperlipidemia, the most common form of dyslipidemia, which plays a major role in the development of atherosclerosis [MATSUZAWA et al. 2004]. But hyperinsulinaemia and central obesity also have a great impact on it as they lead to overproduction of very low-density lipoproteins (VLDL) [HOWARD 1999], which are interacting with other lipoproteins and therefore disturbing the metabolic balance.

As cardiovascular diseases are the number one cause of death in many countries, the management of dyslipidemia forms an important part of the strategies for their prevention [THOMPSON 2004]. To calculate the risk for each person individually the FRAMINGHAM HEART STUDY developed an algorithm, the Framingham Risk Score, which estimates the risk of various cardiovascular diseases. It uses age, gender, total cholesterol, HDL cholesterol, smoker/non smoker and systolic blood pressure, among others, as parameters for the assessment.

At the moment there are two ways of classifying dyslipidemia. One is by aetiology and one by phenotype. As genes are always affected by lifestyle and nutrition the first classification can be a problematic one. Nevertheless, common variants have been found at 30 loci that contribute to polygenic dyslipidemia [KATHIRESAN et al. 2009]. FREDERICKS and LEES developed a classification by phenotype in 1965. They used paper electrophoresis to separate lipoprotein groups and then arranged them into five different groups. More details on that topic were excluded, as they are not necessary for this thesis.

2.2.4. Hypertension

Hypertension is a condition of constantly elevated blood pressure (BP). It is a major risk factor for coronary heart disease, stroke, congestive heart failure, renal insufficiency and peripheral vascular disease [BURT et al. 1995]. There are indications that MetS components like hyperglycemia or systemic inflammation affect the functioning of the vascular endothelium and therefore have an influence on the blood pressure [HENNEMAN 2010].

Nutritional factors, e.g. high salt intake, obesity and insulin resistance have been shown to increase the BP but genes have been discussed to be involved in the development of hypertension, too. At least 10 genes have been shown to raise or lower BP by increasing or decreasing salt and water reabsorption by the nephrons [CARRETERO and OPARIL 2000].

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, which was done in 2003 by the National High Blood Pressure Education Program (NHBPEP) estimates that hypertension affects approximately 1 billion people worldwide with an upward trend. All of these individuals can be classified into groups by level of their BP: normal blood pressure, pre-hypertension, hypertension and hypertension stages I and II. For the MetS definition a BP of ≥ 140/90 mmHg (EGIR, WHO) or ≥ 130/85 mmHg (NCEP ATP III, IDF), respectively, is needed to be considered as hypertension.

2.2.5. Inflammation

Inflammation is a response of the vascular tissue to infection, irritation or any other injury. In this way the body tries to get rid of pathogens and to initiate the healing process. Acute inflammation is crucial for wound healing but if it turns into chronic inflammation it is dangerous and painful. Instead of healing it damages the healthy organs and can lead to various diseases like atherosclerosis, rheumatoid arthritis or inflammatory bowel disease [THEWS et al. 1999].

This happens because lowgrade systemic inflammation is damaging the endothelium, leading to endothelial cell dysfunction and abnormal blood pressure regulation. When the endothelial barrier function is not working properly, harmful substances, like LDL, can enter into the sub-endothelial space. A chronic accumulation and oxidation of LDL as well as an uptake of invading macrophages leads to foam cell formation, which is considered to be the first step of atherosclerotic plaque [THEWS et al. 1999]

Although it is not part of any of the explained definitions, inflammation, as a fifth component of MetS, is also included in this thesis. The reason is that inflammation has been shown to play an important role in developing CVD and T2D [HAFFNER 2006]. An 8-year follow-up of 14.719 initially healthy women, which was conducted by RIDKER et al. (2003), showed that elevated levels of the inflammatory marker C-reactive protein (CRP) are associated with increased risk for CVD and T2D, but also with fasting insulin and microalbuminuria. Although it is not included in any of the definitions, CRP measurements would add important information for easier identification of patients with MetS.

In general it can be said that MetS is an equation with various unknown parts. Inaccurate definitions or unreliable parameters make it hard to answer the question of how many people are really affected by MetS.

2.3. Prevalence

Talking about prevalence of MetS is almost impossible. As mentioned above, different definitions not only make it uneasy to compare individual studies but also make it hard to give exact numbers of prevalence. Looking at the table, the cut-off points and the combination of components do not seem to have large scale variations but when seen graphically the degree of disparity in diagnosing MetS can be seen.

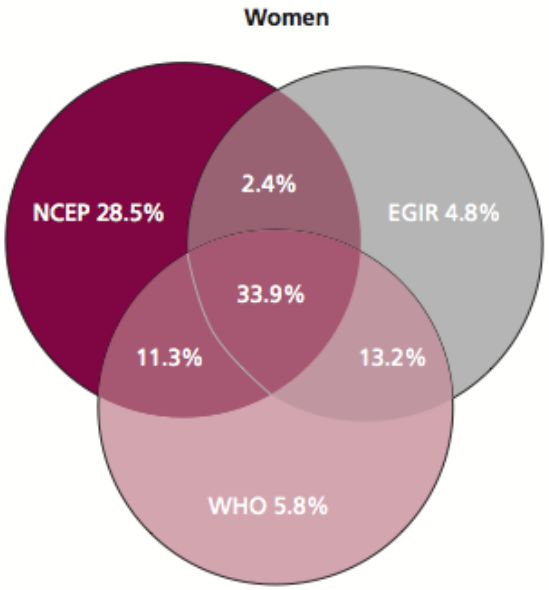

Figure 1: Comparison of the MetS definitions by prevalence [DECODE STUDY GROUP, 2005]

The DECODE Study Group [2005] compared the three definitions with each other in a cohort group of 9140 non-diabetic European subjects (4190 men and 4950 women). The graphic that they generated out of their collected data shows that there is a poor agreement between the definitions. There are 34% of the women (31% of men, data not shown in figure 1) who meet all the three definition criteria. It shows furthermore that 39% of the whole female subjects (37% of the men) meet only one definition. The definition by NCEP ATP III had the highest MetS prevalence identifiying 76% of the subjects as affected with MetS in comparison to EGIR, which identified 54% as affected (WHO: 64%) [DECODE STUDY GROUP 2005].

A study in Pudong, new area of SHANGHAI, collected data of 5583 subjects (2477 males and 3107 females) and compared the NCEP ATP III, the WHO and IDF definition with each other. Although 69% of the men and 64% of the women were classified as being free of MetS only 9% of men and 13% of women met all of the three definition criteria. In this study the NCEP ATP III data showed prevalence for MetS of 28% in men and about 35% for women [XU et al. 2010].

In the USA, FORD et al. (2002) conducted a study on MetS prevalence using NCEP ATP III criteria. They showed that of 4265 men 22% and of 5356 women 24% have MetS, also emphasizing the age of the subjects. Because 44% of men and 42% of women in the 60+ years category have MetS it is evident that age is an important risk factor, which is very alarming. XU et al. (2010) namely mention in their study that the age group 60+ accounts for

more than 20% of overall residents of the city. And in almost all developed countries the same tendency can be seen.

In Europe HU et al. (2004) collected data of 6156 men and 5356 women and evaluated them by WHO criteria with the result that 16% of men and 14% of women have MetS, which may indicate lower prevalence than in China or the USA. But no exact comparison can be made because of different definitions criteria.

Although there are problems because of the inability of comparison between the definitions still some conclusions can be made. There is a high prevalence of MetS, especially in elderly people. Because the average age is constantly rising, the appearance of MetS will increase in the future. The ongoing nutrition transition and sedentary lifestyle retrieve big potential risks for development of MetS, also in young people. So for the future, further research is needed for new therapies, as lifestyle changes are not easy to achieve. Among others, adipose tissue hormones have showed promising results.

3. Adipose tissue and its bioactive peptides

There are different forms of adipose tissue. In general it can be divided into white adipose tissue (WAT) and brown adipose tissue (BAT). In the scope of this work only the first one is considered. It represents the majority of adipose tissue in the organism. Although it consists of numerous cell types, adipocytes are the prevailing one [FANTUZZI 2005]. WAT again can be divided into two subgroups, visceral and subcutaneous fat, by the place where it accumulates (apple-shaped, pear-shaped). In terms of adipocytokine production it is important to know that subcutaneous fat and visceral fat are not equivalent. Visceral fat is likely to have a much more direct signalling and metabolic relation with the liver in comparison to subcutaneous fat because it drains directly into the portal vein [HENNEMAN 2010].

For a long time WAT was considered to be only energy storage. But the identification of its ability to secrete bioactive peptides, which function as hormones, established the tissue as an endocrine organ. Until now it has been shown that adipose tissue has various responsibilities in the human body. It stores triglycerides, responds to nutrient, neural and hormonal signals and secretes adipocytokines that control energy homeostasis, thermogenesis, immunity and neuroendocrine function [KERSHAW and FLIER 2004]. Two of these adipocytokines are leptin and adiponectin, which are the focus of this thesis.

There is a controversial discussion what leptin and adiponectin actually are because they cannot be exactly classified. Suggested groups are adipocytokines, cytokine-like hormones and adipose derived hormones. The problem is that the differences between hormones and cytokines diminish the more it is known about them. To simplify matters, in this thesis, the term adipocytokines will be used. But it is important to know that there are different names when searching for information in databases. Generally, adipocytokines are defined as biologically active polypeptides that are either exclusively or substantially produced by adipocytes and act by endocrine, paracrine and autocrine mechanisms [ROSE et al. 2004].

As already mentioned the focus of the thesis is on leptin, adiponectin and their receptors. To show their relation to MetS it is essential to know the biochemistry behind it. Therefore they are explained in the following subchapters – from the structure to the signalling.

3.1. Leptin

In 1994 ZHANG et al. discovered a murine obese (OB) gene that is responsible for obesity in the ob/ob mouse. Shortly after, the gene was located on chromosome 7 (7q31.3) in humans. Also known as LEP, OBS or FLJ94114 [NCBI Entrez Gene 2010], OB was found to encode a protein, leptin, which has been in the focus of nutrition science ever since.

Leptin is a 16 kD protein that is built of 167 amino acids [KERSHAW and FLIER 2004]. These are arranged in four α-helices (see figure 2), which make it look similar to interleukin-6 (IL-6). Therefore, structurally, it belongs to the type-1-cytokine superfamily [LA CAVA and MATARESE 2004]. The main source of leptin is adipose tissue although it is also secreted by e.g. the gastric epithelium, skeletal muscle and placenta [AHIMA and OSEI 2004].

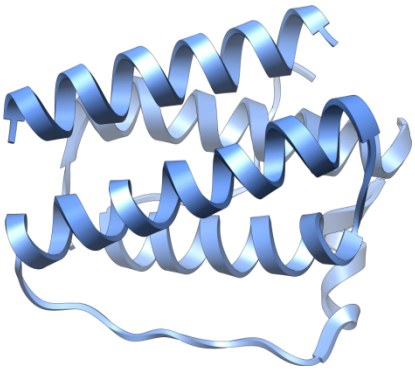

Figure 2: The four α-helices of leptin [VOSS 2007]

Leptin has been shown to be an anorexic peptide that is responsible for the regulation of satiety, hunger and energy homeostasis [OTERO et al. 2006]. Low levels of leptin enhance appetite and decrease energy utilization by initiating endocrine starvation response (see also figure 3) [BATES and MYERS 2004]. Therefore it was expected that leptin may be a good treatment for obese individuals.

Although significant effects have been shown in ob/ob mice, the effect in obese humans failed because leptin deficiencies are not common in human obesity [TARTAGLIA 1997]. In contrast, obese individuals showed increased levels of leptin leading to the conclusion that this hormone serves more as a

signal of energy sufficiency rather than energy excess, and therefore resistance to leptin may be more important than increased or decreased levels [BATES and MYERS 2004]. Consequently, attention was paid to the leptin receptor and the appropriate LEPR gene, pushing the db/db mouse, a model of complete resistance, into the spotlight.

Other causes that lead to non-activity of leptin have also been suggested. It has been linked to decreased transport across the blood brain barrier (BBB), which is due to a deficit in short leptin receptor isoforms, but also to desensitization for the leptin signal [OTERO et al. 2006].

