Elina Suviolahti

SEARCH FOR GENETIC VARIANTS CONFERRING SUSCEPTIBILITY TO OBESITY AND RELATED METABOLIC TRAITS

A C A D E M I C D I S S E R T A T I O N

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ABSTRACT

Obesity increases the risk for several diseases, such as type 2 diabetes mellitus (T2DM), coronary heart disease (CHD), hypertension, osteoarthritis and certain cancers. In addition, obese individuals often have elevated blood pressure, increased levels of serum triglycerides (TG), free fatty acids (FFA) and insulin, as well as decreased serum high density lipoprotein cholesterol (HDL-C) levels. Twin, family and adoption studies suggest a major genetic component in the determination of body weight, although environmental factors also play a critical role in the development of obesity.

In this thesis, we investigated the genetic background of obesity using several different approaches. In the first part of the thesis, two functional candidate genes, Melanocortin-4 receptor (*MC4R)* and Pro-opiomelanocortin *(POMC)* were screened for mutations in obese Swedish individuals. No obesity-causing mutations were identified. However, we detected variations in the *POMC* gene associated with serum leptin levels in lean individuals.

Second, we examined the possible role of the Lipin 1 (*LPIN1*) gene in humans. This gene was originally identified for lipodystrophy, hypertriglyceridemia and insulin resistance in the *fld* mice. Proper function of the *Lpin1* gene is crucial for the normal adipose tissue differentiation, as well as for maintaining the glucose and lipid homeostasis in mice, thus presenting an interesting candidate gene for obesity and related metabolic disorders in humans. In the present study, the expression levels of *LPIN1* in human fat biopsies correlated negatively with blood glucose and serum insulin levels. In addition, genetic variations of the *LPIN1* gene were associated with serum insulin levels and body mass index (BMI) in dyslipidemic families, as well as in obese case and lean control individuals, males contributing the most to the differences. This is the first study suggesting the involvement of the *LPIN1* gene in obesity and glucose metabolism in humans.

Third, we fine mapped the region on chromosome Xq24 previously linked to obesity in obese Finnish families. Initially, linkage and shared haplotypes were identified in obese male sibpairs for this region. Subsequently, significant evidence of association between variations in the Solute carrier family 6 (neurotransmitter transporter)

member 14 (*SLC6A14)* gene and obesity was observed in two different study populations. The *SLC6A14* gene represents the second novel candidate gene for obesity, identified using the positional cloning approach in humans.

In the fourth part of the thesis, we further investigated six chromosomal regions that have been previously linked to premature CHD, familial combined hyperlipidemia (FCHL), low HDL-C and obesity in Finnish families. We identified a novel quantitative trait locus (QTL) for HDL-C on chromosome 10q11. The markers in this region produced evidence of linkage with serum HDL-C levels, as well as provided evidence of association with serum HDL-C and TG levels, suggesting that the locus on chromosome 10q11 harbours variation(s) influencing the serum HDL-C and TG levels.

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TIIVISTELMÄ

Lihavuus on kasvava terveysongelma länsimaissa, koska lihavuus lisää huomattavasti riskiä sydän- ja verisuonitauteihin, aikuistyypin diabetekseen, nivelrikkoon ja tiettyihin syöpiin. Lihavuuteen liittyy myös keskeisiä aineenvaihdunnan häiriöitä, kuten kohonneet veren triglyseridi-, vapaa rasvahappo ja insuliinipitoisuudet, alentunut seerumin HDL-kolesterolipitoisuus sekä insuliiniresistenssiä. Suku-, kaksos- ja adoptiotutkimusten perusteella lihavuuden taustalla arvellaan olevan perintotekijöitä, jotka yhdessä ympäristön ja sosiaalisten tekijöiden kanssa määräävät kehon ilmiasun.

Tämän tutkimuksen tavoitteena oli selvittää lihavuuden sekä siihen liittyvien aineenvaihdunnan häiriöiden molekyyligenetiikkaa suomalaisessa ja ruotsalaisessa väestössä. Väitöskirjatyön ensimmäisessä osassa tutkimme sairaanloisen lihavan ruotsalaisen potilaan joukkoa tarkoituksena selvittää kuinka yleisiä lihavuuden selittäjiä melanokortiini-4 reseptori (*MC4R*)- ja pro-opiomelanokortiini (*POMC*)-geenien mutaatiot ovat. Yhtään lihavuuden selittävää muutosta ei näistä geeneistä tästä potilasjoukosta löytynyt. Sen sijaan yhden nukleotidin muutokset (single nucleotide polymorphism=SNP) *POMC*-geenissä olivat yhteydessä alentuneeseen leptiinipitoisuuteen normaalipainoisilla henkiloillä.

Väitöskirjan toisessa osassa tavoitteena oli selvittää lipin 1 (*LPIN1*)-geenin merkitystä painonsäätelyssä sekä sokeri-ja rasva-aineenvaihdunnan häiriöiden synnyssä suomalaisessa väestössä. *LPIN1*-geeni ilmenee rasvakudoksessa ja on välttämätön rasvasolujen normaalin kehityksen ja erilaistumisen kannalta. *Lpin1*-geenin mutaatio aiheuttaa hiirellä rasvakudoksen puutoksen, korkeita veren triglyseriditasoja (hypertriglyseridemiaa) ja insuliiniresistenssiä. Tässä tutkimuksessa tutkimme *LPIN1*-geenin ilmentymistä rasvakudoksessa sekä *LPIN1*-geenin sisäisiä ja geeniä ympäröivän alueen DNA-muutoksia lihavien ja normaalipainoisten henkilöiden tapaus-verrokkiaineistossa sekä perheaineistossa, jossa on useita familiaarista kombinoitua hyperlipidemiaa (FKH-tautia) sairastavia potilaita jokaisessa perheessä. Havaitsimme *LPIN1*-geenin ilmentymisen rasvakudoksessa olevan yhteydessä seerumin insuliini- ja sokeritasoihin sekä geenin sisäisten DNA-muutosten assosioituvan seerumin insuliipitoisuuksiin sekä

painoindeksiin erityisesti miehillä. Tutkimus on ensimmäisen osoitus *LPIN1*-geenin yhteydestä lihavuuteen ja sokeriaineenvaihdunnan säätelyyn ihmisillä.

Väitöskirjan kolmannessa osatyössä tarkkakartoitettiin suomalaisessa aineistossa lihavuuteen kytkeytynyt kromosomialue kromosomissa Xq24. Tutkimalla suomalaisessa lihavien sisarusparien aineistossa perimän monimuotoisia markkereita sekä geeninsisäisiä SNP:ja alueen lupaavimmista lihavuuden ehdokasgeeneistä rajasimme alueen noin neljän megaemäksen (=4 miljoonaa nukleotidia) laajuiseksi. Tutkiessamme tämän alueen jaettuja haplotyppejä ja testatessamme alueen ehdokasgeenejä tapaus-verrokkiaineistossa löytyi lihavuuteen assosioituva SNP solute carrier family 6 member 14 (*SLC6A14*) -geenin 3'-päästä sekä SNP-haplotyyppi *SLC6A14*-geenin ympäriltä. Myös Suomesta ja Ruotsista kerätyssä replikaatioaineistossa näkyi ero alleelifrekvensseissä lihavien ja normaalipainoisten henkilöiden välillä *SLC6A14*-geenin sisäisen SNP:n kohdalla. *SLC6A14*-geeni on toinen lihavuuteen yhdistetty geeni, joka on löytynyt koko genomin haun tuloksena.

Väitöskirjatyön viimeisessä osassa tutkittiin koko perimän geenihauissa lihavuuteen, sepelvaltimotautiin, FKH-tautiin, pieneen seerumin HDL-kolesterolipitoisuuteen ja korkeaan triglyseridipitoisuuteen kytkeytyviä kromosomialueita (2q31, 8q23, 10q11, 16q24.1-24.2, 20q13.11, Xq24). Edellä mainitut fenotyypit liittyvät myös tiiviisti yhteen; lihavuus altistaa sepelvaltimotautiin ja toisaalta, lihavuuteen, kuten myös sepelvaltimotautiin, liittyvät yleensä kohonneet seerumin kolesteroli- ja triglyseridiarvot ja onkin mahdollista, että näiden fenotyyppien taustalla on yhteisiä altistavia geneettisiä tekijöitä. Totesimme tässä työssä HDL-kolesterolipitoisuuden kytkeytyvän kromosomiin 10q11. Lisäksi kromosomi 10q11 alueen markkerit assosioituivat matalaan HDL-kolesterolipitoisuuteen sekä kohonneisiin triglyseridipitoisuuksiin viitaten mahdollisesti yhteisiin altistaviin DNA-muutoksiin.

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ABBREVIATIONS

BMI	body mass index
CHD	coronary heart disease
CVCD	common variant, common disease
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
FCHL	familial combined hyperlipidemia
FFA	free fatty acid
GAD2	glutamate decarboxylase 1
HDL-C	high density lipoprotein cholesterol
HGP	Human Genome Project
IBD	identical by descent
IBS	identical by state
LD	linkage disequilibrium
LDL-C	low density lipoprotein cholesterol
lod	logarithm of odds
LPIN1	lipin 1
MC4R	melanocortin-4 receptor
NCEP: ATP III	National Cholesterol Education Program's Adult Treatment Panel III
POMC	pro-opiomelanocortin
PPARG	peroxisome proliferative activated receptor-γ
QTL	quantitative trait locus
SLC6A14	solute carrier family 6 (neurotransmitter transporter) member 14
SNP	single nucleotide polymorphism
SSCP	single-strand conformation polymorphism
TC	total cholesterol
TDT	transmission/disequilibrium test
TG	triglyceride
T2DM	type 2 diabetes mellitus
UTR	untranslated region
WHO	World Health Organization
WHR	waist-to-hip ratio

LIST OF ORIGINAL PUBLICATIONS

I. **Elina Suviolahti**, Martin Ridderstråle, Peter Almgren, Mia Klannemark, Olle Melander, Emma Carlsson, Martin Carlsson, Jan Hedenbro and Marju Orho-Melander: The pro-opiomelanocortin gene is associated with serum leptin levels in lean but not in obese individuals. *International Journal of Obesity and Related Metabolic Disorders,* 2003 27:1204-11

II. **Elina Suviolahti**, Karen Reue, Rita M. Cantor, Jack Phan, Maximilliano Gentile, Jussi Naukkarinen, Aino Soro-Paavonen, Laura Oksanen, Jaakko Kaprio, Aila Rissanen, Veikko Salomaa, Kimmo Kontula, Marja-Riitta Taskinen, Päivi Pajukanta and Leena Peltonen. Cross-species analyses implicate Lipin 1 involvement in human glucose metabolism *(submitted)*

III. **Elina Suviolahti**, Laura Oksanen, Miina Öhman, Rita M. Cantor, Martin Ridderstråle, Tiinamaija Tuomi, Jaakko Kaprio, Aila Rissanen, Pertti Mustajoki, Pekka Jousilahti, Erkki Vartiainen, Kaisa Silander, Veikko Salomaa, Leif Groop, Kimmo Kontula, Leena Peltonen and Päivi Pajukanta. The *SLC6A14* gene shows evidence of association with obesity. *Journal of Clinical Investigation,* 2003 112:1762-72

IV. Heidi E. Lilja, **Elina Suviolahti**, Aino Soro-Paavonen, Tero Hiekkalinna, Aaron Day, Kenneth Lange, Eric Sobel, Marja-Riitta Taskinen, Leena Peltonen, Markus Perola and Päivi Pajukanta. Locus for quantitative HDL-cholesterol on chromosome 10q in Finnish families with dyslipidemia. *Journal of Lipid Research,* 2004 45:1876-84

* Publication IV also appears in the thesis of Dr. Heidi Lilja (12/2004)

INTRODUCTION

Obesity, or in other words excess accumulation of body fat, is a serious health problem in the Western societies due to the increased risk for related disorders such as type 2 diabetes mellit us (T2DM, Chan et al. 1994; Colditz et al. 1995), coronary heart disease (CHD, Hubert et al. 1983; Willett et al. 1995; Hu et al. 2005), hypertension (Huang et al. 1998), osteoarthritis (Felson 1996) and certain cancers (Calle et al. 2003). Obese individuals have three times higher risk for CHD (Willett et al. 1995) and up to 40 times higher risk for T2DM when compared to lean individuals (Chan et al. 1994; Colditz et al. 1995). Because of the serious health consequences related to obesity, it is of great interest to discover the factors predisposing to obesity, in order to create efficient prevention and treatment for obesity.