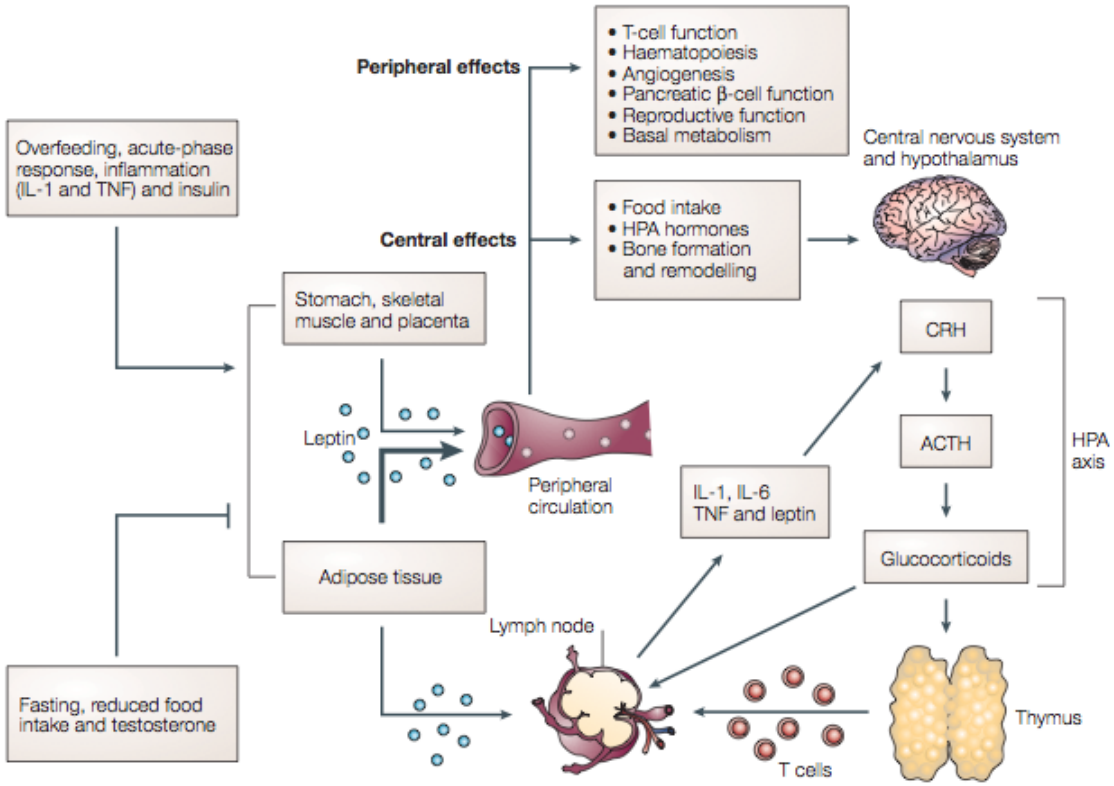

Figure 3: Effects of Leptin in the human body [LaCAVA and MATARESE 2004]

3.2. Leptin receptor

As mentioned above, with time, leptin resistance got more important than the increased or decreased levels of leptin itself. The db/db mice were chosen because they showed early onset obesity like the ob/ob mice and indicated defects in reception of leptin. It was suggested that the db gene encodes either the gene for the leptin receptor or a gene that is encoding an important

component of the leptin signalling pathways [TARAGLIA et al. 1995]. Further studies revealed that there is a mutation in the db gene that can be made responsible for the leptin resistance. In exon 12 of the LEPR gene a G residue is deleted. So the initial sequence AAGGAG turns into AAGAG. It is impossible to say if the nucleotide 2022 or 2023 was deleted but the change causes a substitution of 11 amino acids. As a result, the intracellular domain of the protein is cut [TARTAGLIA 1997; BROWN et al. 2000].

LEPR has been assigned to the class-I-cytokine receptors. It is closely related to gp130, a protein of the IL-6 signalling complex, the leukaemia inhibitory factor receptor and the granulocyte colony stimulating factor receptor [TARTAGLIA et al. 1995]. Until now at least 5 LEPR isoforms have been described and labelled from LEPRa to LEPRe [BJOERBAEK and KAHN 2004]. They all share an identical N terminal ligand-binding domain, which is the extracellular domain, but differ in the C terminal (intracellular) region because of the alternative RNA splicing at the most C-terminal coding exon [TARTAGLIA 1997].

The intracellular domains of LEPR a, c and d are about 30 to 40 amino acids long [BANKS et al. 2000]. Therefore they have no signal transducing capabilities and are called “short receptors”. In general their levels in human tissue are much higher than those of LEPRb, with only one exception - the hypothalamus. Their exact functional roles and the differences among the various forms remain to be defined. It is suggested that they are important for carrying leptin from the blood into the cerebrospinal fluid, from where the adipocytokine can diffuse to the brain centres and perform its functions. It is also proposed that they could be responsible for clearance or as a source of soluble receptor [TARTAGLIA 1997].

LEPRe is the shortest receptor of the group, because it is truncated. It contains only the coding regions for extracellular domains, meaning, it does have neither the transmembrane nor the intracellular domain. So LEPRe functions as a soluble leptin-binding protein [AHIMA and OSEI 2004].

Last but most important is LEPRb, also called the “long receptor”, that is build out of 1162 amino acids [LA CAVA and MATARESE, 2004]. Its C-terminal region exists of about 300 amino acids, which is almost ten times longer than the one of the short forms. LEPRb is the only leptin receptor that has intracellular tyrosine residues and therefore is the only isoform capable of activating a signal transduction [BANKS et al. 2000]. It can be detected in several peripheral tissues but at much lower levels compared to LEPRa. It has been measured in lung, kidney, adrenals and lymph nodes and at lower levels in liver, BAT, WAT, and skeletal muscle. The account of LEPRb is under 5-10% of total leptin receptor expression [BJORBAEK and KAHN 2004].

There are various signalling effects that can emanate from LEPRb when leptin binds to it. Four of them are mentioned in this thesis: The JAK (janus activating kinase)/STAT (signal transducer and activator of transcription) pathway, the p38 mitogen-activated protein kinase (p38 MAPK) and phosphatidyinositol 3 kinase (PI3K) pathway as well as the adenosine monophosphate kinase (AMPK) pathway [ZHANG et al. 2005]. In the leptin signalling chapter the JAK/STAT pathway is explained in detail, as it is the best-known one. P38 MAPK and PI3K are only shortly mentioned. The AMPK pathway is explained in more detail in the adiponectin signalling chapter.

3.3. Leptin signalling

LEPRb is a protein with a fibronectin type III domain and a block of amino acids in the extracellular region. This block contains four conserved cysteine residues and two cytokine-like binding motifs - Trp- Ser- Xaa- Trp- Ser (WSXWS) (see figure 4) [LA CAVA and MATARESE 2004; AHIMA and OSEI 2004].

When Leptin binds to the receptor the “box1” motif recruits cytoplasmic JAK2 [FRÜHBECK 2006]. It binds to the box resulting in its activation, which leads to tyrosine phosphorylation. There are four phosphorylated tyrosine residues (Tyr974, Tyr985, Tyr1077, Tyr1138) on LEPRb that function as docking sites for proteins with Src homology 2 (SH2) domains like STATs [La CAVA and MATARESE 2004; LÖFFLER 2005].

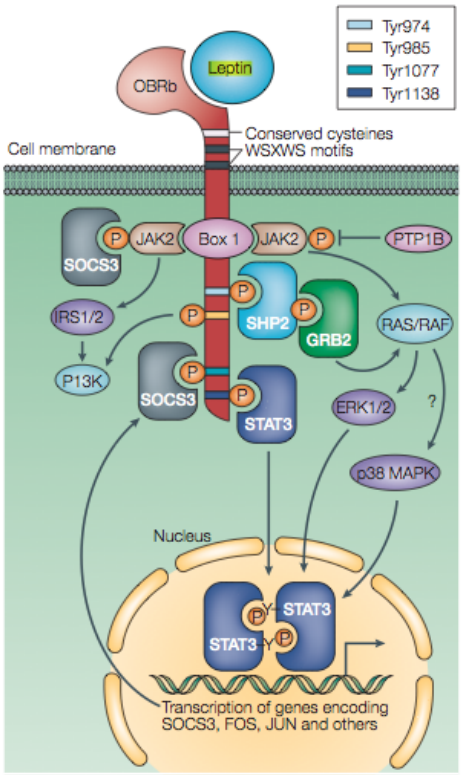

Figure 4: Leptin signalling [LaCAVA et MATARESE, 2004]

Tyr1138 functions as a docking site for STAT3. When STAT3 binds to that site it becomes phosphorylated by JAK2. After phosphorylation STATs dissociate into the cytoplasma and form dimers. In that form these dimers can translocate into the nucleus and induce the expression of the suppressor of cytokine signalling 3 (SOCS3) and other genes. SOCS3 binds to the phosphorylated tyrosines (Tyr 1077 in figure 4) and therewith blocks the leptin signalling. A second negative regulator of leptin signalling is phosphotyrosine phosphatase 1B (PTP1B). It dephosphorylates JAK2, which is then incapable of activation of the signalling pathway. PTP1B is actually a physiological insulin receptor phosphatase, also functioning as a negative regulator of insulin signalling, which shows the proximity of the pathways [LA CAVA and MATARESE 2004; BJORBAEK and KAHN 2004].

Tyr985 and Tyr974 recruit SH2 domain-containing phosphatase 2 (SHP2). This phosphorylates the growth factor receptor-bound protein 2 (GRB2), which is then able to activate extracellular signal-related kinase 1/2 (ERK 1/2) and p38 MAPK cascade over Ras/Raf. In the end of the signalling, the expression of Fos and Jun is induced [LA CAVA and MATARESE 2004; BJORBAEK and KAHN 2004; FRÜHBECK 2006]

JAK2 induces the phosphorylation of IRS 1/2 proteins that activate the PI3K pathway [LaCAVA et MATARESE 2004; LÖFFLER 2005]. As IRS is also used by insulin signalling, it represents the point of crosstalk between leptin and insulin.

As already mentioned the AMPK pathway is explained in more detail in the adiponectin signalling part. The only thing to be mentioned is that leptin decreases AMPK activity in the hypothalamus, which leads to reduced appetite and increased peripheral fatty acid consumption. Negative energy balance and reduction in body weight are the results [LIM et al. 2010].

3.4. Adiponectin

The adipocytokine adiponectin is also called GBP-28 (gelatin binding protein of 28kDa), apM1 (adipose most abundant gene transcript 1), AdipoQ or Acrp30 (adipocyte complement-related protein of 30 kDa) [KERSHAW and FLIER 2004]. It is encoded by the ADIPOQ gene, which is located on chromosome 3 (3q27) [NCBI Entrez Gene, 7.6.2010]. Since it has been characterized in 1995/1996, adiponectin has been in the focus of interest as it has shown possible therapeutic effects for metabolic disorders [YOON et al. 2006].

It is an approximately 30 kDa polypeptide, made of 244 amino acids, that has a N-terminal signal sequence, a variable domain, a collagen-like domain and a C-terminal globular domain [KERSHAW and FLIER 2004]. In contrast to leptin it is specifically and highly expressed in human adipose cells. Adiponectin belongs to the soluble defence collagen superfamily, as it has structural homology to collagen VIII and X as well as complement factor 1q [DIEZ and IGLESIAS 2003]. Existing in a wide range of multimer complexes in plasma, it combines itself via its collagen domain to create 4 major oligomeric forms. It appears as a trimer (globular form), a trimer with a low molecular weight (LMW), a hexamer with medium molecular weight (MMW) and a 12 to 18-mer high molecular weight form (HMW) [KADOWAKI et al.

2006]. With levels of 5-30 µg/ml it is abundant in human plasma [DIEZ and IGLESIAS 2003].

An exact physiological role for adiponectin has not yet been established but hypoadiponectinaemia has been associated with MetS more than any other inflammatory marker [KADOWAKI et al. 2006]. In contrast to leptin, it has been shown that adiponectin is negatively regulated in obesity, T2DM and coronary artery disease, leading to the opinion that this protein has anti-atherogenic and insulin-sensitizing effects [DIEZ and IGLESIAS 2003]. Adiponectin is able to increase tissue fat oxidation. Through that it can reduce the amount of circulating fatty acids, which leads to increased insulin sensitivity. Furthermore it suppresses the cytokine production from macrophages and the expression of adhesion molecules in vascular endothelial cells, which results in the inhibition of the inflammatory process. Therefore it has been concluded that adiponectin also has anti-inflammatory effects [DIEZ and IGLESIAS 2003].

Although it is not known how estrogen and testosterone influence adiponectin levels it has been shown that they are, like leptin, gender-dependent. Studies have reported higher levels in women than in men [DIEZ and IGLESIAS 2003] which might make women more insulin sensitive than men and lower their risk for cardiovascular diseases. How adiponectin exactly works is not fully elucidated. It is known that it activates the AMPK pathway, which increases glucose uptake as well as fatty acid oxidation rates and that it stimulates the peroxisome proliferator activated receptor α (PPARα), which promotes lipid oxidation. These effects are dependent from relative circulating concentrations and different isoforms of adiponectin [KERSHAW and FLIER 2004], but also of its receptors [BEYLOT et al. 2006], which are explained closer in the next section.

3.5. Adiponectin receptor

Two transmembrane domain-containing proteins, which are encoded by genes on chromosome 1 (1q32) and 12 (12p13) have been identified as adiponectin receptors and named AdipoR1 and AdipoR2. However, these two are not like the usually G-protein-coupled receptors (GPCR), as they are structurally and functionally distinct from them. AdipoR have the N-terminus within the cell. Therefore adiponectin has to interact with the C-terminus, which is opposite to the normal GPCRs [MAO et al. 2006]. Another argument showing that these receptors are not GPCRs is that overexpression of AdipoR1 and AdipoR2 has little effect on cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP) and intracellular calcium levels [KADOWAKI et al. 2006].

AdipoR1 is primarily expressed in skeletal muscle and functions there as a high-affinity receptor for globular adiponectin and as a low-affinity receptor for full-length HMW-adiponectin. AdipoR2, in contrast, is primarily expressed in liver where it functions as an intermediate-affinity receptor for both, globular and full-length HMW adiponectin [TAKEUCHI et al. 2007]. Both of them activate sets of signalling molecules such as PPARα, AMPK and p38 MAPK, glucose uptake and fatty oxidation [KADOWAKI et al. 2006].

As a third receptor T-cadherin, which belongs to the cadherin (calcium dependant adhesion molecules) superfamily, has been characterized. It is a unique member of that group as it lacks the transmembrane and the cytoplasmic domain. T-cadherin is only anchored to the surface membrane via a glycosyl phosphatidyl inositol part. As it has no intracellular domain, it is thought that it has no effect on adiponectin cellular signalling. Nevertheless it is able to bind it [KADOWAKI et al. 2006] – its hexamer and HMW isoforms in particular [HUG et al. 2004].

3.6. Adiponectin signalling

Adiponectin signalling differs substantially from the biochemical pathway of leptin because the constitutions of the receptors are unequal. As mentioned above, it is not known if T-cadherin has any signalling effects and therefore it is not explained any further in this thesis. For the other two receptors (AdipoR1 and AdipoR2) various pathways are suggested and explained (see figure 5).

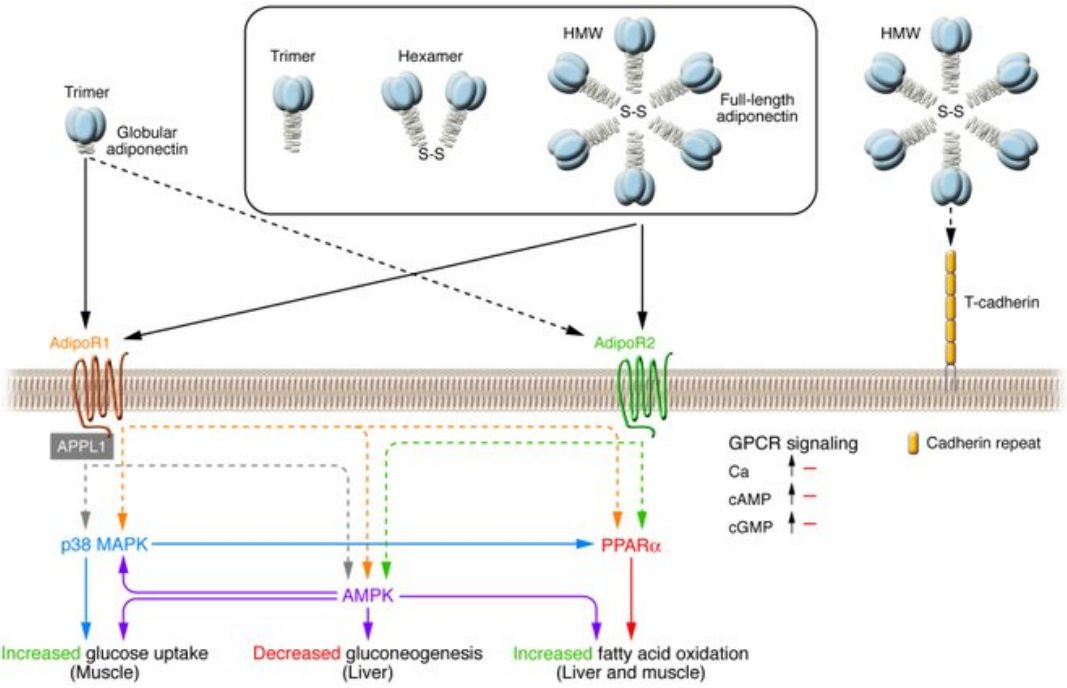

Figure 5: Structural forms of adiponectin and their ways of signalling [KADOWAKI et al. 2006]

Adiponectin is considered a starvation hormone. During fasting conditions high levels of adiponectin are present, which stimulate central and peripheral AMPK, leading to increased food intake and decreased energy expenditure [LIM et al. 2010]. When adiponectin binds to the extracellular C-terminus of the receptor, APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding domain and leucin zipper motif) is recruited to the intracellular N-terminus [MAO et al. 2006]. It is not known how APPL1 activates AMPK, p38 MAPK and Rab5, but it does [DEEPA and DONG 2009]. In this chapter only the AMPK is explained.

If AMPK is activated, adenosine triphosphate (ATP)-consuming processes are shut down for energy saving and catabolic processes are activated to generate ATP [DEEPA and DONG 2009]. In general it can be said that AMPK plays a key role as regulator of cellular energy homeostasis (see Figure 6). It has an influence in the carbohydrate, lipid and protein metabolism and therefore defects in the signalling pathway might play a big role in the MetS. E.g AMPK inactivates 3-hydroxy-3-methlygluataryl (HMG) CoA reductase as well as acetyl CoA carboxylase (ACC), which are the key enzymes of fatty acid and cholesterol synthesis [LIM et al. 2010]. A decrease in ACC activity reduces intracellular malonyl-CoA levels and stimulates carnitin palmitoyl transferase 1 (CPT1), which increases the influx of long chain fatty acids into the mitochondria, where they are oxidized [YOON et al. 2006].

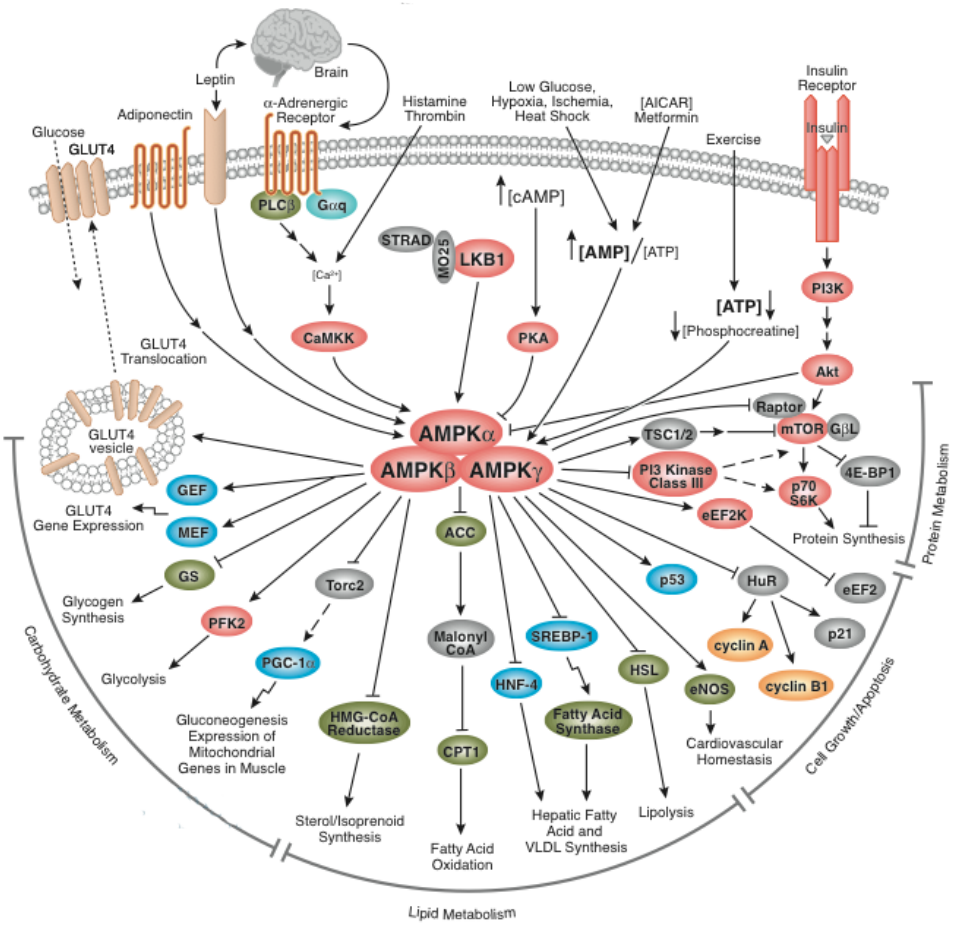

Figure 6: AMPK signalling. [Cell Signaling Technology 2010]

It is clear that little changes in the signalling pathways as well as defects on genes encoding every single parameter might play a significant role in the development of the diseases. Searching for single changes in the DNA that have an effect on the functioning of leptin and adiponectin is the main topic of this thesis. How researchers find these polymorphisms is explained in the following chapter.

4. Genome-wide association studies

4.1. Definition and advantages

“Genome wide association studies (GWAS) use high throughput genotyping technologies to assay hundreds of thousands of single nucleotide polymorphisms (SNPs) and relate them to clinical conditions and measurable traits” [PEARSON and MANOLIO 2008].

Candidate gene association studies and linkage analysis have been the past methods of genetic studies. In contrast to GWAS they are more restricted. For candidate gene association studies it was a precondition to have an idea which genes could have an effect on e.g. the disease development. Linkage studies, on the other hand, had the advantage that genes could be identified without known functional connection to disease. However, they lack statistical power to detect quantitative trait loci (QTL). Furthermore they were performed in families, which means that unrelated individuals could not be compared. GWAS, in contrast, can be performed in unrelated individuals and there is also no need to know the biological function in advance [FARBER and ROSEN 2010].

Also in the case of non-Mendelian conditions, GWAS aroused to be better than family based linkage studies. GWAS have higher power for complex disorders that are influenced by multiple genes and the large size of the chromosomal regions shared between family members [PEARSON and MANOLIO 2008]. When GWAS result in novel loci, the “candidate gene” approach is generally chosen to search for and validate the causal variant [HENNEMAN 2010].

4.2. History

Until 2005 a few “early versions” of GWAS have been performed and published. A study by OZAKI et al., which was accomplished in 2002, is considered to be the first large genome-wide study. Nevertheless, a GWAS by KLEIN et al. (2005), showing that age related macular degeneration associates with variation in the gene for complement factor H, is registered as the first study in the National Human Genome Research Institute (NHGRI) Catalogue of Published Genome-Wide Association Studies [HINDORFF et al. 2010]. The reason therefore is that KLEIN et al. (2005) were the first ones to use microarray technologies instead of high throughput multiplex PCR-Invader assay methods. And they also used random selection of SNPs, covering genes and intergenic regions instead of only focusing on gene-based SNPs that were randomly selected from a gene-based SNP discovery study [KU et al. 2010].

The breakthrough for GWAS was the completion of the International HapMap project (phase I in 2005 and phase II in 2007). That project was a multi-country effort to validate millions of SNPs, to characterize their correlation or linkage disequilibrium patterns in populations of European, Asian and African ancestry and to establish a catalogue of common genetic variants that appear in human beings. This catalogue describes “what these variants are, where they occur in our DNA and how they are distributed among people, within populations and among populations in different parts of the world”. [International HapMap Project 2010]. After the publishing of the HapMap project the number of performed GWAS exploded and a huge amount of data, which has to be dealt with, has been generated ever since. GWAS represent a powerful new tool for identification of genes that are influencing common diseases but also lead to new terminologies. Most important ones are explained below as well as the statistical terms that are used.

4.3. GWAS basics

4.3.1. Single nucleotide polymorphisms

The most important abbreviation in GWAS is SNP, which stands for single nucleotide polymorphism. Every time the DNA replicates itself it makes a couple of mistakes, which are classified into three different groups: bad mistakes, good mistakes and indifferent mistakes [BRODY 2007]. Although 99.9% of the mistakes have no effect (indifferent mistakes) there is this 0.1% part that, nevertheless, represents millions of mistakes among 3.2 billion base pairs that have an effect and are looked at accurately, because they might be the cause of disease or health [KRUGLYAK and DICKERSON 2001].

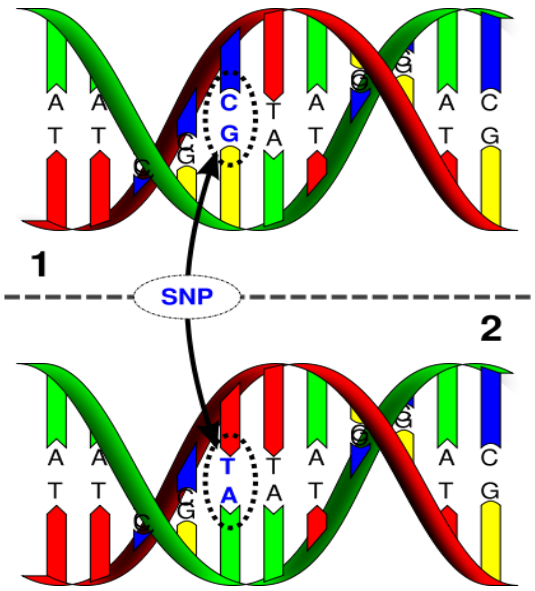

Figure 7: Single nucleotide polymorphism [SCIENCE MARSHALL 2010]

Single Nucleotide Polymorphisms are the most common “mistakes” in the human genome [RAMENSKY et al. 2002]. To be put into that category the variation has to occur in, at least, 1% of the population and has to be stable - otherwise it is called a mutation [KRUGLYAK and DICKERSON 2001].

SNPs occur when the genome sequence is altered. As mentioned above the biggest portion of the bases match but from time to time there are variants where a single nucleotide (A, T, C or G) in the genome sequence is altered and the DNA sequence is changed e.g. from CTA to TTA (see Figure 7). These variations happen due to replication error and imperfect DNA repair. They appear approximately in 1 out of 1000 bases. That leads to the estimation that 20 to 30 millions of different variable sites exist in the population but not all of them are common [BRODY 2007].

Generally SNPs can be divided into two groups – the non-synonymous and the synonymous SNPs. Synonymous SNPs have an altered nucleotide but still encode the same amino acid. E.g. the sequences GGG and GGC both encode the amino acid glycin. But if a polymorphism results in a change in the amino acid sequence of a protein and therefore changes its function, it is called a nonsynonymous SNP [PEARSON and MANOLIO 2008]. If there is a non-synonymous SNP, the impact on the phenotype is much higher than of synonymous ones [RAMENSKY et al. 2002].

As there are huge numbers of SNPs it was needed to develop a database to keep them organised. Almost 12 million human SNPs were assigned a reference SNP (rs) number in the dbSNP database of the National Center for Biotechnology Information and characterized as to specific alleles (alternate forms of SNPs), summary allele frequencies and other genomic information [PEARSON and MANOLIO 2008]. In the results chapter the rs numbers are used when they are known. If not, it was referred to the old descriptions where instead of the number (rs7799039) the correspondent marker is used (G2548A).