Body weight is determined by the complex interplay between genetic and environmental factors. Obesity aggregates in families, but the pattern of inheritance does not in most cases follow any Mendelian form of segregation. Common forms of obesity are thus most likely to be caused by multiple genetic and environmental factors, and by their interactions.

What kind of genetic variants predispose to common complex diseases such as obesity? Multiple common alleles with minor effects have been suggested to form disease susceptibility to common complex diseases (Collins et al. 1997). Alternatively, rare alleles with strong phenotypic effects may underlie the genetic background for common diseases (Pritchard 2001). Genes responsible for common forms of obesity can also represent so-called ‘thrifty genes’ (Neel 1962). These gene variants may have provided a survival advantage during evolution at times when there were frequent periods of food shortage or famines. However, within the last decades, as the food supply has been constant and plentiful for the first time in human history, these gene variants have become detrimental, predisposing to common diseases, such as overweight, obesity and T2DM, typical to Western societies.

The purpose of this study was to investigate the genetic background of obesity. The completion of the Human Genome Project (HGP, Collins et al. 2003) and advances in technologies and statistical methods have provided the basic tools for effective gene identification in complex traits. The following review will focus on the existing knowledge of the genetic background of human obesity and on strategies utilized in the post-genome area to identify genes conferring susceptibility to common forms of obesity and related metabolic disorders.

REVIEW OF LITERATURE

1 OBESITY – PREVALENCE, RISK FACTORS AND HEALTH CONSEQUENCES

1.1 Definition and prevalence of obesity

Obesity can be described as an excess amount of fat tissue accumulated as a result of imbalance between energy intake and energy expenditure. A simple way to measure obesity is by using the body mass index (BMI), which is calculated by dividing person's weight in kilograms by square of person's height in meters. The cut-off points proposed by the World Health Organization (WHO) for defining obesity are shown in table 1 (World Health Organization 1995; World Health Organization 1997).

Table 1. *Definitions of normal weight and obesity according to the World Health Organization (WHO 1995; 1997).*

BMI (kg/m^2)	WHO Classification	Popular description
< 18.5	Underweight	Thin
18.5-24.9	Normal range	Healthy, normal, acceptable
25.0-29.9	Grade 1 overweight	Overweight
30.0-39.9	Grade 2 overweight	Obesity
≥ 40.0	Grade 3 overweight	Morbid obesity

** Body mass index (BMI)*

The BMI is a crude measure of adiposity but correlates well with body fatness (Gray and Fujioka 1991; Strain and Zumoff 1992; Steinberger et al. 2005). Other anthropometric measures include waist and hip circumference or the ratio of them (waist-to-hip ratio = WHR). There are more accurate measures to estimate adiposity, including bioimpedance analysis, under water weighing, dual-energy X-ray absorptiometry, measurements of skin fold thickness, and imaging methods, such as computed tomography and magnetic resonance imaging. Since these techniques are often more laborious and expensive, they are not as popular in routine use, whereas information to calculate the BMI is usually easily available. The BMI also correlates with obesity related risk factors (Willett et al. 1999; Steinberger et al. 2005), making it the most frequently used method to evaluate obesity. Recently, waist circumference has been suggested to be even better predictor of obesity-related diseases than BMI (Janssen et al. 2004). In addition, the cut-off points proposed by

WHO mainly for the Western societies may not be optimal to all different ethnicities (WHO Expert Consultation 2004; Tan et al. 2004).

There are large differences between countries in the prevalence of obesity (Kopelman 2000). However, obesity has become increasingly prevalent both in Western societies and in developing countries (Bjorntorp 1997; Kopelman 2000; Flegal et al. 2002). The prevalence of obesity is high for example in Eastern Europe, Eastern Mediterranean, North, Central and South America (especially in the US, Argentina, Chile, Paraguay and Mexico), as well as in many Western European countries (Kopelman 2000; James et al. 2001; James 2004). There are certain isolated Pacific Islands such as Samoa, Nauru, Tonga, the Cook Islands and French Polynesia where obesity is extremely common with a prevalence close to 75% (Bjorntorp 1997). Within some of these ethnic groups large physical size is still considered as a mark of beauty and social status.

The marked increase in the prevalence of obesity in the US during the last 20 years is well documented in the reports of the Behavioral Risk Factor Surveillance System, conducted annually in the US by the Centers for Disease Control and Prevention (*http://www.cdc.gov/nccdphp/dnpa/obesity/trend/)* (Li et al. 2005). Data collected for 1999-2002 estimates that 30.4% of the adult US population has a BMI more than 30 kg/m^2 and is thus considered obese (Hedley et al. 2004). This number has almost doubled when compared to the results from the same survey in 1976-1980.

In Finland, according to the national FINRISK 2002 survey, 20.4% of men and 18.9% of women had a BMI more than 30 kg/m^2, and were thus co nsidered obese (Laatikainen et al. 2003). The mean BMI in men was 27 kg/m^2 and in women 25.9 kg/m^2 in 2002, see figure 1. There are interesting gender-differences in trends of obesity in Finland, as the mean BMI in males has been increasing steadily since 1972, and on average the obesity of men exceeds the obesity of women (Lahti-Koski et al. 2001). In females the mean BMI actually dropped in 1970's, but has increased again since 1980's. This is opposite to what is seen in most other Western countries where women are generally both more obese and overweight than men (Flegal et al. 2002; Baskin et al. 2005). The increased prevalence of obesity coincides with industrialization and automation of societies. Compared to the past decades, the physical effort in daily labors has been minimized, as the work has become increasingly sedentary. It can be speculated that during the last couple of decades highly educated Finnish women have recognized the social pressure for slimness and learned to control their weight (Sarlio-Lahteenkorva et al. 2004). At the same time the social pressure has not been as strong on men, and this combined with a decrease in the energy expenditure at work has resulted in the tendency of men to continue gaining weight.

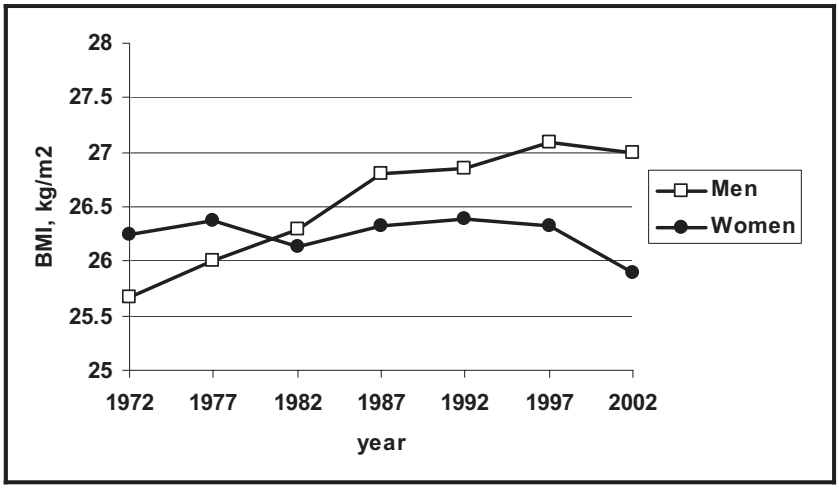

Figure 1. *The mean body mass index (BMI) among men and women in Finland during the years 1972-2002 (Lahti-Koski et al. 2001; Laatikainen et al. 2003).*

1.2 Factors predisposing to obesity

Body weight is the result of the complex interplay between genetic, environmental and psychosocial factors acting through the physiological mediators of energy intake and energy expenditure (figure 2). Environmental factors must play a significant role in obesity, as evidenced by increasing prevalence of obesity in the last decade. A sedentary life style and low physical activity promote obes ity (Rissanen et al. 1991). Body weight also increases with age (Rahkonen et al. 1998). Of the dietary factors, high fat content and energy density have been associated with obesity (Lindroos et al. 1997; Bray and Popkin 1998; McCrory et al. 2000; Bray et al. 2004). An association between low socioeconomic status and obesity has also been reported (Kahn and Williamson 1990; Rissanen et al. 1991; Rahkonen et al. 1998; Sarlio-Lahteenkorva et al. 2004). In addition, overweight individuals more often have difficulties controlling eating, have stronger feeling of hunger, and they tend to engage in emotional eating (Lindroos et al. 1997; Hakala et al. 1999).

However, in a similar, shared environment some people are likely to become obese, whereas others are not. Twin, family and adoption studies suggest a major genetic component in the determination of body weight (see page 34) (Stunkard et al. 1986; Stunkard et al. 1990; Sorensen et al. 1992a; Sorensen et al. 1992b; Vogler et al. 1995; Allison et al. 1996b; Maes et al. 1997; Rice et al. 1999). Cur rently, obesity is thus seen as a complex disorder with an individual's genetic background affecting the susceptibility, but ultimately genetic, physiological and psychosocial factors acting together to determine the body composition (figure 2).

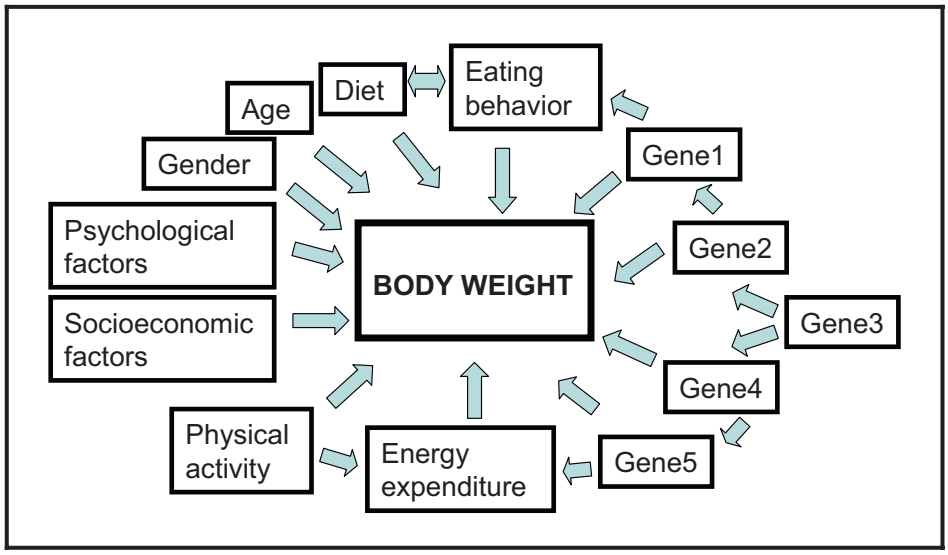

***Figure 2**. Factors affecting body weight.*

1.3 Health consequences of excess body weight

The increased public and scientific attention to obesity is largely due to its health consequences. Total mortality associated with BMI shows a J shaped curve (figure 3), meaning that overweight and obesity, as well as underweight, are associated with increased total mortality (Manson et al. 1995; Flegal et al. 2005). Increased death in underweight individuals is largely due to smoking (Manson et al. 1995). The increased death in obese individuals can be explained by chronic diseases that are more common in obese than in normal weight individuals (Willett et al. 1999; Kopelman 2000). T2DM (Chan et al. 1994; Colditz et al. 1995; Rimm et al. 1995), CHD (Hubert et al. 1983; Willett et al. 1995; Hu et al. 2005), hypertension (Huang et al. 1998), cholelithiasis (Maclure et al. 1989), certain forms of cancer (Calle et al. 2003) and osteoarthritis (Felson 1996) are the most common disorders associated with obesity.