4.3.2. Linkage disequilibrium

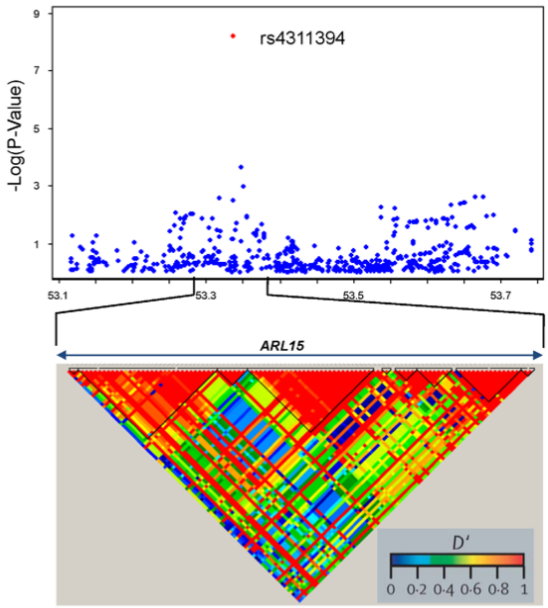

Figure 8: Linkage disequilibrium plot of the ARL15 gene [RICHARDS et al. 2009]

Linkage disequilibrium is defined as "association between two alleles located near each other on a chromosome, such that they are inherited together more frequently than expected by chance" [PEARSON and MANOLIO 2008]. This means that variations are inherited in groups, which is very important for GWAS (especially in terms of time and costs) because it significantly reduces the number of SNPs that need to be genotyped [KU

et al. 2010]. Consequently one SNP can be used as a proxy for others, which is the concept of tag SNPs [MANOLIO 2007].

R^2 or D´ express the correlation between SNPs. Both represent the proportion of variation of one SNP explained by the other. The results range from 0 (no association, which are the blue areas in figure 8) to 1 (perfect correlation, which are the red areas in figure 8) [PEARSON and MANOLIO 2008]. To simplify the display, association statistics are typically shown as the *–log_{10} of the p-value* of each SNP versus its chromosomal location, also called the "Manhattan" plot (upper part of figure 8).

The degree of linkage disequilibrium can vary between populations. The further the SNPs are separated the less certain the relationships between them becomes. The distance can extend from hundreds to thousands of bases [BRODY 2007]. The blocks of identical genome sequences are shorter the less closely people are related. To say it the other way round: the blocks are long in identical twins where, in fact, the entire genome is the same initially. In siblings, parents and offspring the blocks are about 10 millions base pairs. GWA technology allows studies in unrelated people assuming that blocks of about 10000 base pair lengths are common [BRODY 2007].

4.3.3. Statistical terms

Statistical knowledge is crucial to analyze GWAS. The most important terms are explained in this subchapter. The first to mention is the *p-value*, which stands for the probability of finding a result by chance alone. In the case of GWAS the conventional level of significance ($p<0.05$) would show that 50 000 out of 1 million SNPs are associated with the disease, almost all falsely positive. To solve this problem the *Bonferroni correction* is applied. There the conventional p-value is divided by the tests performed. In the case of 1 million SNPs a p-value of 10^{-8} ($P=0.05/10^6$) is needed to identify associations that are unlikely to be due to chance alone [PEARSON and MANOLIO 2008]. As the number of tested SNPs varies the p-values also alter. Recent studies just use probability values between 10^{-4} and 10^{-7} to minimize false association. The Bonferroni correction has been criticized because it

assumes that each SNP is independently associated with a disease but it is well known that some SNPs are in linkage disequilibrium [DING and KULLO 2009].

Another measurement for linkage, which has nothing to do with LD, is the *LOD score*. LOD (logarithm of odds) estimates whether two loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together as a package. A LOD score of three or more is generally taken to indicate that two gene loci are close to each other on the chromosome [DORAK 2010].

If there are results the *Odds ratio* is always calculated. It stands for the approximate relative risk and measures the magnitude of the association between the disease and the sought polymorphism. In the case of GWAS, odds ratios are typically modest, often in the range of 1.2 to 1.3. If the disease is rare the odds ratio approximates the risk but always overestimates the effect [MANOLIO 2007]. Usually at least 1000 cases and 1000 controls are needed to detect ORs > 1.5 with at least 80% power. For ORs higher than 1.5 much larger sample sizes are needed.

Hardy Weinberg equilibrium is a concept of stability of gene proportions in an ideal population. It explains the distribution of 2 alleles (with frequencies p and q) and their stable distribution from generation to generation as well as their genotypes that occur at frequencies of p2, 2pq and q2 for the major allele homozygote, heterozygote and minor allele homozygote respectively [HARDY 1908]. Although there is no ideal population, this concept is still practically used to detect genotyping errors.

Replication studies are done as a test to exclude false-positive associations, but in GWAS, they often lead to failure of replication of the initial results. What also happens is the appearance of so-called flip-flop results. This means that an allele, initially protective, happens to be the risk allele in the replication study. Therefore all detected results have to be handled with care.

4.4. Genotyping platforms

As mentioned shortly earlier in this thesis, GWAS use microarray technology for SNP detection. But the two commonly used brands, Affymetrix and Illumina, use different ways of approach. Illumina uses probes that are based on haplotype- tagging SNPs, which were identified in the HapMap project. Affymetrix arrays, in contrast, are for random SNPs that are chosen to cover the genome, supplemented by tagSNPs. The amount of tested SNPs is around 1 million. The Affymetrix‘s Genome Wide Human SNP Array 6.0 e.g. contains >906 000 SNPs [Affymetrix.com, Illumina.com].

The core of microarray technology is the preferential binding of complementary single stranded nucleic acid sequences. DNA microarrays are glass or silicone chips that consist of thousands of arrayed spots of specific DNA sequences, which are called probes. On these chips the target has to be applied.

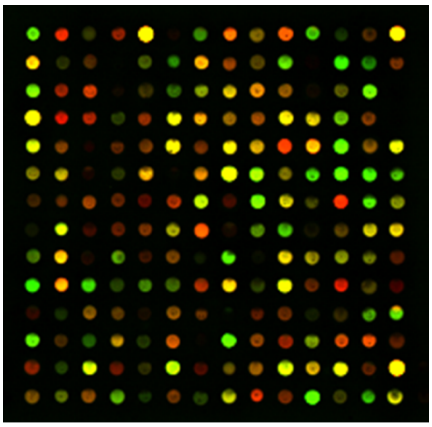

Figure 9: Microarray picture. [ANDERSON 2010]

For easier comparison the way of functioning is explained in comparing diseased and healthy subjects. The first step is extracting the mRNA out of the cells of both populations, as it gives information about which genes are frequently read. With the help of mRNA the complimentary DNA (cDNA) can be reconstructed. In this case the healthy people are coloured green, the diseased ones red. Subsequently, the red and the green cDNA have to be mixed and applied on the platforms. As mentioned before every single spot on the chip is made of DNA and is able to build pairs with the cDNA, if they are complementary. This binding of probes and targets is called hybridization. If the two strands are absolutely complementary they will bind to each other readily. But if there are single inconsistencies they will make the binding less favourable or not bind at all. So on the chip there are spots that bind only the green cDNA, some only the red one, but some bind both of them [CAMPBELL 2001].

Next step is washing the chip to remove all the cDNA that has not found a complementary strand and then to analyse it. The chip is scanned by a green and red laser, which reveals two individual pictures that are combined later on. The spots where the green cDNA has found her docking station are glowing in a green light and the landing places for the red cDNA are glowing in a red light. Spots that bound red and green cDNA appear as yellow dots (see figure 9) [CAMPBELL 2001]. From the combined picture it can be seen which genes are more expressed in diseased people or in the case of GWAS, which SNPs appear in unhealthy people but do not in healthy subjects. These are then tested for causation of the disease and/or to which part they play a role.

5. Literature research results

The search for significant GWAS results was conducted by searching in the HuGe Navigator GWAS interrogator. This is a database that includes all the GWAS that have tested more than 100.000 SNPs in the initial stage and found SNPs with a p-value < $5x10^{-5}$. To fine-tune the query results a filter was used. In that way it was possible to limit the GWAS to the wanted gene. Disadvantages of this searching machine are the cut-off points. Not every GWAS tested more than 100.000 SNPs and therefore not every study needed a p-value < $5x10^{-5}$ to acquire significant results. So those studies are excluded in the Interrogator. SNPs that were probably close to the significance level are therefore not included although they might be important. To look for these "lost SNPs" PubMed was used as the main searching machine usually with specific catchwords, which are mentioned when used. Interesting SNPs, revealed from the studies, are looked one by one to search for further results e.g. through the NCBI Entrez SNP site or again PubMed. Common polymorphisms, reported not only by GWAS but also from different kind of studies were included as they show what has already been done and what results have been obtained until now. If studies that were used by researchers had a "short" name e.g. HYPEST (HYPertension ESTonia), it has been written that way. The meaning of the shortenings can be looked up in the list of abbreviations. Other used databases were Scopus and ScienceDirect but in most cases gave the same or less results than PubMed. The only database that gave the same or more results, but was to be treated with care, was Google Scholar.

Generally an attempt was conducted to get full access to all the studies. As many of them are new (from 2009/2010), and were not even printed but published online, authors were asked for full access. Where it was not allowed abstracts were used. The same is valid for older studies, where full access was not granted. It was attempted to provide a complete overview of the topic but this thesis makes no claim to be complete.

5.1. LEP

5.1.1. LEP – GWAS results

The search for leptin polymorphisms with the GWAS interrogator revealed no hits, which means that none of the performed studies had tested more than 100.000 SNPs and/or got significant results at a p-value level of $<5x10^{-5}$ so far.

Searching PubMed for further GWAS results with the keywords “genome-wide association” and “LEP” yielded only one hit - a study searching for associations between polymorphisms and hypertension. It has been performed by SOBER et al. (2009) with the aim to see how well previously reported SNPs are represented on the arrays, as well as to find the polymorphism with the strongest signal for association. They included 5467 participants from three different studies (KORA n=1644, HYPEST n=1823 and BRIGHT n=2000) in which they analyzed SNPs from 160 genes that were connected to hypertension earlier on, for their association with blood pressure. Rs10954174 on the LEP gene has been associated with diastolic BP at a p-value level of $5.20x10^{-5}$ (see Table 2), which makes it not being significant for the GWAS Interrogator but coming quite close to the cut-off point. The search for further results for that particular SNP revealed no hits in other studies.

5.1.2. LEP - Further results

As there are no other GWAS results, outcomes from studies with other designs have been searched for too. Generally it has to be pointed out that these studies have declared their results as significant when reaching a p-value<0.05. As only a few SNPs and/or smaller groups of people have been investigated, the general level of statistical significance may be enough.

To continue the results from hypertension, combinations of the catchwords “leptin”, “polymorphism”, ”association” and “hypertension” have been used to find studies dealing with that topic. MA et al. (2009) have conducted a study

searching for polymorphisms that are associated with SBP, DBP and plasma leptin levels. 29 SNPs within and around LEP were selected, 20 within the gene itself. 6 SNPs (rs13245201, rs7799039, rs6467166, rs12536535, rs10244329 and rs11763517) have shown associations with blood pressure/ hypertension but only in postmenopausal women. The search for more results on rs13245201 has shown no further hits, whereas rs7799039 has been mentioned by other studies (see below and Table 2). Rs6467166 has shown again no hits as well as rs12536535, rs10244329 and rs11763517.

GANELHU et al. (2009) analyzed whether the rs7799039 polymorphism is associated with hypertension in 140 obese Brazilian women. Their findings have shown that the variant is linked to higher blood pressure levels and therefore might be an important mediator of hypertension in obese women. The same SNP has also been analyzed by BEN ALI et al. (2008) who have conducted a study where 229 Tunisian obese subjects were compared to 251 Tunisian normal weight subjects with the aim to look for association with hypertension. As a result they have shown that rs7799039 is associated with blood pressure, but only in obese male patients.

Studies dealing with associations of the LEP gene polymorphisms and obesity itself have been found in PubMed through the combination of catchwords “leptin”, “polymorphism”, ”association” and “obesity”. JIANG et al. (2004) have carried out a study looking for polymorphisms associated with BMI. They have genotyped 29 SNPs within (20 SNPs) and around (9 SNPs) the LEP gene. Out of the 20 SNPs within the gene, 18 were associated with the BMI (see Table 2) at a level of $p<0.05$, but merely in men. Only one SNP, rs2167270, has reached a p-value of 0.049 in women. The search for other studies dealing with one or more of the named SNPs has revealed positive hits for a couple of polymorphisms. However they are not mentioned here as they deal with cancer or spina bifida. Rs7799039 and rs2167270, in contrast, have been associated with various MetS features in numerous studies and therefore a couple of them are presented subsequently.

One of those studies has been carried out by CONSTANTIN et al. (2010). They have analyzed 202 subjects (obese and non-obese) for linkage of

rs7799039 to obesity. In contrast to JIANG et al. (2004), the LEP polymorphism has shown no significant associations. FURUSAWA et al. (2010) have also analyzed if there is a connection between the same polymorphism and obesity. But the SNP has not been associated with neither BMI nor obesity.

Studies dealing with insulin resistance have been found with the catchwords “leptin”, “polymorphism”, ”association” and “insulin resistance”/”T2D”. Most of them were excluded because they were dealing with the receptor instead but were included in the LEPR results chapter later on. The one study indeed dealing with LEP has been carried out by HAN et al. (2008). In 752 Korean women, rs28936687 and 4998A>C have been identified as marginally associated with T2D. Similar results were achieved when looking for associations of LEP with inflammation. Generally, all results were considering the LEPR gene polymorphisms and therefore were excluded in this part.