The risk for CHD was two times higher in overweight women compared to lean individuals (BMI < 21 kg/m^2) and more than three times higher in women with BMI > 29 kg/m^2 (Willett et al. 1995). The risk for T2DM goes up even more dramatically with increasing BMI. Obese females with BMI > 31 kg/m^2 have about 40 times higher risk for T2DM compared to lean individuals with BMI < 22 kg/m^2 and more than 90 times higher risk when BMI exceeds 35 kg/m^2 (Colditz et al. 1995). In males, the association between obesity and T2DM has been detected, too; when BMI exceeds 35 kg/m^2, the risk for T2DM is more than 40 times greater when compared

to lean individuals (Chan et al. 1994). The significant increase in risk for T2DM can be seen even in normal weight pe ople, especially in the case of women; the risk for T2DM is increased five times with BMI 24-25 kg/m^2 compared to women with BMI < 22 kg/m^2 (Chan et al. 1994; Colditz et al. 1995). The weight change also affects the risk for T2DM; loss of approximately 10 kg decreases the risk 1.4 times, whereas gaining the same amount of weight increases the risk 2.2 times. (Tuomilehto et al. 2001). The types of cancers associated with obesity include breast cancer in postmenopausal women, as well as cancers of colon, endometrium, kidney, oesophagus, gastric, pancreas, gallbladder and liver (Calle et al. 2003; Calle and Kaaks 2004).

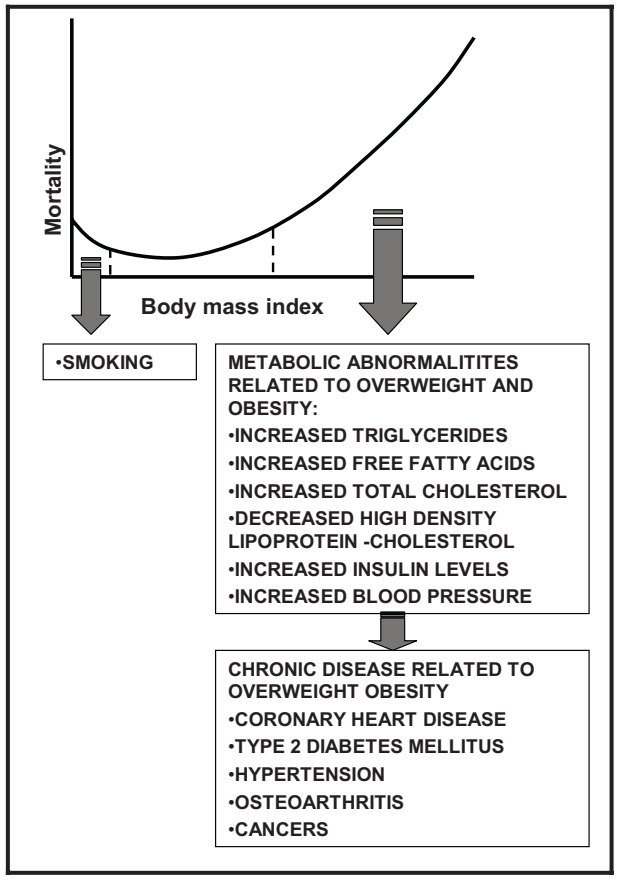

Figure 3. *The association between body mass index (BMI) and total mortality and factors suggested explaining it.*

The location where fat accumulates in body has an effect on disease susceptibility, the abdominal fat being more risky than subcutaneous fat (Janssen et al. 2004; Borodulin et al. 2005). In this respect, measuring the waist circumference or the WHR may be more accurate in defining the individual's risk.

Because of the serious health consequences related to overweight and the large resources that the obesity-related diseases require on the health care system, it is of great interest to discover the mechanisms that predispose to obesity, as well as to create efficient prevention and treatment for obesity.

2 METABOLIC DISORDERS RELATED TO OBESITY

2.1 Metabolic consequences of obesity

Obese individuals often have elevated insulin levels and are insulin resistant (Pascot et al. 2001). A major contributor to the insulin resistance is excess free fatty acids (FFA, Eckel et al. 2005). FFAs are derived from the triglyceride (TG) stores in the adipose tissue through the action of hormone sensitive lipase and from the TG-rich lipoproteins by the action of lipoprotein lipase. Normally, insulin inhibits the lipolysis in adipose tissue. Thus, more fatty acids are released from the adipose tissue when insulin resistance develops. The lipotoxic effects of excess FFAs may occur via increased fatty acid oxidation instead of the utilization of glucose for energy in peripheral tissues (Randle et al. 1963). Alternatively, FFAs may inhibit insulin signaling including phosphorylation of the insulin receptor substrates and, thus, decrease glucose transport across the cell membrane (Dresner et al. 1999).

Obese individuals often have elevated serum TG (Devroey et al. 2004), total cholesterol (TC, Gregg et al. 2005) and FFA (Pankow et al. 2004), as well as decreased high density lipoprotein cholesterol (HDL-C) levels (Devroey et al. 2004). In the circulation, most lipids are transported as water-soluble lipoproteins that consist of a hydrophopic core of TGs and cholesterol-esters surrounded by more hydrophilic surface of cholesterol, apolipoproteins and phospholipids. Lipoproteins are separated into five major classes according to their densities. These classes include chylomicrons, VLDL, intermediate density lipoproteins (IDL), LDL and HDL. Chylomicrons mainly deliver the exogenous lipids to peripheral tissues, whereas the VLDL is the transporter of the endogenously produced TGs and cholesterol. After losing its TGs by the action of lipoprotein lipase, VLDLs are transformed into IDL and further to cholesterol-rich LDL particles. HDL particles are mainly responsible for the reverse cholesterol transport, i.e., they transport the excess cholesterol from the peripheral tissues to liver. Nascent HDL particles are

synthesized in liver and intestine. These discoidal lipid-free HDL particles absorb intracellular cholesterol from the peripheral tissues and mature while acquiring cholesterol esters via esterification of free cholesterol by lecithin:cholesterol acyltransferase.

Dyslipidemic features in obese individuals are related to both lipoprotein concentrations, as well as to lipoprotein composition (Eckel et al. 2005). Increased fatty acid availability in the liver results in increased synthesis of TGs, which further promotes the formation of apolipoprotein B-containing VLDL particles. Decreased HDL-C levels in the circulation are associated with increased TG levels (Despres et al. 2000). This may have resulted from the action of cholesterol ester transfer protein, which mediates the exchange of TG from VLDL to HDL and cholesterol esters from HDL to VLDL (Eckel et al. 2005). The net effect is the enrichment of HDL particles with TGs and depletion of cholesterol esters, in addition to reduced size of HDL particles, which may enhance the clearance of the HDL particles from the circulation. The content of LDL particles is modified resulting in formation of small dense LDL particles that are more readily oxidized and highly atherogenic.

Visceral fat has been implicated as a specific source of FFAs (Moller and Kaufman 2005). It is more metabolically active and has higher rate of lipolysis than subcutaneous fat, possibly explaining why central obesity could be even better predictor of obesity-related diseases than BMI alone. Interestingly, normal-weight individuals with insulin resistance have increased visceral adipose tissue (Ruderman et al. 1998). Increased waist circumference is associated with increased obesity-related health risks in overweight, obese, as well as in normal individuals (Janssen et al. 2004). The metabolic effects of increased visceral fat may be me diated throught the production of adipocyte-derived proteins, including for instance adiponectin and resistin.

The hypertension associated with obesity may be mediated through elevated insulin levels and insulin resistance (Huang et al. 1998). In normal weight individuals, insulin is a vasodilator, but the vasodilating effect may be lost in obesity (Eckel et al. 2005). This combined with enhanced renal sodium retention in obese individuals and increased symphathetic nervous system activity may drive hypertension.

2.2 The metabolic syndrome

Obesity, especially central obesity, insulin resistance, dyslipidemia and hypertension co-occur in the same individuals more often than expected by chance alone and are collectively referred to as the metabolic syndrome (Eckel et al. 2005). Diagnostic criteria for the metabolic syndrome have been suggested by four different committees: the WHO (Alberti and Zimmet 1998; Alberti et al. 2005), the European Group for the Study of Insulin Resistance (Expert Panel on Detection Evaluation, and Treatment of High Blood Cholesterol in Adults 2001), the National Cholesterol

Education Program's Adult Treatment Panel III (NCEP: ATP III, Balkau and Charles 1999) and the International Diabetes Federation (IDF, *http://www.idf.org/)*. They agree on the essential diagnostic components including glucose intolerance, obesity, hypertension and dyslipidemia, but differ in the emphasis on different elements and their cut-off points. Criteria from the WHO and the European Group for the Study of Insulin Resistance highlight insulin resistance as the core component, whereas, the NCEP: ATP III considers that all components are equally important. IDF focuses on central obesity and has suggested ethnic-specific cut-off points for waist circumference. These criteria are presented in table 2.

Table 2. *The three different criteria for the metabolic syndrome suggested by the World Health Organization (WHO), the European Group for the Study of Insulin Resistance and the National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III).*

World Health Organization	**European Group for the Study of Insulin Resistance**	**National Cholesterol Education Program's Adult Treatment Panel III**	**International Diabetes Federation**
• Diabetes or impaired fasting glycaemia or impaired glucose tolerance or insulin resistance (hyperinsulinaemic, euglycaemic clamp-glucose uptake in lowest 25%) • Plus 2 or more of the following - Obesity: BMI > 30 or WHR > 0.9 (male) or > 0.85 (female) - Dyslipidaemia: TGs ≥ 1.7 mmol/L or HDL-C < 0.9 (male) or < 1.0 (female) mmol/L - Hypertension: blood pressure > 140/90 mm Hg - Microalbuminuria: albumin excretion > 20 µg/min	• Insulin resistance - hyperinsulinaemia: top 25% of fasting insulin values from non-diabetic population • Plus 2 or more of the following - Central obesity: waist circumference ≥ 94 cm (male) or ≥ 80 cm (female) - Dyslipidaemia: TGs > 2.0 mmol/L or HDL-C < 1.0 - Hypertension: blood pressure ≥ 140/90 mm Hg and/or medication - Fasting plasma glucose ≥ 6.1 mmol/L	• 3 or more of the following: - Central obesity: waist circumference > 102 cm (male), > 88 cm (female) - Hypertriglyceridaemia: TGs ≥ 1.7 mmol/L - Low HDL-C: < 1.0 mmol/L (male), < 1.3 mmol/L (female) - Hypertension: blood pressure ≥ 135/85 mm Hg or medication - Fasting plasma glucose ≥ 6.1 mmol/L	• Central obesity: waist circumference with ethnic-specific cut-off points • Plus 2 or more of the following -Hypertriglyceridaemia: TGs ≥ 1.7 mmol/L or medication - Low HDL-C: < 1.03 mmol/L (male), < 1.29 mmol/L (female) or medication - Hypertension: blood pressure ≥ 135/85 mm Hg or medication - Fasting plasma glucose ≥ 5.6 mmol/L or diagnosed T2DM

Abdominal obesity or the so-called hypertriglyceridemic waist phenotype has also been suggested to be the central component of the metabolic syndrome (Frayn 2005), while others consider insulin resistance to be the culprit of the condition (Eckel et al. 2005). Thus far the key component of the metabolic syndrome is still not clear. In figure 4 Eckel et al. (2005) depict the role of excess FFAs in promoting

insulin resistance as a starting point of the metabolic abnormalities. Excess FFAs released from the adipose tissue promote glucose, TG and very low density lipoprotein production in the liver. This is associated with decreased HDL-C and increased low density lipoprotein cholesterol (LDL-C) in the circulation. Increased FFA levels reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. This in turn increases TG accumulation in muscle tissues and reduces the glucose portioning to glycogen. Increased plasma glucose levels promote insulin secretion from the pancreas resulting in hyperinsulinemia. Hyperinsulinemia may also enhance sodium reabsorption and increase the activity of sympathetic nervous system contributing to hypertension.

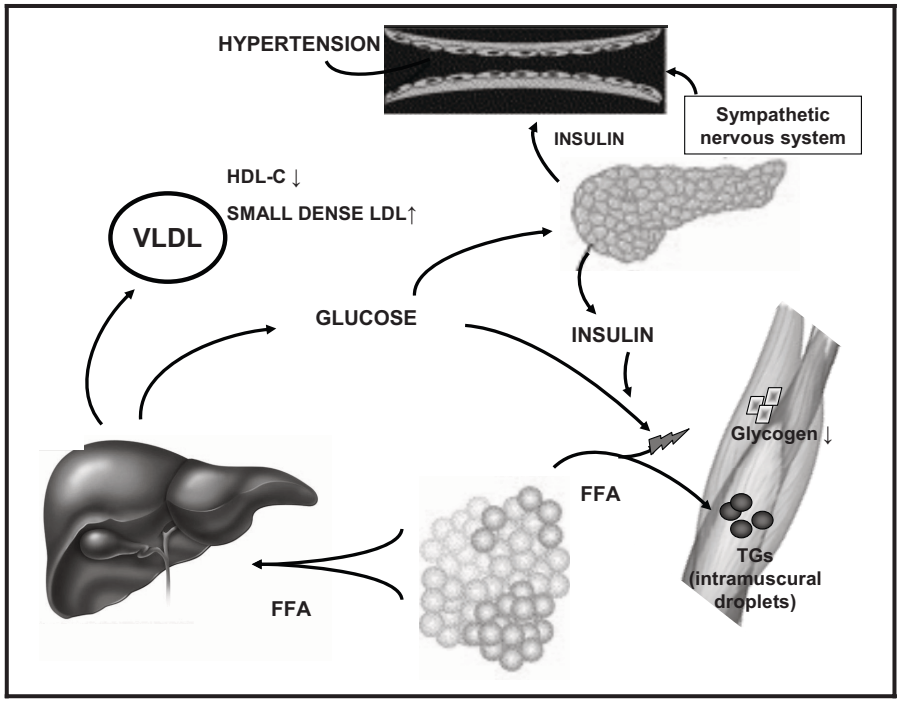

Figure 4. *The key organs in development of the metabolic syndrome. The release of excess free fatty acids (FFA) from visceral or peripheral adipose tissue has effects on lipid and glucose metabolism in multiple organs. In the liver, FFAs promote the production of glucose, triglycerides (TG) and very low density lipoprotein (VLDL). This reduces high density lipoprotein-cholesterol (HDL-C) levels and increases the amount of small dense low density lipoprotein (LDL) particles in the circulation. In muscle, FFAs inhibit insulin-mediated glucose uptake, which in turn increases TG accumulation in muscle. Increased plasma glucose levels increase insulin secretion from the pancreas, which may promote sodium reabsorption and contribute to hypertension. Modified from Eckel et al. (2005).*

2.3 Phenotypic overlap between obesity, T2DM and atherosclerosis

Clinical diagnosis of the metabolic syndrome has been suggested as a means to identify individuals susceptible to obesity, T2DM, familial combined hyperlipidemia (FCHL) and CHD. The metabolic syndrome was approximately five times more common in overweight (BMI > 25 kg/m^2) and 30 times more common in obese individuals (Katzmarzyk et al. 2005). In young adults, small increases in BMI, as well as insulin resistance, were associated with the metabolic syndrome (Weiss et al. 2004). On the hand, CHD events were three times more likely in individuals with the metabolic syndrome (Isomaa et al. 2001; Lakka et al. 2002). Patients with the metabolic syndrome were five to eight times more likely to develop T2DM (Laaksonen et al. 2002). In addition, abnormal lipid levels were observed in more than half of the premature CHD patients, the low HDL-C levels being the most common dyslipidemia (Genest et al. 1992). To conclude, there is phenotypic overlap between obesity, T2DM, CHD and the metabolic syndrome, as these conditions occur in an individual more often than expected by chance.

3 STRATEGIES TO IDENTIFY GENES PREDISPOSING TO OBESITY AND RELATED METABOLIC DISORDERS IN THE POST-GENOME AREA

3.1 Complexity of contributing factors in obesity and related metabolic disorders

3.1.1 Allelic spectra of disease variants

Multiple genetic and environmental risk factors most likely confer susceptibility to complex diseases, including obesity and related metabolic disorders. Each risk factor may only have a minor-to-modest effect on this susceptibility. In addition to the phenotypic complexity due to the overlap of obesity and related metabolic disorders, many other factors complicate the identification of the genetic background of obesity. These factors, listed in table 3, hamper the identification of genes for other complex traits, too.

Table 3. *Multiple factors complicate the identification of the underlying genes for complex disorders. These factors are related to the unknown allelic architecture of the predisposing alleles, difficulties in defining the phenotype, technical issues in genotyping, and statistical analyses.*

Unknown allelic spectra	Unknown allele frequency
	Unknown mode of inheritance
	Unknown quantity of risk provided
	Epistasis
	Genetic heterogeneity
Phenotype	Difficulties in diagnosis
	Late onset of the disease
	Pleiotrophy
	Phenocopies
	Incomplete penetrance or variable expression of the phenotype
	Quantitative phenotypes
Technical issues	Affordable large-scale genotyping methods
Statistical analyses	Multiple testing
	Limited statistical power
Publication bias	A tendency to publish results that appear significant, because negative or near neutral results do not arouse enough interest

It is still debatable what kind of allelic spectrum of the disease alleles underlies common complex diseases (Botstein and Risch 2003; Pajukanta 2004). Common complex diseases do not follow classic Mendelian inheritance patterns that are the dominant, recessive or X-linked mode of inheritance. Thus, they are unlikely to be caused by single gene defects. Several different hypotheses of the allelic spectra of the common complex diseases have emerged. The most well-known hypothesis suggests that the disease-predisposing alleles occur frequently in the population and each of them contributes little to disease susceptibility. This is known as the common variant, common disease (CVCD) hypothesis (Collins et al. 1997). The alternative heterogeneity model suggests that rare alleles with strong phenotypic effects may underlie the genetic background for common diseases. According to the so-called neutral hypothesis, the allelic spectrum of common diseases is similar to that of the allelic spectrum of all the variants in the genome (Wang et al 2005). The potential allelic spectra according to these three hypotheses are presented in figure 5.

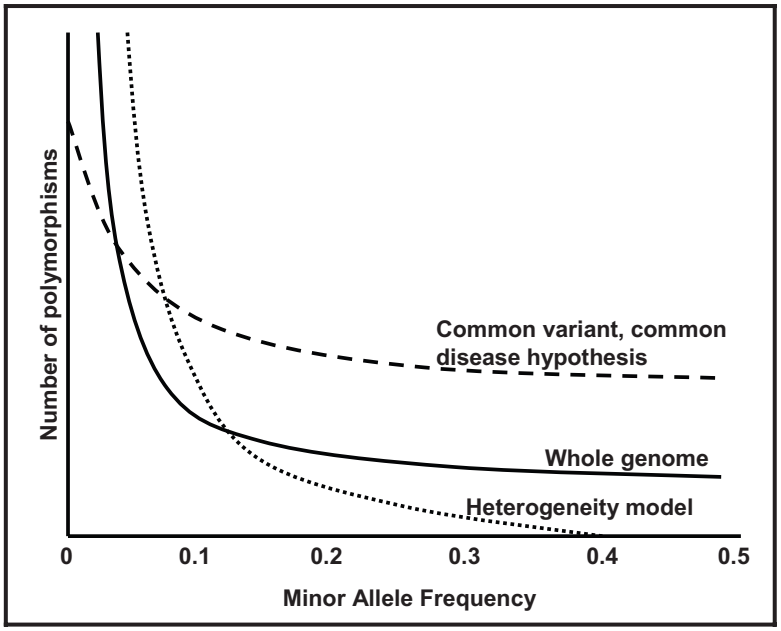

Figure 5. *The depiction of three hypothetical distributions of the allelic spectra of the common diseases. The common variant, common disease (CVCD) hypothesis suggests a large number of high frequency variants. According to the classical heterogeneity model, more rare variants are expected and, thus, more genetic heterogeneity is expected in the population. The solid line shows an estimated allelic spectrum in the whole genome, regardless of whether the variants are disease-causing or not. Modified from Wang et al. (2005).*

In addition to the sample size requirements, the allelic spectrum of the disease in question is important in sampling strategies. Different patterns of decline in disease risk among relatives can be expected for diseases with major gene effects compared to multiple genes with minor effects. In theory, the correlation of traits between related subjects should decrease linearly with distancing of relatedness, if few rare variants with additive and independent effects underlie the disease (Wang et al. 2005). In contrast, in the case of multiple alleles with minor and potentially interdependent effects, the risk may decline more rapidly with a decrease in relatedness. In addition, more genetic heterogeneity is expected in pedigrees when more distant relatives are included.

3.1.2 Genetic forces driving the allelic spectra

Very few disease genes for complex diseases have been identified so far, making it difficult to evaluate the accuracy of each model (for examples of the genes associated with obesity and related metabolic disorders, please see pages 39 and 40).

Certain evolutionary phenomena may have driven the allelic spectra in different directions. Negative selection reduces the frequency of the alleles that have deleterious effects on the phenotype. As a result of the negative selection, the disease alleles should have a low frequency. Positive selection refers to the effect of evolutionary forces that favor certain variants and increases their allele frequencies. Positive selection should result in the common variants conferring more frequently to disease susceptibility. In addition, certain variants that cause disease in homozygous form are known to be beneficial in the heterozygous state, i.e., possess a heterozygote advantage. Because of the heterozygote advantage these variants remain frequent in the population. For example, the variant causing sickle cell anemia in homozygotes is protective against malaria infection in heterozygotes (Ashley-Koch et al. 2000). Random genetic drift has little effect in a large population, whereas, in a small isolated population genetic drift or random sampling of gametes may have an impact on allele frequencies. The knowledge of the allelic spectra typical of common complex diseases would be very helpful when designing statistically powerful studies for disease gene identification.

3.1.3 The risk associated with the disease variants

Another important feature of a disease variant is the amount of risk that it carries. The associated risk has been in most cases small for the disease variants identified so far, with magnitudes of odds ratios of 1,1-2 (Lohmueller et al. 2003). For example, the common pro12-allele of the pro12ala polymorphism in the Peroxisome proliferative activated receptor-γ (*PPARG*) gene has been associated with T2DM (Altshuler et al. 2000). The carriers of the more common pro12-allele have been estimated to exhibit a 1.25-fold increase in risk for T2DM, as reported in a meta-analysis evaluating 16 previously published studies (Altshuler et al. 2000). The small increase in disease risk makes the search for variants difficult, since large sample sizes are necessary to detect statistically significant differences. Although risk for an individual is small, the population attributable fraction may, however, be large when taking into account the allele frequency of a variant. Thus, the variant may explain a considerable portion of the risk in population. In the case of pro12ala polymorphism of the *PPARG* gene, the population attributable fraction is estimated to be as high as 25%, because of the high frequency of the disease predisposing allele (Altshuler et al. 2000).