The search for the catchwords “leptin”, “polymorphism”, ”association” and “lipid” has also revealed only few fitting results. The use of “HDL”, “LDL” or “lipoproteins” as substitutes for “lipid” did not change the outcome significantly. One of the studies that appeared was conducted by OKADA et al. (2010). They have performed a research looking for association of rs2167270, rs7799039, 188C>A, 633C>A and serum lipids in Japanese obese children. But none of the polymorphisms has shown any significant relationships.

The search for polymorphisms that are causing changes in the leptin levels are usually variations from the LEPR gene and are therefore shown afterwards. The LEP gene was only mentioned by MA et al. (2009) but they have found no significant associations between LEP and plasma leptin levels when looking at them. Only rs28954369 and rs2060715, which is not located in LEP gene but in an intergenic region, came close to the level.

SNP	Other name	Authors	Associated with
rs10954174		SOBER et al. 2009	Diastolic BP
rs13245201		MA et al. 2009	BP in post-menopausal women
rs7799039	G2548A	MA et al. 2009 GANELHU et al. 2009 BEN ALI et al. 2008 JIANG et al. 2004 CONSTANTIN et al. 2010 FURUSAWA et al. 2010 OKADA et al. 2010	BP in post-menopausal women BP in obese women BP in obese men BMI in men No association with obesity No association with obesity or BMI No association with serum lipids
rs6467166		MA et al. JIANG et al. 2004	BP in post-menopausal women BMI in men
rs12536535		MA et al. JIANG et al. 2004	BP in post-menopausal women BMI in men
rs10244329		MA et al. JIANG et al. 2004	BP in post-menopausal women BMI in men
rs11763517		MA et al.	BP in post-menopausal women
rs28954369		MA et al. 2009	Tendency to accociation to leptin levels
rs10249476		JIANG et al. 2004	BMI in men
rs1349419		JIANG et al. 2004	BMI in men
H1328083		JIANG et al. 2004	BMI in men
rs12535708		JIANG et al. 2004	BMI in men
rs11770725		JIANG et al. 2004	BMI in men
rs12535747		JIANG et al. 2004	BMI in men
rs13228377	G1387A	JIANG et al. 2004	BMI in men
rs2167270	A19G	JIANG et al. 2004 OKADA et al. 2010	BMI in men No association to serum lipids
rs2278815		JIANG et al. 2004	BMI in men
H1432616		JIANG et al. 2004	BMI in men
H1432615		JIANG et al. 2004	BMI in men
rs11763517		JIANG et al. 2004	BMI in men
rs11760956		JIANG et al. 2004	BMI in men
rs10954173		JIANG et al. 2004	BMI in men
rs28936687		HAN et al. 2008	T2D
	4998A>C	HAN et al. 2008	T2D
	1887C>A	OKADA et al. 2010	No association with serum lipids
	633C>A	OKADA et al. 2010	No association with serum lipids

Table 2: Overview of all mentioned LEP polymorphisms and their associations

Polymorphisms that have an impact on the leptin level but are not located on the LEP or LEPR gene have also been reported. DO et al. (2008) have conducted a GWAS within the WTCCC considering 908 individuals who feature an association of rs17817449, which is located on the FTO gene, with plasma leptin levels at the $p<0.05$ level ($p=0.036$). Additional genes containing polymorphisms that have been associated with leptin levels and MetS features are located e.g. on the tumor necrosis factor α receptor gene

(TNF-α-R2) (A3 allele) [FERNANDEZ-REAL et al. 2000], on the β-3-adrenergic receptor gene (Trp64Arg) [LIN et al.1999] or on the peroxisome proliferator-activated receptor gene (PPAR) (C/T exon 6) [MEIRHAGHE 1998].

Genes that do not have an influence on leptin levels but an effect on MetS by modulating the leptin signalling pathways have also been included into the search. A gene that is thought to play a role in the regulation of body weight and fat through leptin is the SH2B gene. A study by JAMSHIDI et al. (2007) in 2455 white female twins in the UK (Twins United Kingdom) has revealed that the tagging SNP rs7498665, which represents 5 common SNPs in perfect LD (r^2 = 1), has shown an association when it was linked to body fat and serum leptin levels (p-value = 0.04 in both). A further study has been carried out by OLIVIER et al. (2004). They have reported that 54281T>A on the PTPN1 (Protein-tyrosine phoshatase, non receptor type 1) is associated with hypertension (p=0.02).

Obesity e.g. has been associatied to polymophisms on the SOCS3 gene by TALBERT et al. (2009). They have conducted a study in 1425 Hispanics investigating the associations of the SNPs in the SOCS3 gene, which is a leptin signalling inhibitor, with BMI, visceral adipose tissue and WC. Rs9914220 has been associated to all three features (p-values from 0.003 to 0.017). JAK2 variants have been linked to body fat but also to insulin sensitivity and lipid profile. As explained in the leptin signalling subchapter, JAK2 is crucial for the functioning of leptin, which might explain the effects. Two polymorphisms located on it have been related to MetS features. Rs7849191 has been associated with body fat and WC (p=0.03 and 0.027) while rs3780378 to total cholesterol, LDL cholesterol and triglycerides (p=0.014, 0.012 and 0.023) [GE et al. 2008].

SNP	Other name	Gene	Authors	Associated with:
rs17817449		FTO	DO et al. 2008	Leptin levels
	A3 allele	TNFalphaR2	FERNANDEZ-REAL et al. 2000	Leptin levels, insulin resistance
	Trp64Arg	Beta-3- adren. Recep.	LIN et al. 1999	Leptin levels
	C/T exon 6	PPAR	MEIRHAGHE et al. 1998	Leptin levels
rs7498665		SH2B	JAMSHIDI et al. 2007	Body weight
	54281T>A	PTPN1	OLIVIER et al. 2004	Hypertension
rs9914220		SOCS3	TALBERT et al. 2009	BMI, visceral adipose tissue, WC
rs7849191		JAK2	GE et al. 2008	Body fat and WC
rs3780378		JAK2	GE et al. 2008	Total cholesterol, LDL cholesterol, triglycerides

Table 3: Overview of all mentioned polymorphisms associated with leptin levels or MetS features not located on the LEP gene

5.1.3. LEP - Discussion

In general it can be said that polymorphisms on the LEP gene do not play a big role in the development of MetS, as the results are not as fruitful as expected. Only rs7799039 has shown some promising results. Associations with BP and BMI have been reported but then again these findings have been disproved by other studies. Reasons for these outcomes might be diverse.

Most of these studies had not many subjects for investigation. Inclusion criteria e.g. for insulin resistance are often not mentioned. But it is also possible that variations in the LEP gene just do not play a role. It has been observed earlier that leptin levels are high in obese people leading to the conclusion that defects of function are due to the leptin receptor and not to a lack of leptin. Leptin deficiencies that lead to morbid obesity are very rare [FAROOQI and O`RAHILLY 2009] and cannot be accounted for general development of MetS.

Outcomes showing that some polymorphisms are sex-specific indicate that endogenous hormones might be important, too. Testosterone and oestrogen might play a crucial role. These data are supported by MARTIN et al. (2002). They concluded that it is not only due to the different fat amounts in men and women but also due to the different expression of genes in the sexes. It has

been shown that testosterone plays a role in modulating leptin levels. A suppression results in an increase in leptin [ELBERS et al. 1997]. But whether the modulation effects of these hormones on leptin levels can explain the differences between prevalence of e.g. hypertension in men and women remains to be investigated.

Another possibility of failure of detection might be due to underrepresentation of the SNPs on the arrays. With time these platforms contain more and more SNPs but maybe some important polymorphisms are still missing.

It is also supposable that polymorphism on the LEP gene might be associated with LEPR SNPs and that only in combination they are causing MetS.

5.2. LEPR

5.2.1. LEPR - GWAS results

The search for significant LEPR results in GWAS revealed substantial hits. The Interrogator revealed 4 studies, which reached significant results under the needed criteria of 100.000 genotyped SNPs and a p-value of $5x10^{-5}$. These findings are summarized in Table 4 for easier overview.

One of the studies has been conducted by SUN et al. (2010), aiming to discover if there are polymorphisms in the LEPR gene that function as determinants of the plasma soluble leptin receptor. They have analyzed 1504 women from the Nurses‘ Health Study and detected 26 SNPs that are significantly associated with LEPRe (p-value < $5x10^{-8}$) all mapping to the LEPR gene. Additional 106 SNPs have been included because of analysis of imputed genotypes on autosomal chromosomes. Of the total 132 SNPs three SNPs remained associated with LEPRe at the 0.05 level: rs2767485 (p=9.1 x 10^{-9}), rs1751492 (p=0.0105), rs4655555 (p=0.0267). These results have been replicated in a sample of young males (n=875) residing in Cyprus. As full access to the study was not allowed, p-values of the replication study were not available. This is important to know as the GWAS interrogator

revealed rs1751492 as the only SNP reaching the GWAS significance level with a p-value of $6x10^{-13}$. Further search for the mentioned SNPs has revealed no more hits.

The other three studies with significant results have shown associations with CRP levels, which points out the linkage of leptin and inflammation. RIDKER et al. (2008) have evaluated 336.108 SNPs in 6345 women from the Women‘s Genome Health Study. The initial group contained 4418 study participants and then promising results were replicated in a second group of 1927 participants. 46 SNPs have been associated with CRP at a genome wide level of significance ($p=5x10^{-8}$). Nine of these SNPs (rs1892534 $p=6.5x10^{-21}$, rs2889195 $p=2.78 \times 10^{-20}$, rs2211651 $p=3.10 \times 10^{-20}$, rs12753193 $p=3.27x10^{-17}$, rs2186245 $p=1.27x10^{-13}$, rs12022410 $p=7.47x10^{-13}$, rs7539471 $p=1.87x10^{-10}$, rs4291477 $p=7.65x10^{-10}$ and rs4655537 $p=3.28x10^{-9}$) are clustered in the LEPR gene. RIDKER et al. (2008) have also reported that 1.6% of the CRP level variation has been due to the rs1892534 polymorphism, which is also the only SNP mentioned in the GWAS interrogator. Additional search for the individual SNPs has revealed hits for rs1892534. These studies have not been included though because of dealing with irrelevant topics. The other polymorphisms showed either no results or again no results that were adequate.

The second study has been conducted by ELLIOTT et al. (2009) with the aim to find genetic loci that are influencing CRP levels and are causing the risk of CHD. 17977 participants from five studies (LOLIPOP, NFBC, CoLaus, GEMS and DESIR) were analyzed and an additional 14747 LOLIPOP participants (not included in the initial GWAS) were used for the replication study. ELLIOTT et al. (2009) have found 160 SNPs in five different loci that have been associated with the CRP levels at a significance level of $p<5x10^{-8}$. One of those SNPs was on the LEPR gene locus. This polymorphism, rs6700896, has been shown to be strongly associated with reduced CRP levels ($p= 1.6x10^{-21}$) and increased CHD (coronary heart disease) risk. These have been completely new findings suggesting that CRP does not mediate CHD.

The third study and the last with significant results has been carried out by SABATTI et al. (2008). 329.091 SNPs were analyzed in 4763 individuals from the NFBC1966 for association in 9 metabolic traits (BMI, TG, HDL, LDL, glucose, insulin, CRP, systolic BP and diastolic BP). In the main analysis 21 SNPs have shown an association with a p-value $<5x10^{-7}$. Rs12753193, one of those SNPs, is located on LEPR and has been linked to C-reactive protein levels with a p-value of 3.76×10^{-7}.

GWAS that have been conducted but did not reach the significance level of $5x10^{-5}$ and/or did not test 100.000 SNP as well as other types of studies have been looked for in PubMed. The catchwords “genome-wide association” and LEPR revealed, next to already above described studies, the study by SOBER et al. (2009) which was explained closer in the LEP GWAS results part. Next to LEP polymorphisms, this study group also analyzed 2 LEPR SNPs (rs10889553 and rs17097182). Although none of the p-values remained significant after the Bonferroni correction ($p<0.05/2319$, $p=2.15x10^{-5}$), the association between rs10889553 and systolic BP ($p=4.5x10^{-5}$) was close to the cut-off point. The search for other studies concerning LEPR polymorphisms and hypertension revealed no further GWAS hits but results from studies with other design.

SNP	Other name	Authors	Associatied with:
rs2767485		SUN et al. 2010	Plasma soluble leptin receptor
rs1751492		SUN et al. 2010	Plasma soluble leptin receptor
rs4655555		SUN et al. 2010	Plasma soluble leptin receptor
rs1892534		RIDKER et al. 2008	CRP levels
rs2889195		RIDKER et al. 2008	CRP levels
rs2211651		RIDKER et al. 2008	CRP levels
rs12753193		RIDKER et al. 2008 SABATTI et al. 2008	CRP levels CRP levels
rs2186245		RIDKER et al. 2008	CRP levels
rs12022410		RIDKER et al. 2008	CRP levels
rs7539471		RIDKER et al. 2008	CRP levels
rs4291477		RIDKER et al. 2008	CRP levels
rs4655537		RIDKER et al. 2008	CRP levels
rs6700896		ELLIOTT et al. 2009	reduced CRP levels, increased CHD risk
rs10889553		SOBER et al. 2009	systolic BP
rs17097182		SOBER et al. 2009	systolic BP

Table 4: Overview of all mentioned LEPR polymoprhisms from GWAS and their associations

5.2.2. LEPR - Further results

To continue with the significant CRP results, like those from the GWAS, a study by ZHANG et al. (2007) is presented. This research group has namely identified rs3790432 and rs1805096 in 630 Caucasians as being associated with fibrinogen and CRP – both markers for inflammation. Further results for these SNPs were only interesting for rs1805096. An appearing study, by PHILLIPS et al. (2010), is mentioned in the dyslipidemia part also, as it additionally reports rs8179183, too.