3.2 Human Genome Project

One major obstacle or at least laborious task in disease gene mapping used to be the creating of complete genetic and physical maps of the region of interest. However, this problem has recently been largely solved, as the public Human Genome Project

(HGP) and the private Celera Company have provided a comprehensive map of the human genome (Venter et al. 2001; Collins et al. 2003).

The HGP started nearly two decades ago when an international multicenter program set an ambitious goal to define the nucleotide sequence of the human genome, in order to understand the genes and the genetic constitution of human beings. In the beginning, a lot of emphasis was placed on developing and improving technologies for genome research and providing better genetic and physical maps of the genome. When genome sequencing had proceeded to the point where 90 percent of the genome's three billion base-pairs were sequenced, first part of the results was published in February 2001 (Lander et al. 2001). This was accompanied with the surprising news that the human genome only consisted of approximately 30,000 genes (Lander et al. 2001). At the same time, the sequence of the human genome was also published by the Celera company (Venter et al. 2001), which recently made the sequence information freely available.

This enormous sequencing project was completed in 2003, two years ahead of the initial schedule, and an accurate and complete human genome sequence was made available to the research community (Collins et al. 2003) *http://www.ncbi.nlm.nih.gov*). The surprisingly low number of human genes, current estimation 20.000-25.000 (International Human Genome Sequencing Consortium 2004), as compared to other species, suggested that the function and understanding of the genome is far beyond the sequence itself (Carninci et al. 2005; Katayama et al. 2005).

3.3 Statistical methods of gene identification

3.3.1 Phenotypes

The phenotypic traits of interest are usually qualitative, i.e., the disease is present or not (for example obesity) or quantitative, i.e. the trait values are continuous (for example BMI). Certain cut-off points can be applied to the quantitative traits to convert them into qualitative. For example, individuals with BMI equal to or more than 30 kg/m^2 can be considered obese (WHO 1995; 1997).

3.3.2 Linkage analysis

A commonly used approach in disease gene mapping is to study families with multiple affected individuals, in order to identify chromosomal regions linked to disease. In linkage studies, the chromosomal location of the gene predisposing to the disease is searched by monitoring co-segregation of a genetic marker with the trait in

pedigrees. Statistical approaches in linkage studies can be divided into parametric and non-parametric.

Linkage analysis determines whether the disease and the polymorphic marker loci co-segregate in a pedigree more often than if the marker was not located physically near the disease mutation. The overall likelihood of the data for two alternative assumptions is calculated in the linkage analysis; first, that the two loci are linked with the given recombination fraction (θ); second, that they are not linked. The logarithm to the base 10 of the ratio of these two likelihoods is the logarithm of odds (lod) score (Z):

$$Z(\theta) = \log_{10} \frac{L(linkage)}{L(no\ linkage)}.$$

For unlinked loci, θ is expected to equal 0.5. The most likely distance between two loci is the recombination fraction at which the lod score peaks. For monogenic disorders, a lod score of 3.0 corresponds to a p-value of 0.001 and is considered significant evidence of linkage, whereas a lod score of -2.0 indicates significant evidence of exclusion of linkage (Ott 1991).

In parametric analyses, also called model-based methods, three parameters need to be estimated prior to any analysis: mode of inheritance, frequency of the disease gene in the population and the penetrance of the disease gene. Parametric linkage analyses have been successfully used in mapping genes for monogenic diseases. However, estimation of the necessary parameters for common complex diseases can be difficult. Errors in estimating the parameters will hamper the possibilities to detect true signals (Clerget-Darpoux et al. 1986).

The major advantage of non-parametric analysis or allele-sharing methods is that they are not dependent on the estimates needed for the parametric linkage analysis. Instead, these methods are based on allele sharing between affected siblings. Under random Mendelian segregation, sibling pairs can share 0, 1 or 2 copies of alleles at any locus with a distribution of 25%, 50% and 25%, respectively. The distribution in the allele sharing can be monitored using a chi-squared test. Excess allele sharing between affected siblings suggests an involvement of the chromosomal region in disease susceptibility. Any two copies of the allele are called identical by state (IBS). If the shared allele is known to be inherited from the common ancestor, the allele is called identical by descent (IBD). Unfortunately, in late-onset diseases the parental information of the affected siblings is often not available, making the IBD estimation difficult. Therefore, a large number of affected siblings is often needed for a powerful affected sibpair analysis.

Quantitative trait locus (QTL) analysis can be performed in order to avoid the arbitrary cut-off points necessary to defining affected and non-affected individuals for continuous variables such as BMI or plasma glucose levels. The QTL analysis is based on the assumption that phenotypic similarity between related individuals is correlated with alleles shared at the locus determining the trait. It has been suggested that QTL analysis will better utilize full information in continuous traits. Ideally, study samples for QTL analysis are not collected to bias towards either end of the continuum of the variable, but instead represent the wide variation present in the general population. For the most part, two methods have been used in the QTL analysis; Haseman-Elston regression (Haseman and Elston 1972) and variance component analysis (Amos 1994; Almasy and Blangero 1998; Blangero et al. 2001). The advantage of the variance component methods is that they can include covariates, gene-environment interactions and other confounding factors within the model.

3.3.3 Association analysis

In genetic association analysis, the allele or genotype frequencies are compared between a group of affected individuals and a control group. Two types of settings for an association study are usually designed: case-control studies and family-based association studies. In classical case-control studies, a sample of unrelated affected individuals and a sample of well-matched unrelated controls are studied. The case-control study designs have been criticized for their potential spurious association due to population stratification. Population stratification refers to the existence of subgroups in the sample, for instance, the mixing of two or more ethnicities may lead to false positive findings (Schulze and McMahon 2002). In addition relatively large sample sizes are required to detect significant difference.

To circumvent the problem of selecting controls, family-based association studies were developed. In the Haplotype Relative Risk approach, the alleles transmitted to affected offspring are designated as case alleles and the "internal" control group is derived from the alleles that are not transmitted from the parents (Falk and Rubinstein 1987; Terwilliger and Ott 1992). The transmission/disequilibrium test (TDT) also utilizes the "internal" control group, but by extracting data only from heterozygous parents (Terwilliger and Ott 1992; Spielman et al. 1993). In the late-onset disease, a difficulty for these types of analyses arises when the parents of the affected individuals are not available. The sib-TDT has been developed to avoid the problem of missing parental information. The sib-TDT utilizes information from unaffected siblings instead of parents (Boehnke and Langefeld 1998). The original TDT has also been extended to accommodate multiple loci, extended pedigrees and quantitative traits (Schulze and McMahon 2002).

Previously, association studies have predominantly been applied to candidate gene studies or fine mapping of a linked region. Advanced genotyping technologies and

data from the HapMap project are making the genome-wide association analyses using dense single nucleotide polymorphism (SNP) maps both affordable and feasible. The genome-wide association analyses may be efficient in detecting the effects of the common variants with modest effects (Risch and Merikangas 1996). However, at the moment genome-wide association analyses are facing a number of challenges, including issues of multiple testing, SNP selection, as well as selection of the study sample (Risch and Merikangas 1996).

3.4 Linkage disequilibrium, founder populations and mixed populations

When alleles of two loci are inherited together with a higher incidence than expected by chance, they are in linkage disequilibrium (LD). There are two different and frequently used metrics to measure LD; D' and r^2 (Zondervan and Cardon 2004). They are both related to the deviation of haplotype frequency in the equilibrium state (D). r^2 is a measure of correlation between a pair of variables, and it is of particular importance in genetic mapping as it is inversely related to the required sample size in association analysis (Wang et al. 2005).

LD is the feature that makes it possible to detect linkage or association between a genetic marker and a disease without genotyping the actual causative marker. Small, isolated founder populations have turned out to be beneficial in isolating genes for rare Mendelian disorders due to the reduced genetic heterogeneity in these populations (Peltonen et al. 1999). Fewer disease alleles exist in founder populations, and the shared haplotypes around the disease mutations are usually extended. As a result, the disease causing mutation in affected individuals may be in complete LD with another variant. However, the mutation usually leads to such dramatic changes in the gene function that it is possible to pinpoint the actual mutation among the harmless polymorphisms by performing functional studies.

The founder populations may also exhibit beneficial features for isolating genes for complex disorders, although, the situation may be more complicated (Peltonen et al. 2000). In rare monogenic disorders just one or few founders usually introduced the disease allele into the population and, thus, the LD around the mutation is extended. The common alleles may have entered the gene pool so many times that the length of the shared haplotype around the disease alleles may be nearly indistinguishable from the more mixed populations (Kruglyak 1999; Shifman et al. 2003; Hirschhorn and Daly 2005). However, there is a potential advantage when studying complex diseases in isolated populations and it is the similarity in environmental factors and life style that people share (Peltonen et al. 2000).

LD that is beneficial in the initial identification of the chromosome region linked or associated with a complex disease, may become a problem when defining the actual causative variant. Causative variants for complex disorders are expected to result in less dramatic changes in gene function. Unfortunately, the tools today are often not efficient enough to separate the causative variant(s) from the harmless ones in LD using functional studies. Overall, data suggest that several isolated populations have modestly extended general LD around common alleles (Kaessmann et al. 2002; Hirschhorn and Daly 2005). For this reason it may be optimal to use isolated founder populations in the initial studies to define the chromosomal region carrying a gene predisposing to the disease. To further restrict the associated region and to reveal the causative variant, it may be helpful to study more mixed populations. This was demonstrated in the positional cloning of the gene predisposing to FCHL on chromosome 1q21-23 (Pajukanta et al. 2004). The LD extended to 46 kb in the region associated with the disease in Finns. A subsequent study in the more mixed Mexican population restricted the region significantly to 14 kb (Huertas-Vazquez et al. 2005).

3.5 HapMap project and haplotype blocks

Starting in October 2002 the International HapMap project set a goal of producing the "next-generation" map of the human genome to facilitate and accelerate the discovery of genes related to common complex disorders (The International HapMap Consortium 2003). The aim was to provide a limited number of tag-single nucleotide polymorphisms (tag-SNPs) for genotyping, in order to cover most of the variation in genome without genotyping redundant SNPs. Within three years the HapMap project was to provide a SNP and haplotype map of the human genome at an extraordinary level of precision. As realized earlier, recombinations in the genome do not happen evenly, but instead occur more in so-called recombination hot-spots, resulting in “blocks” across the genome (Daly et al. 2001; Gabriel et al. 2002). Within one block there is little evidence of historical recombination. SNPs within a block are in considerable LD and, thus, to obtain maximal information of the genetic variation, it is not necessary to genotype all the SNPs in the block. The idea of the haplotype blocks and the way in which the HapMap project is expected to facilitate the identification of disease genes is presented in figure 6a and b.

Unlike the original HGP, the HapMap project has faced a lot of criticism regarding the usefulness of the information provided by the project, taking into account the fact that some genomic regions fit better to the block theory than others (Wall and Pritchard 2003b; Wall and Pritchard 2003a). This may imply that usefulness of the haplotype block method will be uneven throughout the genome (Weiss and Terwilliger 2000; Couzin 2002). By now, there are already some interesting new gene discoveries, the identification of which considerably relied on HapMap data and on haplotype block structure around the associated region for instance the

identification of the complement factor H gene predisposing to macular degenaration (Edwards et al. 2005; Haines et al. 2005; Klein et al. 2005).

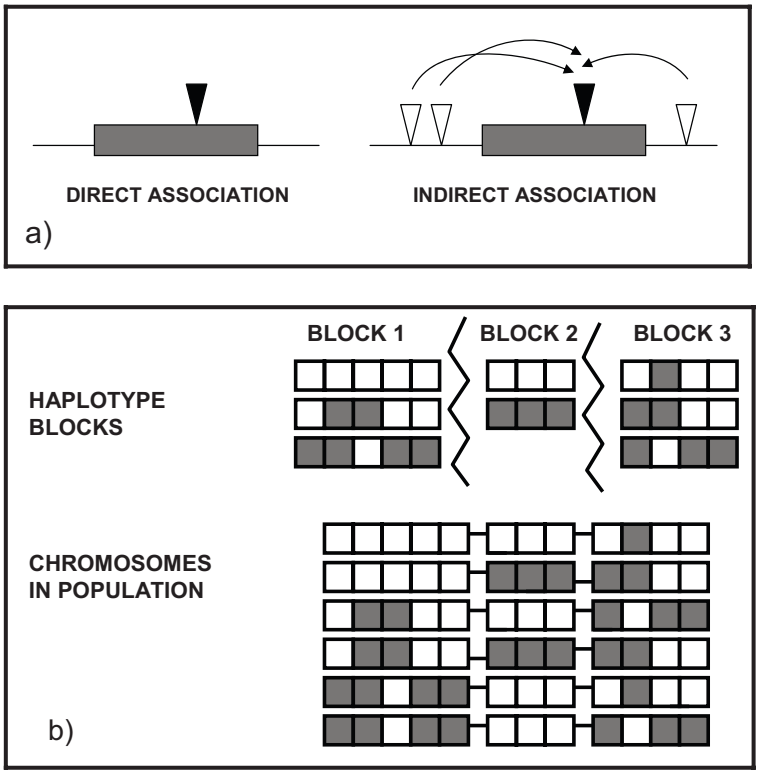

Figure 6. *The idea behind the HapMap project lies in the linkage disequilibrium (LD) between nearby single nucleotide polymorphisms (SNPs) and in the blocks (haplotype blocks) that these SNPs cluster in. An increased amount of LD and a reduced amount of historical recombination can be seen between the SNPs in the haplotype blocks. To identify the SNP causing the disease of interest, the SNP itself does not necessarily need to be genotyped (direct association). Instead, the SNP in LD with the causative one may give a signal of association (indirect association) (a). The knowledge of the haplotype blocks across the genome will help choose SNPs for genotyping, in order to get a maximum amount of information of the variation between individuals with minimal amount of genotyping (b). Modified from Hirschhorn and Daly (2005) and Cardon and Abecasis (2003).*

3.6 Expression studies

The gene expression is the major determinant of the phenotype and function of a living organism. The profile of expressed genes in a particular cell is highly dynamic

and changes rapidly in response to cellular events and external stimuli. Methods have been available to measure the gene expression of a limited number of genes at a time. Recently, DNA microarray technology has provided a tool to monitor the expression pattern of a whole genome simultaneously on a single chip (Lockhart and Winzeler 2000). In this process total RNA from a particular tissue sample is extracted, reverse transcribed and hybridized on a slide containing oligonucleotides corresponding to thousands of different genes. Using microarray technology it is possible to determine a set of genes expressed or turned off together. Consequently, the technique should allow identification of the genes that are differentially expressed in healthy and diseased individuals and, thus, to get a clue of the pathways important in the disease process.

3.7 Comparative genomics

After completion of the enormous HGP, genomic sequences of many other organisms have been completed and released. By the end of September 2005 complete genome sequences of altogether 283 organisms were publicly available, including 3 animals in addition to human, 2 plants, 9 fungi, 5 protists and 263 prokaryotes. In addition to these complete sequences, draft sequences of many other mammals are also available such as the draft sequences of chimpanzee, rat, dog and cow (*http://www.ncbi.nlm.nih.gov/genomes/static/gpstat.html*).

It is apparent that exonic regions of the genes are highly conserved between different species. In addition to exons, certain intronic and intergenic sequences harbor regions that are highly conserved between species. These conserved regions are likely to contain important regulatory regions. Comparing sequences of co-regulated genes within species may also lead to the detection of conserved regions that carry a binding site for a transcription factor or motifs, i.e., clusters of binding sites for transcription factors. Although regulatory regions probably constitute only a small proportion of all the non-coding sequences, they still determine the level, location and chronology of gene expression (Pennacchio and Rubin 2001).

A number of transcription factor binding sites have been described in the literature. Great effort has been made to collect these empirically tested transcription factors and their binding sites to a single catalogue, for example TRANSFAC (Wingender 1988). This information can be used to search for new binding sites of previously known transcription factors. The difficulty in the identification of the new sites lies in the nature of the transcription factor binding sites; they are typically short sequences that carry a small invariant core sequence (4-6 bases) surrounded by a variable number of degenerate nucleotides. Full control of the gene expression is considered to involve multiple transcription factors, including both enhancers and silencers that together coordinate the activity of the gene.

MicroRNAs are an interesting genomic feature of gene regulation that was recently identified (Ambros 2004; Ying and Lin 2004). They are short fragments, approximately 22 nucleotides, encoded by microRNA genes and transcribed to RNA, but not translated to protein. MicroRNAs regulate gene expression by binding to the untranslated regions (UTR) of the messenger RNAs and, consequently, promote their degradation. To identify typical microRNA binding sites, Xie et al. (2005) compared UTRs of all the known human and mouse genes. They found multiple conserved sequences with strong directional bias to forward strand and to 8-base length (Xie et al. 2005). This could be reminiscent of a feature of mature microRNAs that tend to start with a U base followed by a complementary sequence of seven bases, suggesting that most of the identified sequences actually could represent microRNA binding sites.

4 GENETIC VARIATIONS ASSOCIATED WITH OBESITY

4.1 The thrifty gene hypothesis

The ”thrifty gene” hypothesis was initially introduced by James Neel in 1962 as an attempt to explain the increase in T2DM prevalence (Neel 1962). He suggested that genes or genotypes responsible for improved energy storage during famine and starvation provided a survival advantage at a time when humans were hunter-gatherers, and there have been periods of time when the food supply was plentiful followed by periods of famine. All food in the stone-age was obtained via extensive physical activity. Thus, the lives of our ancestors alternated between shortage and abundance, the latter possibly occurring after successful hunting tending to lead to reduced physical activity. Excessive energy consumed during this period was stored as TGs in adipose tissue (and glycogen in muscles) referred as thrifty storage. When this was followed by decreased amounts of food available with possible famine, considerable physical activity was needed to provide food again. Individuals with maximal energy storing capabilities during time of abundance, combined with their economical usage of the stored energy during famine, were probably the most capable of surviving the physical rigors of life. Genes or genetic variations enhancing these features were restored in the human genome during the evolution.

Dramatic changes have occurred in the process of food supply within the last thousands of years, which is still a short period in evolutionary terms. Nowadays food supply is constant and plentiful, obtainable with minimal physical effort, consequently creating a so-called “obesogenic” environment. Most of the job descriptions of people in Western societies do not include physical labor, as neither do leisure time activities. Therefore, the body of the human being designed to

function in the cycles of abundance and shortage have stalled to the abundance step prepared to take on the next shortage. When the shortage or extensive physical activity never arrives, the properties of efficient energy storage become detrimental, predisposing to diseases typical to Western societies, such as overweight, obesity and T2DM. Thus, genes previously beneficial are now causing diseases.

In accordance with the thrifty gene hypothesis, it has been suggested that excess fat should not be considered as a disease, i.e., a biological abnormality of an individual, but instead as a collective adaptation to the pathological pressure of the environment to eat too much and exercise too little (Bell 2005).

4.2 Genetic epidemiology of obesity and related metabolic disorders

Obesity aggregates in families, but the pattern of inheritance does not in most cases follow any Mendelian segregation. This suggests a complex mode of inheritance, and the proportion of obesity due to genes is somewhat difficult to predict. To quantify the familial aggregation, the recurrence risk λ_R can be estimated (Risch 1990a). λ_R is the disease risk for a relative of an affected person divided by the disease risk in the general population. The λ_R value does not directly reflect the genetic proportion, since obesity may aggregate in families due to shared genes or shared environment. If λ_R varies according to the degree of genetic relatedness, being larger for closer relatives than for more distant relatives (for example monozygotic twins versus dizygotic twins and other first degree relatives, or first degree relatives versus second degree relatives), it suggests that there are genetic components behind the disease (Risch 1990b; Risch 1990a). The recurrence risk for siblings (λ_S) is often used and it is obtained by dividing the risk ratio for siblings by the risk in the general population. Heritability (h^2) refers to the actual genetic proportion of the disease risk and can be derived from λ_R values obtained from different kinds of relatives. In a broad sense, heritability can be defined as the proportion of the total phenotypic variability V_P (=variance due to additive genetic effects V_A, dominant genetic effects V_D and environmental effects V_E) due to genetic effect V_G (= additive and dominant effects). In a narrow sense, heritability is the proportion of the total phenotypic variability V_P due to the additive genetic effect V_A.

For obesity, λ_R has been calculated for different kinds of relatives using varying population percentiles or BMI cut-offs (Allison et al. 1996a; Lee et al. 1997). The risk for obesity (defined as 90th BMI percentile or BMI $\geq$ 30 kg/m^2) was two to three times higher for a person with family history of obesity. The risk increased with the severity of obesity, with estimates between λ_R of 3-6 for the 95th percentile cut-off point or for BMI $\geq$ 40 kg/m^2.

Heritability values have been estimated from twin, family and adoption studies, the estimates varying from 5% to as high as 90% (Loos and Bouchard 2003). Large variation in the heritability estimates may partly be explained by the study design and by the kinds of relatives upon which the results are based. Studies on monozygotic and dizygotic twins or monozygotic twins reared apart give the highest heritability estimates, of the order of 70% (Stunkard et al. 1990; Allison et al. 1996b; Maes et al. 1997). Adoption studies suggest the lowest heritability with the values clustering around 30% (Stunkard et al. 1986; Sorensen et al. 1992a; Sorensen et al. 1992b; Vogler et al. 1995). Results from the family studies are intermediate between the twin and adoption studies (Rice et al. 1999). Certain diseases and traits that co-occur with obesity also show high heritability (table 4). Twin studies also suggest a considerable genetic component to eating behavior (Tholin et al. 2005).

Table 4. *Heritability estimates and population prevalence of different traits related to obesity.*

Trait	Population prevalence	Heritability
Obesity	30%	30-70 (Loos and Bouchard 2003)
T2DM	5-10%	40-80 (Permutt et al. 2005)
Metabolic syndrome	7-43%	24-61 (McQueen et al. 2003; Eckel et al. 2005; Lin et al. 2005)
TG levels	Quantitative trait	40-80 (Lander et al. 2004)
TC levels	Quantitative trait	40-60 (Lander et al. 2004)
HDL-C levels	Quantitative trait	45-75 (Lander et al. 2004)
Leptin levels	Quantitative trait	40-60 (Comuzzie et al. 1997; Rotimi et al. 1999)
Eating behavior	Quantitative trait	45-60 (Tholin et al. 2005)

* Abbreviations: high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), type 2 diabetes mellitus (T2DM)

Gene-diet and gene-environment interactions have been suggested in the etiology of obesity. Obesity and T2DM are very common among Pima Indians living in United States, and they provide an example of the gene-environment interactions (Ravussin et al. 1994). Pima Indians living in a remote mountain area in Mexico have lower prevalence of obesity and T2DM than those living in more obesogenic areas in the United States. A gene-diet interaction has been observed in experimental studies performed on monozygotic twins (Bouchard et al. 1990; Hainer et al. 2000). These studies show that monozygotic twin pairs have less variation in response to positive or negative energy balance compared to the variation between twin pairs.

4.3 Genes associated with human obesity and related metabolic traits

Numerous studies using both candidate gene and genome-wide approaches have attempted to identify genes predisposing to obesity. The latest Human Obesity Gene Map published in March 2005 was the 11th in a series of reviews collecting and reporting the literature published on genes related to obesity (Perusse et al. 2005). The findings on obesity genes so far provide support for both the CVCD hypothesis and for the classical heterogeneity model.