It has to be emphasized that there are four common polymorphisms on the LEPR gene - rs1137100 (Lys109Arg), rs1137101 (Gln223Arg), rs790419 (Ser343Ser) and rs8179183 (Lys656Asn) - which have been in the focus of researchers. They are mentioned here because they are used in most of the studies like in the one that has been conducted by LINSHUAN et al. (2008). They have investigated the association of rs1137101 with hypertension in patients with IGT in 572 subjects (252 with normal glucose tolerance, 320 with IGT). The results have shown that the polymorphism is associated with hypertension but only in IGT male patients. Similar results have been shown by RUOWANG et al. (2008), who also demonstrated that rs1137101 might be the causation of hypertension in obese patients.

Studies concerning associations of LEPR with obesity have been found through the catchwords “leptin receptor”, “obesity”, “polymorphism” and “association”. MARTI et al. (2009) have carried out one of the detected studies. In their case-control study with obese subjects (n=159) and normal weight controls (n=154) they have genotyped the four common polymorphisms of the LERP gene (rs1137100, rs1137101, rs790419 and rs8179183). No significant case-control differences and no associations between the SNPs and obesity or serum leptin levels have been found. These findings are supported by CONSTANTIN et al. (2010) and their study, already mentioned in the LEP results. They have reported that in their 202 Romanian subjects rs1137101 might also not be considered as a genetic risk factor for obesity. PYRZAK et al. (2009) have shown also that in their case-

control study there is no association of LEPR rs1137101 polymorphism with leptin, obesity or metabolic disturbances.

A study by FURUSAWA et al. (2010), in contrast, has shown associations between the polymorphisms and obesity. Their research group has carried out a study in 809 individuals from the Pacific Islands (Micronesian, Polynesian and Melanesian populations) where they were, among others, looking for associations of known LEPR polymorphisms (rs1137100 and rs1137101) with obesity. Their results have shown that carriers of rs1137101 had significantly higher body weight (p=0.0009) and BMI (p= 0.0022) as well as that they were more obese (p=0.0222). These findings are supported by BEN ALI et al. (2009). In their case-control study with 391 obese and 302 normal weight subjects they have pointed out that rs1137101 influences plasma leptin levels and is also associated with BMI in obese patients. DUARTE et al. (2007) have also reported an association of LEPR and body weight regulation. Their case-control study has revealed that rs1137101 is related to BMI increase. GALLICHIO et al. (2009) support these findings by showing that rs1045895 and rs1137101 are associated with BMI change.

It has already been mentioned in this thesis that the function of leptin does not depend on the amount of leptin, as obese people have a high level of leptin, but on the functioning of the leptin receptor. Polymorphisms in the LEPR gene that might have an effect on leptin levels have also been searched for. RIESTRA et al. (2010) have reported that rs1137101 is significantly associated with serum leptin levels (p=0.016) but only in girls. RAGIN et al. (2009) support these findings in their study, with 1418 healthy subjects from various ethnic groups. They have suggested rs1137101 to be associated with circulating plasma leptin levels but only in postmenopausal Caucasian women. POPRUK et al. (2008) have also shown an effect of a polymorphism on leptin levels in Thai children, although not from rs1137101 but from rs8179183. They have reported an association of rs8179183 with cholesterol and LDL levels.

Further studies concerning polymorphisms and their effects on lipids were found with the catchwords “leptin receptor”, “polymorphism”, “association”

and “lipid”. OKADA et al. (2010), already mentioned above, have conducted a study where they have analyzed 136 obese Japanese children looking for associations between LEPR polymorphisms and serum lipids. They have reported that LEPR gene SNPs might partly contribute to the profiles as rs1137100 and rs790419 showed significant effects but rs1137101 e.g. did not. VAN DER VLEUTEN et al. (2006) have carried out a study where they looked at 644 subjects of which 158 had hyperlipidemia. As a result, rs1137101 has been pointed out to have an association with HDL-C. PHILLIPS et al. (2009), already mentioned above, have investigated the relationships among LEPR polymorphisms and various MetS features. Subjects with rs3790432 had higher risk for developing MetS and this polymorphism has shown associations with plasma fatty acids.

Studies dealing with insulin resistance have been found by combinations of the catchwords “insulin”, “polymorphism”, “association” and “leptin receptor”. DE LUIS et al. (2008) have analyzed 233 obese non-diabetic subjects and reported rs8179183 to be associated with higher levels of insulin and leptin but only in males. SALOPURO et al. (2005) have conducted a study where they have evaluated the association between LEPR polymorphisms and diabetes risk as well as bodyweight in 507 individuals with IGT. Rs1137100 and rs1137101 have been linked to higher risk for T2D. Both of these studies support the first findings by CHIU et al. (2004). They have namely reported an association of rs1137101 and insulin sensitivity as well as glucose clearance.

Associations between the rs1137101 polymorphism of the leptin receptor and the MetS in general was reported by GOTTLIEB et al. (2009) in free-living elderly in Brazil.

Other genes that might influence the leptin receptor have only been reported by SANTANIEMI et al. (2004). In their study they showed two polymorphisms of tyrosine phosphatase 1B interact with LEPR rs1137101, which results in influencing the BMI and explaining 3% of its variation.

SNP	Other name	Authors	Associated with:
rs1137100	Lys109Arg	MARTI et al 2009	No association with obesity or leptin levels
		FURUSAWA et al. 2010	No association with obesity
		OKADA et al. 2010	Lipid profiles
		SALOPURO et al. 2005	T2D
rs1137101	Gln223Arg	LINSHUAN et al. 2008	Hypertension in male IGT patients
		RUOWANG et al. 2008	Hypertension in obese patients
		MARTI et al. 2009	No association with obesity or leptin levels
		CONSTANTINI et al. 2010	No association with obesity
		PYRZAK et al. 2009	No association with obesity
		FURUSAWA et al. 2010	Higher body weight
		BEN ALI 2009	Higher body weight and leptin levels
		DUARTE et al. 2007	BMI increase
		GALLICHIO et al. 2009	BMI increase
		RIESTRA et al. 2010	Leptin levels in girls
		RAGIN et al. 2009	Leptin levels in postmenopausal women
		OKADA et al. 2010	no association with lipid profiles
		VAN DER VLEUTEN et al. 2006	HDL- cholesterol
		SALOPURO et al. 2005	T2D
		CHIU et al. 2004	Insulin sensitivity and glucose clearance
		GOTTLIEB et al. 2009	Metabolic Syndrome
rs790419	Ser343Ser	MARTI et al. 2009	No association with obesity or leptin levels
		OKADA et al. 2010	Lipid profiles
rs8179183	Lys656Asn	MARTI et al. 2009	No association with obesity or leptin levels
		POPRUK et al. 2008	Leptin levels
		POPRUK et al. 2008	Cholesterol and LDL levels
		DE LUIS et al. 2008	Higher linsulin and leptin levels in males
		PHILLIPS et al. 2010	Plasma fatty acids, MetS
rs3790432		ZHANG et al. 2007	CRP levels
rs1805096		ZHANG et al. 2007 PHILLIPS et al. 2010	CRP levels Plasma fatty acids, MetS
rs1045895		GALLICHIO et al. 2009	BMI increase

Table 5: Overview of all mentionend LEPR polymorphisms and their associations

5.2.3. LEPR - Discussion

As with the LEP results the findings are varying but it has to be said that the outcomes are more promising. Nevertheless, considering the results of the individual SNPs it remains hard to say if LEPR really plays a role in the development of the MetS. Rs1137101 has been the most investigated and the most discussed LEPR polymorphism. It has been linked to hypertension, obesity, leptin levels, lipid profiles but most of these associations have been refuted again. The most promising results refer the polymorphisms influencing CRP levels, which is the correlation of LEPR and inflammation. Therefore it might be important to add CRP as an additional factor to MetS definition.

The reason for these unsatisfying outcomes might be the same as for LEP. Again, the inclusion criteria of patients can be questioned as well as the amount of tested subjects. Not many GWAS are available and studies with other study designs just might not have enough people to have significant outcomes without false positive results. It can also occur that the polymorphisms on the arrays are just not representing the important polymorphisms leading to the fact that those cannot be found. If there are some results shown it is also a question which p-values are necessary to consider those significant. Not every study is corrected for the tested SNPs and some just take given significance levels and apply them to their studies. This might include many false-positive results but also exclude promising candidates.

Influences of other genes have not been reported yet. Above a possible interaction of LEPR and TP1B is pointed out. A further possibility would also be that not the LEP and LEPR polymorphisms are causing e.g. obesity but rather the other way round. Unhealthy eating habits and obesity could influence the expression of genes and the signalling pathways leading to numerous small dysfunctions that affect the communication of cells and in the end result in a vicious cycle that ends in a cluster of diseases like MetS. Leptin and its corresponding receptor play a major role in cell communication

when people are “normal” but probably are constricted through westernized lifestyle. In that case genes would play no role at all.

An interesting study has been published by DEMOOR et al. (2009). This GWAS has analyzed the associations of LEPR polymorphisms and leisure time behaviour like exercise participation for example. Rs12405556 reached a p-value of 9.7×10^{-4}. This means that if there are genes that influence our urge to move they could be the ones that are indirectly causing the MetS. But it is more likely that they have only a small size effect.

5.3. ADIPOQ

5.3.1. ADIPOQ - GWAS results

The search for GWAS results in the HuGE Navigator for the ADIPOQ gene revealed three studies presenting four significant SNPs, all of them being associated with adiponectin levels.

HEID et al. (2010) have reported one of the significant polymorphisms. They have conducted a study with the aim to find SNPs associated with plasma adiponectin levels, which might explain the difference between men and women. 4659 subjects (w=2562, m=2097) that derived from ERF, KORA and MICROS have been tested in the first stage for 2.585.854 SNPs. 40 of these were considered to be interesting because they had combined p-values $<1x10^{-4}$ and a minor allele frequency (MAF) greater than 5%. Additionally, 73 SNPs, associated with MetS parameters before, have been selected and studied for their association with plasma adiponectin. The primarily selected 40 SNPs have been checked in stage 2 by carrying out a replication study based on 7 cohort studies (CoLaus, Framingham, GEMS, ALSPAC, TWINS UK, InChianti and BLSA). Only one SNP has reached genome-wide significance, namely rs17366568 with a combined p-value of $4.3x10^{-24}$, which has been consistent in women ($p=8.7x10^{-17}$) and men ($2.5x10^{-11}$). Although the p-values were different in the sexes the differences in plasma adiponectin levels could not be explained by the top SNP as it revealed a p-value of 0.62 suggesting that the differences seem to be more influenced by sex hormones

than by the gene itself. It is interesting to know that rs17366568 is completely independent of all the other SNPs in the ADIPOQ region. Many SNPs that are located in the same region have a weak r^2 even if they are located on the same LD block. The percentage of plasma variance ranged from 3.9% to 6.7% depending on whether only the rs17366568 has been considered or all the SNPs with MAF >5% within the LD blocks have been included. An association with any of the additionally included 73 polymorphisms did not reveal any convincing results with plasma adiponectin levels. Further outcomes for this SNP are mentioned later on.

The second study, which has revealed significant results, has been carried out by LING et al. (2009). Like HEID et al. (2009) they also aimed to identify genes influencing adiponectin levels. The GEMS study has included 1845 subjects (997 cases, 989 controls), which have been tested for the desired associations. Out of 10 strongly associated SNPs, five were located on genes. Three of them, rs6773957 ($p=4.78x10^{-8}$), rs3774261 ($p=5.15x10^{-8}$) and rs17366568 ($p=3.70x10^{-6}$) were directly in the ADIPOQ gene. Rs3774261 and rs6773957 have remained associated in controls (p=0.004 and p=0.003) but only rs6773957 was mentioned in the Interrogator. These two SNPs revealed to be highly correlated ($r^2=0.98$). Rs3774261 accounted for 1.2% of the total variance in plasma adiponectin level in the pooled sample of cases and controls. Associations of these two ADIPOQ SNPs with other MetS parameters have not achieved any statistical significance (all p>0.001). Rs6773957 is additionally mentioned in the further results for LEPR polymorphisms.

The third study, which has reported two significant results in the GWAS Interrogator has been conducted by RICHARDS et al. (2009). They meta-analyzed three GWAS (TwinsUK, GEMS, CoLaus) initially containing 8531 participants for circulating adiponectin levels. The most strongly associated SNPs ($p<11x10^{-4}$, n=250) have been chosen and tested in five additional cohorts (BLSA, EPIC-Norfolk, Framingham, INCHIANTI, ALSPAC) containing 6202 subjects in total. 5 SNPs have achieved genome-wide significance ($p< 5x10^{-8}$) and have been tested for their association with MetS and its traits in further independent cohorts. Associations with T2D have been tested in the

DIAGRAM consortium (10.128 subjects), those with insulin resistance in MAGIC (24.188 people), those with CHD in a consortium of 8 cohorts and those with BMI in the GIANT cohort (32.527 subjects). In total, 4 SNPs have shown genome-wide significance: rs6444175 ($p=1.2x10^{-21}$), rs266717, rs1426810 ($p=2.2x10^{-18}$), rs1648707 ($p=3.0x10^{-12}$). The most strongly associated SNP has been rs266717 with a p-value of $9.2x10^{-19}$. The findings have been consistant through all stages. Only rs6444175 has demonstrated heterogeneity, and therefore, although the p-value is better than in rs266717, it is not the most strongly associated polymorphism. Interestingly the GWAS HuGE Navigator only mentions rs266717 and rs1648707 to have reached significance level. These four reported polymorphisms have also been tested for MetS traits but none of the SNPs in the ADIPOQ locus has shown a significant relationship to T2D or CHD although rs1648707 has had some positive results (CHD p-value=0.04 and T2D p-value=0.046). Rs1648707 is in moderate linkage disequilibrium with rs266729 ($r^2=0.74$), which has been associated with adiponectin levels earlier on [MENZAGHI et al. 2007], but not with T2D. Further results are only added for rs6444175 later on.

Further GWAS hits have been searched for with combinations of catchwords in PubMed, without any results.

SNP	Other name	Authors	Associated with:
rs17366568		HEID et al. 2010	Adiponectin levels
rs3774261		LING et al. 2009	Adiponectin levels
rs6773957		LING et al. 2009	Adiponectin levels
rs6444175		RICHARDS et al. 2009	Adiponectin levels, no association to T2D or CHD
rs1426810		RICHARDS et al. 2009	Adiponectin levels, no association to T2D or CHD
rs1648707		RICHARDS et al. 2009	Adiponectin levels, possible association with T2D and CHD
rs266717		RICHARDS et al. 2009	Adiponectin levels, no association to T2D or CHD

Table 6: Overview of all mentioned ADIPOQ polymorphisms in GWAS and their associations

5.3.2. ADIPOQ - Further results

Like in the chapters dealing with LEP and LEPR, further study designs and their outcomes have been included into ADIPOQ results due to unavailable,

missing or insufficient GWAS achievements. Generally it can be said that, like for LEPR, common SNPs have been reported, namely rs17300539, rs266729, rs2241766 and rs1501299 [CHIODINI et al. 2010], which were investigated more closely. Most of the studies were dealing with obesity, insulin resistance and adiponectin levels. Therefore results for hypertension, dyslipidemia or inflammation are scarce or not available, showing that that those associations have not been analyzed until now.

To continue with polymorphisms influencing adiponectin levels a study by HENNEMAN et al. (2010) is presented. They have conducted an investigation where they have evaluated the associations of MetS and its features with 10 earlier reported ADIPOQ SNPs. Their family-based population (ERF) has included 1,258 women and 967 men, which have revealed significant associations of plasma adiponectin with the ADIPOQ variants rs17300539 ($p=9.3x10^{-5}$) and rs182052 ($p=3.0x10^{-4}$). For the first one (rs17300539) an association with plasma insulin is reported additionally. Similar findings have been reported by HIVERT et al. (2008) before. In their study 2.543 individuals from FOS have been analyzed and rs17300539 and rs822387 have been reported to be associated with adiponectin levels (p=0.0005 and p=0.001). Furthermore, these two polymorphisms have been shown to be in strong LD ($r^2=0.8$). Additionally rs6773957 and rs6444175, from an untranslated region (3`UTR), were associated with adiponectin levels, too (p=0.002 and p=0.04). The latter SNP and rs1501299 have demonstrated a state of strong linkage disequilibrium ($r^2=0.92$) but rs1501299 has lacked to show significant association with adiponectin levels (p=0.1). Linkage to MetS was only shown by a nonsynonymous SNP (rs17366743), which has been associated with diabetes incidence (p=0.004).

A few opposing results have been shown by HEID et al. (2006) and MENZAGHI et al. (2007). The first ones also reported rs822387 to be associated with higher adiponectin and in high LD with rs17300539. But in contrast to HIVERT et al. (2008) they reported that no association of rs17366743 and MetS has been observed. MENZAGHI et al. (2007) have also declared rs17300539 to have an effect on adiponecintaemia. Furthermore rs1501299 has been shown to have an effect on adiponectin

levels, insulin resistance and CAD (coronary aretery disease). Additionally rs1501299 has been reported to be in LD with a 3´UTR variant. Rs266729 has been identified to be associated with higher adiponectin levels.

VIMALESWARAN et al. (2008) have analyzed 2.000 people affected with T2D and 2.000 with normal glucose tolerance with the aim to find variants that contribute to the development of T2D in Asian Indians. They found a polymorphism, rs17846866, contributing to development of T2D, obesity and hypoadiponectinemia.

Searching for results concerning adiponectin polymorphisms and obesity, similar to the chapters before, the catchwords “adiponectin”, “polymorphism”, “association” and “obesity” have been used. Looking through various studies especially two SNPs have been in the focus of investigation - rs2241766 and rs1501299. The diverse results of those are explained subsequently. It has also to be mentioned that generally these studies have dealt with insulin resistance, too. Therefore both features of MetS, obesity and insulin resistance, are analyzed and presented simultaneously.

YU et al. (2010) wanted to know if there is an association of rs2241766 and adolescent obesity. For that, 47 obese and 50 subjects with normal weight have been analyzed showing no association. These findings are supported by the study of LEE et al. (2010), who have analyzed the associations of rs2241766 and rs1501299 with obesity-related phenotypes in 1260 twin pairs but they did not find any bond. An association of rs2241766 with IR, in contrast, has been shown to exist [YU et al. 2010]. These data have been refuted by CHIODINI et al. (2010). In their case-control study with 2008 Italians, the SNPs rs17300539, rs266729 and rs2241766 had no significant associations with T2D or myocardial infarction unlike rs1501299 polymorphism mentioned above, which has been linked to decreased risk of myocardial infarction (p=0.01).

Similar results to those of CHIODINI et al. (2010) have been reported before by JANG et al. (2006). They aimed to determine whether polymorphisms of the ADIPOQ gene contribute to IR and CVD in non-obese and non-diabetic

Korean men and have reported that rs1501299 rather than rs2241766 is associated with several components of MetS and CVD risk, including IR, triglyceride concentration, and low-density lipoprotein particle size. PANAGOPOULOU et al. (2009) have confirmed these findings by analyzing 48 obese children from Greece for their association of rs2241766 and rs1501299 with IR. Rs2241766 has not been associated with decreased risk for IR but rs1501299 has been reported to possibly be protective against IR. MOHAMMADZADEH and ZARGHAMI (2009) confirm these results by reporting that in their study rather rs2241766 than rs1501299 has been associated with risk in T2D in obese individuals.

The search for studies dealing with hypertension or blood pressure revealed only a few results. Again a combination of catchwords (“adiponectin”, “polymorphism”, “association” and “hypertension”/”blood pressure”) has been used to find these. ANTONOPOULOS et al. (2009) have conducted a study to show if the rs2241766 polymorphism is associated with lower risk of arterial hypertension and decreased CVD risk. They have been able to point out that the SNP has an effect on adiponectin expression but only in healthy individuals, suggesting that higher adiponectin levels might prevent the appearance of CAD in healthy people but as soon as they are diseased the effect disappears.

IWASHIMA et al. (2004) have investigated the associations of I164T and rs1501299. Whereas the latter has not been associated with adiponectin concentrations or hypertension the first one showed a lowering effect on adiponectin levels and was associated with appearance of hypertension. In the study by RONCONI et al. (2010) previous results for the two SNPs were approved. Rs2241766 has been shown to have a protective role, like reported by ANTONOPOULOS et al (2009), while rs1501299 appeared to have a worsening effect or no effect at all.

Studies dealing with polymorphisms of adiponectin and their associations with dyslipidemia have been identified by using combinations of the catchwords “adiponectin”, “polymorphism”, “association” and “HDL” or “LDL”. BERTHIER et al. (2005) have examined the associations of adiponectin

polymorphisms and lipoprotein levels. Their data showed that carriers of rs1501299 had higher LDL and lower HDL levels but subjects with rs2241766 had higher plasma adiponectin. These results have been supported by the findings of JANG et al. (2005) and JOHANSSON et al. (2009). The first ones have reported an association of rs1501299 with lower plasma adiponectin and significantly higher concentrations of LDL and triacylglycerol but no effect of rs2241766. The latter group has shown again a linkage of rs1501299 with LDL cholesterol.

Although adiponectin and inflammation have been linked to each other, no polymorphisms have been investigated and reported until now.

Influence on the adiponectin levels, not due to the ADIPOQ locus itself have been reported recently. RICHARDS et al. (2009) have identified a novel intronic SNP, rs4311394, which is located in the ARL15 (ADP-ribosylation factor like 15) gene. The polymorphism has been associated with decreased adiponectin levels ($p=2.9x10^{-8}$) and linked to CHD (various cohorts, n= 22421, p-value= $8.5x10^{-6}$), to an increased risk of T2D (DIAGRAM consortium, 10128 individuals, $p=3.2x10^{-3}$) as well as to increased fasting insulin (MAGIC consortium, 24614 subjects, 2.3x10-3). The GIANT consortium has demonstrated a moderate but non-significant association with BMI (p=0.016).

SNP	Other name	Authors	Associated with:
rs17300539	11391G>A	HENNEMAN et al. 2010	Adiponectin levels, plasma insulin
		HIVERT et al. 2008	Adiponectin levels
		HEID et al. 2006	No associations wit MetS
		MENZAGHI et al. 2007	Adiponectinaemia
		CHIODINI et al. 2010	No association with T2D or myocaridal infarction
rs266729	11377C>G	MENZAGHI et al. 2007	Adiponectin levels
		CHIODINI et al. 2010	No association with T2D or myocaridal infarction
rs2241766	45T>G	YU et al. 2010	no association with obesity but with IR
		LEE et al. 2010	no association with obesity
		CHIODINI et al. 2010	No association with T2D or myocaridal infarction
		JANG et al. 2006	Suggested association with IR, TG concentration, LDL size
		PANGOPOULOU et al. 2006	No associaiton with decreased risk of IR
		MOHAMMADZADEH and ZARGHAMI 2009	increased risk of T2D
		ANTONOPOULOS et al. 2009	Decreased cardiovascular risk
		RONCONI et al. 2010	protection of hypertension
		BERTHIER et al. 2005	Adiponectin levels
		JANG et al. 2005	no asociation with adiponectin levels
rs1501299	276G>T	LEE et al. 2010	no association with obesity
		CHIODINI et al. 2010	Decreased risk of myocardial infarction
		JANG et al. 2006	IR, TG concentration, LDL size
		PANGOPOULOU et al. 2006	Protection against IR
		MOHAMMADZADEH and ZARGHAMI 2009	suggested risk of T2D
		IWASHIMA et al. 2004	No association with adiponectin levels or hypertension
		RONCONI et al. 2010	No association with adiponectin levels or hypertension
		BERTHIER et al. 2005	Lipoprotein levels
		JANG et al. 2005	Lower Adiponectin levels, high LDL level
		JOHANSSON et al. 2009	LDL cholesterol
		HIVERT et al. 2008	No associations with adiponectin levels
		MENZAGHI et al. 2007	Adiponectin levels, insulin resistance, CAD
rs182052		HENNEMAN et al. 2010	Adiponectin levels
rs822387		HIVERT et al. 2008	Adiponectin levels
		HEID et al. 2006	Adiponectin levels
rs6773957		HIVERT et al. 2008	Adiponectin levels
rs6444175		HIVERT et al. 2008	Adiponectin levels
rs17366743		HIVERT et al. 2008	Diabetes
		HEID et al. 2006	No associations wit MetS
rs17846866		VIMALESWARAN et al. 2008	T2D, obesity, hypoadiponectinaemia
	I64T	IWASHIMA et al. 2004	Low adiponectin levels and hypertension

Table 7: Overview of all mentioned ADIPOQ polymorphisms and their associations

5.3.3. ADIPOQ - Discussion

So far adiponectin has been the most promising object of investigation as a lot of positive finding have been achieved. Especially the search for SNPs associated with adiponectin levels has been fruitful, as many results have been reported. Polymorphisms concerning MetS or any of its features however gave more inconsistent results. Rs2241766 has been reported to be associated with IR but also as not having any associations with T2D. For rs1501299 similar results have been achieved so protection against IR was suggested as was a higher risk to get T2D. Therefore, also for ADIPOQ, the question of how big its influence in the development of MetS is and if there actually is one at all, remains.

Adiponectin has been shown to be negatively regulated in obesity, meaning that the more obese a person is the lower the adiponectin levels are. In contrast to leptin, a lack of adiponectin is associated with features of MetS. HEID et al. (2010) have shown that rs17366568 alone is accountable for 4% of the adiponectin level variance. Including other SNPs on the same LD block the influence has been up to 7%. Therefore people possessing one or all of these SNPs might be more protected from developing MetS than the ones without them. Nevertheless, the effect could not be proved as most of the studies revealed no association of MetS with that SNP. It has also been surprising that none of the 73 SNPs, which were additionally tested by HENNEMAN et al. (2010), did reveal any convincing associations although they have been reported to have a strong link to MetS.

Next to the difficulties of study design and statistics that have already been mentioned in the LEP and/or LEPR results discussion, it is also possible that other genes have an influence on adiponectin as well and if so, the question arises how they influence it. For ARL15 it is still unclear how it interacts with adiponectin to influence the disease risk. But it might be a connection between insulin and adiponectin, where adiponectin acts through an insulin dependant pathway, which involves but does not depend entirely upon adiponectin [RICHARDS et al. 2009]

5.4. ADIPOR1, ADIPOR2 and CDH13

5.4.1. ADIPOR1, ADIPOR2 and CDH13 - GWAS results

The search for significant results in the HuGE Navigator GWAS Interrogator has revealed no significant hits for ADIPOR1 or ADIPOR2. The search for polymorphisms in CDH13, the gene encoding one of the three receptors, revealed two studies dealing with MetS with significant results.