4.3.1 Monogenic forms of obesity

Table 5 shows the genes identified so far harboring variants that either segregate with obesity in families, or the variants are only found in obese individuals, or the variants have been causally linked to obesity (Perusse et al. 2005). However, obesity is seldom caused by a single gene defect in general population (Bell et al. 2005).

Table 5. *Genes causing monogenic forms of obesity in humans.*

Gene	Reference
Leptin (LEP)	(Montague et al. 1997; Strobel et al. 1998; Ozata et al. 1999)
Leptin receptor (LEPR)	(Clement et al. 1998)
Pro-opiomelanocortin (POMC)	(Krude et al. 1998; Challis et al. 2002)
Prohormone convertase-1 (PC1) = Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)	(Jackson et al. 1997; Jackson et al. 2003)
Melanin-concentrating hormone receptor 1 (MCHR1) = G protein-coupled receptor 24 (GPR24)	(Gibson et al. 2004)
Melanocortin-3 receptor (MC3R)	(Lee et al. 2002)
Melacortin-4 receptor (MC4R)	(Vaisse et al. 1998; Yeo et al. 1998; Perusse et al. 2005)
Corticotropin-releasing hormone receptor-1 (CRHR1)	(Challis et al. 2004)
Corticotropin-releasing hormone receptor -2 (CRHR2)	(Challis et al. 2004)
bHLH-PAS transcription factor (SIM1)	(Holder et al. 2000; Faivre et al. 2002)

The cloning of the *ob* gene in the mouse and its human homologue, Leptin (Zhang et al. 1994) provided the first example of a causal relationship between a mutation and obesity. Two different mutations disrupting the structure of the Leptin gene have so far been identified in 6 morbidly obese children (Montague et al. 1997; Strobel et al. 1998; Ozata et al. 1999). When these children were treated with the recombinant leptin protein, their weight was dramatically reduced (Farooqi et al. 1999).

Unfortunately, the lack of or reduced amount of leptin protein in obese individuals does not seem to be a common cause of obesity. Rather, obese individuals often have elevated serum leptin levels and leptin resistance (Considine et al. 1996; Ren 2004).

In addition to genetic defects mostly affecting body weight, numerous syndromes featuring obesity as one of the symptoms have been mapped to certain chromosomal loci, and for some of these the underlying gene has been identified (Perusse et al. 2005). Of these syndromes, the Prader-Willi syndrome is the most common one affecting every 16,000-25,000 newborn a year (Burd et al. 1990; Butler 1990). The Prader-Willi syndrome is an imprinting disorder that is usually caused by a deletion of a paternally inherited chromosome 15q region. In addition to defects in the nuclear genome, mutations in the mitochondrial genome have been associated with obesity-related disorders (Wilson et al. 2004).

Of these rare forms of obesity, mutations in *MC4R* are by far the most common, and their prevalence has been estimated to be as high as 4% among obese children (Farooqi et al. 2000; Vaisse et al. 2000; Perusse et al. 2005). The degree of obesity in individuals carrying an *MC4R* mutation varies and these individuals are usually also taller. A recent meta-analysis suggests that the common allele of the val103ile variant in the coding region of the *MC4R* is associated with obesity, whereas the rare allele (ile103) with a frequency of 4% was more common in lean individuals (Geller et al. 2004). The ile103 allele was also associated with lower BMI in a large population-based sample (Heid et al. 2005).

4.3.2 Common forms of obesity

Multiple genome-wide scans have been performed for obesity and traits related to body composition (Perusse et al. 2005). Typically in complex disorders, the first identification of a region linked to the disease does not automatically lead to replication in a follow-up study performed in another study sample. However, evidence of linkage (lod score > 3.0 or $p < 0.001$) for obesity and traits related to body composition has been identified in several studies for certain chromosome regions, see figure 7.

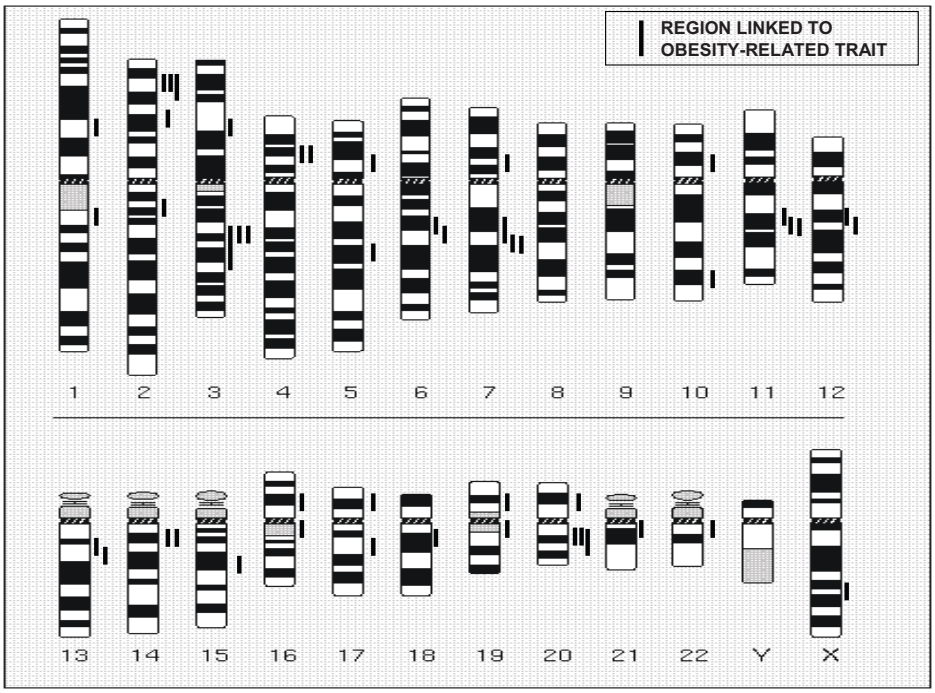

Figure 7. The chromosomal regions showing evidence of linkage (lod score > 3.0 or p < 0.001) to obesity, BMI, body composition, waist circumference, leptin levels and adiponectin levels in genome-wide scans. References: (van der Kallen et al. 2000) chr 1 and 10; (Ng et al. 2004) chr 1; (Comuzzie et al. 1997) and (Deng et al. 2002) and (Chen et al. 2005a) chr 2; (Moslehi et al. 2003) chr 2, 3 and 11; (Palmer et al. 2003) chr 2 and 12; (Kissebah et al. 2000) chr 3 and 17; (Watanabe et al. 2000) chr 3 and 13; (Wu et al. 2002) chr 3; (Stone et al. 2002) and (Arya et al. 2004) chr 4; (Comuzzie et al. 2001) chr 5 and 14; (Gorlova et al. 2003) chr 5, 16 and 20; (Fox et al. 2004) and (Meyre et al. 2004) chr 6; (Borecki et al. 1994) chr 7 and 20; (Feitosa et al. 2002) chr 7 and 13; (Adeyemo et al. 2003) chr 7 and 11; (Platte et al. 2003) chr 7; (Hager et al. 1998) chr 10; (Hanson et al. 1998) chr 11; (Li et al. 2004) chr 12 and 21; (Chagnon et al. 2001) chr 14 and 19; (Chagnon et al. 2000) chr 15 and 18; (Geller et al. 2003) chr 16; (Bell et al. 2004) chr 17 and 19; (Lee et al. 1999) and (Hunt et al. 2001) chr 20; (Martin et al. 2002) chr 22; (Ohman et al. 2000) chr X.

Multiple genes have been associated with common forms of obesity, although only few of them have been replicated in other studies (Perusse et al. 2005). This can be explained by several circumstances including the small risk that the disease-associated variant presents, small sample sizes of the studies, and LD between the actual causative variants and the variants tested in the study. The gene-gene or gene-environment interactions and phenotypic heterogeneity may also complicate the analysis if the study populations have different haplotype backgrounds or different

environmental exposures. Despite these difficulties, the initial associations of some genes with obesity or related phenotypes have been replicated. The genes for which at least five different studies found association with obesity or obesity related phenotypes include Adiponectin, Adrenergic, beta-2- and beta-3- receptors (ADRB2 and ADRB3), Guanine nucleotide binding protein (G protein), beta polypeptide 3 (GNB3), Interleukin 6 (interferon, beta 2) (IL6), Insulin, Leptin (LEP), Leptin receptor (LEPR), Lamin A/C (LIPE), Nuclear receptor subfamily 3, group C, member 1 (NR3C1), *PPARG*, Tumor necrosis factor TNF superfamily, member 2 (TNF), as well as Uncoupling protein proteins 1, 2 and 3 (mitochondrial, proton carrier) (UCP1, UCP2 and UCP3) (Perusse et al. 2005).

Recently marked progress has been made in the identification of obesity-predisposing genes using genome-wide linkage and subsequent fine mapping studies (Boutin et al. 2003; Bell et al. 2005; Meyre et al. 2005a). The benefit of the positional cloning strategy is that it does not rely on any pre-existing knowledge of the genes that underlie the investigated trait. Particularly, for conditions such as obesity this may be useful since there is as yet limited information available, for instance regarding the appetite regulation. The first candidate gene for obesity identified through the genome wide approach was Glutamate decarboxylase 1 (*GAD2)* on chromosome 10p12 (Boutin et al. 2003). It encodes the glutamic acid decarboxylase enzyme GAD65 and may be connected to obesity via the hypothalamic regulation of food intake. *GAD2* is involved in the formation of the γ-aminobutyric acid (GABA) from the glutamic acid. GABA functions together with neuropeptide Y in the paraventricular nucleus to increase food intake. Later, *GAD2* polymorphisms were associated with childhood obesity, birth weight and binge eating (Meyre et al. 2005b). However, a recent replication study involving German, American and Canadian populations did not confirm the association with obesity (Swarbrick et al. 2005).

Another interesting example is the Ectonucleotide pyrophosphatase/ phosphodiesterase 1 *(ENPP1)* gene in chromosome 6q22-23. This 6q22-23 region has been linked to obesity (Atwood et al. 2002; Meyre et al. 2004), BMI (Atwood et al. 2002), insulin secretion (Duggirala et al. 2001; Abney et al. 2002) and T2DM (Ehm et al. 2000; Ghosh et al. 2000; Demenais et al. 2003; Xiang et al. 2004). Recently, variants in *ENPP1* were identified to be associated with obesity and T2DM (Meyre et al. 2005a). *ENPP1* encodes a prohormone convertase-1 enzyme which inhibits the insulin receptor kinase activity and subsequent cellular signaling of insulin (Maddux et al. 1995; Maddux and Goldfine 2000). *ENPP1* is also involved in the post-translational processing of the propeptide that is encoded by the *POMC* gene. Splicing of the propeptide produces adrenocorticotropin, β-lipotropin, α-, β- and γ-melanocyte-stimulating hormones.

4.3.3 Genes conferring susceptibility to obesity-related metabolic traits

In addition to genes primarily identified to cause or increase susceptibility to obesity, gene variations have been identified that contribute to variation in TG, HDL-C and TC levels or predispose to diabetes. Examples of such genes are given in table 6.