LEVY et al. (2007) conducted a study with the participants of the Framingham Heart Study analyzing 70.987 SNPs for their association with hypertension. Among the other genes found to be associated with hypertension they also identified rs3096277, a polymorphism on the CDH13 gene, to be significantly linked to systolic and diastolic BP ($p= 1x10^{-9}$).

Similar findings, but with another SNPs, were achieved by ORG et al. (2009). They tested 1644 participants of the KORA S3 study and their associations of 395.912 SNPs with SBP, DBP and hypertension. The initial results have been replicated in KORA S4 as well as HYPEST. Among the significant results was rs11646213**,** a variation on the CDH13 gene, showing associations with DBP ($p=5.55x10^{-5}$), SBP ($p=0.007$) and hypertension ($p=5.3x10^{-8}$).

SNP	Other name	Authors	Associated with:
rs3096277		LEVY et al. 2007	Systolic and diastolic BP
rs11646213		ORG et al. 2009	Diastolic BP and hypertension

Table 8: Overview of all CDH13 polymorphisms and their associations

5.4.2. ADIPOR1, ADIPOR2 and CDH13 - Further results

To give an overview what is known about ADIPOR1, ADIPOR2 and CDH13 polymorphisms beyond the GWAS results also a PubMed search was conducted. Combinations of catchwords (“adiponectin receptor”, “adipor” or “CDH13” together with “polymorphism”, “association” and/or the MetS trait) was used to find the appropriate studies. Generally it can be said that primarily associations of ADIPOR1/ADIPOR2 with insulin resistance have been reported.

RASMUSSEN-TORVIK et al. (2009) have reported various SNPs being associated with insulin sensitivity but dependant on ethnicity. Rs7539543 on the ADIPOR1 gene, for example, has been significant only in Whites. Rs1342387 from ADIPOR1 has also been significant but then again only in African-Americans. The SNP on the ADIPOR2 gene rs12826079 has shown a p-value of <0.05 but again only in Whites. A further study concerning insulin sensitivity has been conducted by RUCHAT et al. (2008). They have analyzed 100G>T and 3882T>C from the ADIPOR1 and 35361A>G and -1352G>A from the ADIPOR2 gene and their associations with adiponectin plasma levels, indicators of glucose tolerance, insulin sensitivity (IS) and insulin secretion. Only the 3882T>C SNP on ADIPOR1 has been associated with fasting glucose (p=0.03), the homeostasis model assessment for insulin resistance (p=0.04) and an index of insulin secretion (P30/G30, p=0.02). No evidence of association has been found with plasma adiponectin levels.

The study by POTAPOV et al. (2008) has shown three SNPs of which two (rs11061971 and rs16928751), both located in the ADIPOR2 gene, have been associated with higher risk of diabetes. The third SNP, rs22753738, from the ADIPOR1 gene has not shown any associations. KIM et al. (2009) have selected 7 SNPs from ADIPOR1 and 4 SNPs from ADIPOR2 and looked for their associations with T2D. None of the SNPs has shown any linkage. However rs75172865 from ADIPOR1 has been associated with

lower insulin resistance. 63442G and rs1044471 from the ADIPOR2 have been linked to lower waist circumference.

Studies concerning dyslipidemia have been found when using the general catchwords in combination with “lipid”, “HDL”, “LDL” or “lipoprotein”. BROEDL et al. 2006 have been looking for association of ADIPO2 variants with triglyceride levels. They have found 795G/A, 870C/A and 963C/T to be in perfect linkage disequilibrium (r^2=1) and additionally that they are associated with higher plasma adiponectin levels and decreased fasting triglyceride, VLDL-triglyceride and VLDL-cholesterol level. No association, however, has been observed between the AdipoR2 SNP cluster and glucose metabolism. FERGUSON et al. (2010) have conducted a study where they wanted to see if there are associations between SNPs on the ADIPOR1/ ADIPOR2 gene and plasma fatty composition. In their study only rs10920533 has been linked to levels of plasma saturated fatty acids. HALVATSIOTIS et al. (2010) have investigated the association of ADIPOR2 and CAD with the result that rs767870 possibly affects ADIPOR2 levels and consequently indirectly influences the development of CAD. KOTRONEN et al. (2009) have reported that rs767870 is associated with fat accumulation in the liver.

LING et al. (2009) have also checked ADIPOR1 and ADIPOR2 SNPs in their study, which was described in more detail above, and their association to adiponectin levels. But they have not been able to demonstrate any relation. Their search for association with the third adiponectin receptor, **T-cadherin**, was more fruitful. They found a SNP that is located within CDH13, **rs7195409**, which has been associated with adiponectin levels (p=2.0x10-5). Generally, next to the GWAS results, this was the only study dealing with CDH13 and MetS.

SNP	Other name	Authors	Associated with:
ADIPOR1			
rs7539543		RASMUSSEN-TORVIK et al. 2009	Insulin sensitivity in Whites
rs1342387		RASMUSSEN-TORVIK et al. 2009	Insulinsensitivity in African-Americans
rs22753738		POTAPOV et al. 2008	No association of higher risk with diabetes
rs75172865		KIM et al. 2009	Lower insulin resistance
	100G>T	RUCHAT et al. 2008	No associations with insulin sensitivity
	3882T>C	RUCHAT et al. 2008	Fasting glucose, insulin sensitivity
ADIPOR2			
rs12826079		RASMUSSEN-TORVIK et al. 2009	Insulin sensitivity in Whites
rs11061971		POTAPOV et al. 2008	Higher risk of diabetes
rs16928751	795G>A	POTAPOV et al. 2008 BROEDL et al. 2006	Higher risk of diabetes Higher plasma adiponectin, lower fasting TG, VLDL - TG and VLDL cholesterol level
	63442G	KIM et al. 2009	Lower WC
rs1044471		KIM et al. 2009	Lower WC
	35361A>G	RUCHAT et al. 2008	No associations with insulin sensitivity
	1352G>A	RUCHAT et al. 2008	No associations with insulin sensitivity
	870C/A, Ile290Ile	BROEDL et al. 2006	Higher plasma adiponectin, lower fasting TG, VLDL - TG and VLDL cholesterol level
rs9805042	963C/T	BROEDL et al. 2006	Higher plasma adiponectin, lower fasting TG, VLDL - TG and VLDL cholesterol level
rs10920533		FERGUSON et al. 2010	Plasma saturated fatty acids
rs767870		HELVATSIOSIS et al. 2010	AdipoR2 levels
		KOTRONEN et al. 2009	Fat accumulation in liver
CDH13			
rs7195409		LING et al. 2009	Adiponectin levels

Table 9: Overview of all ADIPOR1, ADIPOR2 and CDH13 polymorphisms and their associations

Additional results have been reported by SCUTERI et al. (2007), who have carried out a GWAS focusing on associations between genetic variants in the FTO gene and obesity related traits. Their study population existed of 6148 subjects from an isolated population from Sardinia, which had 362.129 SNPs checked for association with BMI, hip circumference and weight. An additional 74 candidate genes have been analyzed as they might also influence obesity and related traits. Within that study ADIPOR1 and ADIPOR2 have shown a moderate association with BMI, hip circumference and weight (ADIPOR1: p-values = 0.013, 0.027 and 0.016, ADIPOR2: p-values= 0.018, 0.019 and 0.013).

5.4.3. ADIPOR1, ADIPOR2 and CDH13 - Discussion

Generally it can be said that, like for the previously reported polymorphisms on other genes, no real statement can be made. Only one SNP, rs16928751, has been mentioned twice and even for that one opposing results have been reported.

For ADIPOR1, ADIPOR2, CDH13 further research is definitely needed if results are to be confirmed. It has been very surprising that GWAS have reported significant results for CDH13 only, as this receptor is new and not much effect has been thought to be coming from it. But it remains to be elucidated how this receptor transduces signals when adiponectin binds to it.

6. Conclusion

Although the attention to MetS has risen constantly over the years and a lot of research has been done to understand this cluster of diseases it is still an equation with various unknown parts and therefore a subject of frequent research.

As mentioned in the first chapter of the thesis there is no standardized definition available for MetS. But this would be crucial for enabeling the comparison of study data and for lowering the degree of disparity in diagnosing the cluster of diseases. But not only MetS as a whole has been shown to be problematic. Not fully elucidated pathogenesis of each of the components, undefined cut-off points, the exclusion of inflammation in the general definition as well as the use of imperfect surrogates only emphasize that there is still a lot to be investigated and learned about MetS.

The aim of this thesis was to show the role of leptin and adiponectin as well as their receptors in the development of MetS. Especially the SNPs of their encoding genes and their associations with MetS features have been in the focus. And this has not been unproblematic. To the already existing problems of MetS a new field of research has been added, therefore all results have to be interpreted with care. When it is not sure that the person is diseased or not, it is not possible to tell if the present associations are true or falsely positive. Whereas the literature research revealed almost no results for LEP polymorphisms, LEPR, ADIPOQ and adiponectin receptor SNPs have been more promising. But neither trailblazing nor even meaningful results were present pointing out that there are still gaps in the knowledge that have to be filled before more can be said.

If GWAS and the research of polymorphisms should be kept up depends on the point of view on the topic. These kinds of studies are still in the fledging stages and there might be positive results in the future. However, as always in science, those are not guaranteed. If the main goal is to attach functions to the findings and fill in the gaps of knowledge then GWAS should definitely be

kept up. If it is only for assessing the possible risk for an individual to get the disease, then, at the moment, GWAS do not seem promising. In the case of MetS, it can generally be said that genetic screenings have no informative value at the moment. The only way of preventing the development of the disease cluster is the integration of better health habits in every day life.

7. Summary

The attention to metabolic syndrome has risen constantly over the years as the disease is leading to disability and premature death in an increasing number of affected people. Next to environmental factors, genes and gene variations have been considered to play a role in causation. Leptin and adiponectin, two adipocytokines, as well as their receptors, have been the focus of this thesis as they (together with their polymorphisms) have been linked to metabolic syndrome features. Genome-wide association studies have been a novel approach to find these genetic variations and until now they have reported numerous outcomes. The literature research at hand revealed various results pointing out that current knowledge is not enough to explain to which extent the adipocytokines play a role in the development of MetS. Genetic screenings for risk assessement cannot be recommended at the moment. The incorporation of better health habits to prevent the metabolic syndrome and/or any of its features should be in the focus instead.

8. Zusammenfassung

Das Metabolische Syndrom hat über die Jahre immer mehr die Aufmerksamkeit auf sich gezogen, da es zu Invalidität und vorzeitigem Tod bei einer steigenden Anzahl an Betroffenen führt. Neben der Umwelt sind immer wieder die Gene und deren Veränderungen worden, die Krankheit zu verursachen. Leptin und Adiponectin, zwei Adipozytokine, sowie ihre Rezeptoren sind das Hauptaugenmerk dieser Diplomarbeit, da sie und ihre genetischen Polymorphismen mit dem Metabolischen Syndrom in Verbindung gebracht worden sind. Genomweite Assoziationsstudien stellen eine neue Vorgehensweise dar, diese genetischen Veränderungen zu finden. Die Ergebnisse der vorliegenden Literaturrecherche sind sehr inkonsistent ausgefallen und weisen darauf hin, dass nach dem derzeitigen Wissensstand nicht gesagt werden kann in welchem Ausmass die Adipozytokine in der Entstehung vom MetS eine Rolle spielen. Im Moment können keine genetischen Screenings zur Risikobewertung empfohlen werden. Das Einfügen von gesunden Verhaltensweisen in das Alltagsleben und nicht ein genetisches Screening sollten im Mittelpunkt der Prävention vom Metabolischen Syndrom sein.

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Curriculum vitae

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Education

10/2005 – 10/2010	**Institute for Nutrition Science, IfEW, University of Vienna**, Vienna/A, Diplomstudium (approximately equivalent to master`s degree)
08/1997 – 06/2005	**Secondary school „Liechtensteinisches Gymnasium“** , Vaduz/FL, General qualification for university entrance type E (bussiness sciences)
08/1991 – 06/97	**Primary school „Primarschule Resch**, Schaan/FL

Practical experience

04/09 – 07/09	**Clinic „Hochried“,** Murnau/D –Nutrition therapy with obese children and adolescents
10/08, 02/2009	**SIPCAN save your life**, Wien/A – Collaboration in the project „Gesund essen an Wiener Schulen. Gescheite Jause – Coole Pause“. Amelioration of nutrition habits of school children and of the offer at their school buffet
09/2008	**Hilcona AG,** Schaan/FL – temporary employment in the food processing industry: Production area.
08/2008	**Doctor`s practise „Dr. med. Branko Grizelj“,** Schaan/ FL – Administration and reception of patients
02/ 2006	**Labaratory medicine centre „Dr. Risch“,** Schaan/FL, Practical training in clinical chemistry, haematology, immunohaematology, special labaratory, infection serology, and microbiology
2005 - 2008	**Ballet school „Tabesca“,** Walenstadt/CH - Dance lessons for 5 – 11 years old children
2001 - 2005	**Leisure centre „Resch“**, Schaan/FL – Organisation of dance workshops for children and adults

Further skills

Languages	German & Croatian – First languages English – Fluent in writing and speech French - Advanced Italian – High shool level
Computer literacy	MS Office – Advanced Adobe (Photoshop, Illustrator) – good knowledge

Hobbies

Volleyball, dancing, climbing, skiing, swimming