Table 6. *Examples of genes with common variations associated with obesity-related metabolic traits.*

Gene	Chromosome	Trait	Population
ATP-binding cassette, sub-family A (ABC1), member 1 (*ABCA1)*	9q31	HDL-C	(Cohen et al. 2004; Frikke-Schmidt et al. 2004)
Upstream transcription factor 1 (*USF1)*	1q21	TG FCHL	(Pajukanta et al. 2004; Huertas-Vazquez et al. 2005)
Lipoprotein lipase (*LPL)*	8p22	TG	(Hokanson 1997; Wittrup et al. 1999)
Apolipoprotein E *(APOE)*	19q13.2	TC TG	(Zannis and Breslow 1981; Dallongeville et al. 1992)
APOC3/A4/A5 gene cluster	11q23	TG	(Pennacchio et al. 2001; Talmud et al. 2002)
Peroxisome proliferative activated receptor-γ *(PPARG)*	3p25	T2DM	(Deeb et al. 1998; Altshuler et al. 2000)
Calpain 10 (*CAPN10)*	2q37.3	T2DM	(Horikawa et al. 2000; Weedon et al. 2003)
Hepatocyte nuclear factor 4-α (*HNF4A)*	20q13	T2DM	(Love-Gregory et al. 2004; Silander et al. 2004)

* Abbreviations: familial combined hyperlipidemia (FCHL), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), type 2 diabetes mellitus (T2DM)

4.4 Animal models of obesity and related traits

Numerous naturally occurring monogenic and polygenic obese mouse strains have been identified that manifest increases or decreases in adiposity, as well as show features related to body weight regulation, such as eating behavior or energy metabolism (Robinson et al. 2000; Perusse et al. 2005). Transgenic and knock-out animal models of obesity have also been created using recombinant DNA technology. Animal studies have provided a large amount of valuable information regarding the physiology and molecular mechanisms of body weight regulation. Some of them have lead to the identification of mutations in the corresponding human homologue or in the genes acting on the same pathway, for example genes in

the leptin-melanocortin pathway (O'Rahilly et al. 2003), see table 7. However, identification of a mutated gene in an animal model does not automatically lead to detection of a defect in the human homologue, which may reflect the heterogeneous phenotype and genetic heterogeneity in humans and also reflect the difficulties of performing studies that are as tightly controlled as animal studies.

Table 7. *Genes in the leptin-melanocortin pathway that were identified in spontaneous mouse strains or studied in recombinant mice models, and the related human gene harbouring obesity-associated variations.*

Mouse strain	**Mouse gene**	**Human gene**
ob/ob	Leptin (*Lep*)	Leptin (*LEP*)
db/db	Leptin receptor (*Lepr*)	Leptin receptor (*LEPR*)
fat/fat	Carboxypeptidase E (*Cpe*)	Prohormone convertase-1 (PC-1)
agouti	Agouti Signaling Protein (*ASP*)	Agouti-related protein (*AGRP*)
mahogany	Attractin (*Atrn*)	Attractin (*ATRN*)
MC4R-KO	Melanocortin-4 receptor (*Mc4r*)	Melanocortin-4 receptor (*MC4R*)
POMC-KO	Pro-opiomelanocortin (*Pomc*)	Pro-opiomelanocortin (*POMC*)

* knock-out (KO)

AIMS OF THE PRESENT STUDY

The aim of the present study was to investigate the genetic background of obesity and related metabolic disorders by addressing the following specific aims:

- To investigate the prevalence of monogenic forms of obesity caused by mutations in the Melanocortin-4 receptor gene (*MC4R*) on chromosome 18q22) and Pro-opiomelanocortin gene (*POMC*) on chromosome 2p23.3, and to test the common variants in these genes for association with obesity related traits

- To examine in humans the possible role of the Lipin 1 gene (*LPIN1*) on chromosome 2p25.1 previously identified for lipodystrophy, hypertriglyceridemia and insulin resistance in mice

- To fine map six chromosomal regions: 2q31, 8q23, 10q11, 16q24.1-24.2, 20q13.11 and Xq24 previously linked to obesity, CHD, FCHL, high serum TG and low HDL-C levels.

MATERIALS AND METHODS

Materials or methods	Original publication
Study samples	
Swedish cases and controls	I, III
Obesity families	III
Finnish cases and controls	II, III
Low HDL-C families	II, IV
FCHL families	II, IV
DNA isolation	I-IV
Single-strand conformation polymorphism (SSCP) analyses	I
SNP genotyping	I-III
Sequencing	I, III
Microsatellite marker genotyping	III, IV
Fat biopsies and RNA extraction	II
Expression array analysis of adipose tissue	II
Statistical analyses	
Regression analysis	I
Chi-squared tests	I-III
Affected sibpair analyses	III
LD estimation	II, III
Family based association tests	II
Analysis of Covariance	II
QTL analyses	IV

1 STUDY SUBJECTS

Five different study samples from the following sources were included in this study. The Swedish obese cases and lean controls were collected at the Obesity Clinic in Malmö, Sweden (study I and III). The Finnish obese families were collected at the Helsinki University Central Hospital, the Tampere University Hospital, and the Peijas Hospital in Vantaa, as well as from the participants of the Finnish Twin Cohort (study III) (Kaprio 1994). The Finnish obese cases and lean controls were recruited from the Helsinki University Central Hospital, the National FINRISK97 cohort (Vartiainen et al. 2000) and the Botnia study (Parker et al. 2001; Lindgren et al. 2002) (study II and III). The low HDL-C families and FCHL families were collected at the Helsinki and Turku University Central Hospitals as part of the EUFAM study (study II and IV). Each study subject provided a written informed consent prior to participating in the study. All samples were collected in accordance with the Helsinki declaration, and the ethics committees of the participating centers approved the study design.

1.1 Family samples

1.1.1 Obese families

The family sample for obesity (BMI $\geq$ 30 kg/m^2) included 218 obese sibpairs from 184 Finnish nuclear families. Of these sibpairs, 54 were recruited through a proband attending the weight-reduction program at the Helsinki University Central Hospital. These families had at least one additional obese sibling with a BMI $\geq$ 32 kg/m^2. Another 46 sibpairs from 34 families were ascertained through the Finnish Twin Cohort. These sibpairs were dizygotic twins, with their obese siblings all having a BMI $\geq$ 32 kg/m^2. In addition, 93 sibpairs from 79 families were collected in the weight-reduction groups at the Helsinki University Central Hospital, the Tampere University Hospital, and the Peijas Hospital, as well as screened from the participants of the Finnish Twin Cohort (Kaprio 1994). The probands from the weight-reduction groups enrolling in the study had a sibling with a BMI $\geq$ 30 kg/m^2. Of the dizygotic twins, both twins had a BMI $\geq$ 30 kg/m^2. We also included 25 Finnish obese sibpairs (BMI $\geq$ 30 kg/m^2) from the families ascertained for FCHL or low serum HDL-C levels.

Altogether 467 individuals were genotyped. Of these, 398 individuals had a BMI $\geq$ 30 kg/m^2 and were coded as affected (157 males / 241 females, age = 50.7 $\pm$ 8.4 years, BMI = 35.8 $\pm$ 5.9 kg/m^2). In addition, 59 unaffected family members were

genotyped to increase the phase information. On average, 2.2 affected members were present in these families.

1.1.2 FCHL and low HDL-C families

The proband in each FCHL and low HDL-C family was diagnosed for premature CHD. In the FCHL families, the proband had also elevated TG and/or TC levels according to the Finnish 90th age- and sex-specific population percentile based on the population surveys FINRISK and LASERI (Porkka et al. 1994; Vartiainen et al. 1994; Vartiainen et al. 2000). In addition to the proband, at least two affected family members were required in each of the families before any family was included in the study. The inclusion and exclusion criteria for the probands are presented in table 8. The family members were coded as affected if they had the combined hyperlipidemia, i.e., had elevated TG and TC levels or if either of these traits exceeded the 90th population percentile level. These criteria follow the original diagnostic criteria for FCHL, as suggested by Goldstein et al. (1973).

Table 8. *Diagnostic criteria for the familial combined hyperlipidemia (FCHL) probands (Pajukanta et al. 1998; Pajukanta et al. 1999).*

INCLUSION CRITERIA
Age 30-55 years for males, < 60 years for females
Premature coronary heart disease (angiographically verified > 50% stenosis in at least one coronary artery or survived myocardial infarction)
TC and/or TGs > age-sex specific Finnish 90th percentiles
At least one first degree relative affected by FCHL (high TC and/or high TGs)[1]
EXCLUSION CRITERIA
Diabetes mellitus, type 1
Severe renal or hepatic disease
Hypothyreosis
Familial hypercholesterolemia[2]

[1]If the proband had only one elevated lipid trait, a first-degree relative had to have the combined phenotype. [2]Familial hypercholesterolemia was excluded by determining the low density lipoprotein -receptor status of the proband by the lymphocyte culture method (Cuthbert et al. 1986). Abbreviations: familial combined hyperlipidemia (FCHL), total cholesterol (TC), triglyceride (TG)

In the low HDL-C families, the proband had premature CHD, as well as HDL-C levels below the 10th age and sex-specific population percentile values (Porkka et al. 1994; Vartiainen et al. 1994; Vartiainen et al. 2000). The inclusion and exclusion criteria for the probands are listed in table 9. In addition to the proband, at least two members in these families were affected.

Table 9. *Diagnostic criteria for the low high density lipoprotein cholesterol (HDL-C) probands (Lilja et al. 2002; Soro et al. 2002).*

INCLUSION CRITERIA
Age 30-60 years
Premature coronary heart disease (angiographically verified > 50% stenosis at least in one coronary artery or survived myocardial infarction*)
HDL-C < 10th age-sex specific Finnish population percentiles
At least three accessible first-degree relatives
At least one first degree relative affected by low HDL-C
EXCLUSION CRITERIA
Diabetes mellitus, type 1 diabetes mellitus and type 2 diabetes mellitus
Body mass index > 30 kg/m^2
Severe renal or hepatic disease
Hypertriglyseridemia (TG > 2.3 mmol/l for both genders)
Hypercholesterolemia (TC > 6.3 mmol/l for males, > 6.0 mmol/l for females)

**Following three criteria had to be fulfilled for the myocardial infarction diagnosis: (1) typical clinical symptoms, (2) definite electrocardiographic findings, according to the Minnesota coding (WHO criteria) (Rose et al. 1982), and (3) elevated levels of the creatine-kinase enzyme (CK) and its cardiac isoenzyme, CK-MB. Abbreviations: familial combined hyperlipidemia (FCHL), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG)*

All accessible family members from the FCHL and low HDL-C families were included in the study and examined for BMI, serum TG, TC, HDL-C, high density lipoprotein cholesterol particles 2 and 3, apolipoprotein A1, apolipoprotein A2, LDL-C, apoliporotein B, LDL particle size, insulin levels, blood glucose levels, as well as for hepatic and lipoprotein lipase activities. The combined study sample of 53 FCHL and 39 low HDL-C families comprised 1109 genotyped individuals. Because T2DM and obesity were not excluded in the FCHL families, some patients also fulfilled the criteria for the metabolic syndrome. The characteristics of the pedigrees are shown in table 10.

Table 10. *Charasteristics of the FCHL and low HDL-C families to show the number of generations, number of affected subjects and affected sibpairs for TG, TC, FCHL, and HDL-C.*

	53 Finnish FCHL families	39 Finnish low HDL-C families
No. of individuals, M/F	431/430	258/276
No. of probands, M/F	35/18	32/7
No. of families in each group/Mean no. of individuals of which DNA is available in each family type:		
2 generations	3/6	1/6
3 generations	42/17	32/13
4 generations	7/24	5/20
5 generations	1/51	1/33
No. of affected subjects, M/F; Mean no. of affected subjects in families is given in parenthesis:		
TG > 90th percentile	91/111 (4)	10/39 (1)
TC > 90th percentile	110/116 (4)	21/24 (1)
FCHL[1]	132/162 (5)	29/54 (2)
HDL-C < 10th percentile	117/120 (4)	95/79 (5)
No. of affected sibpairs (independent):		
TG > 90th percentile	66	8
TC > 90th percentile	59	4
FCHL[1]	111	12
HDL-C < 10th percentile	72	62

[1]TG and/or TC > 90th percentile. Abbreviations: M = male; F = female; FCHL= familial combined hyperlipidemia; HDL-C = high density lipoprotein cholesterol; TG = triglycerides; TC = total cholesterol

1.2 Case-control study sample for obesity

For the initial association study (III), one obese male from each of the 184 original nuclear families ascertained for obesity was selected when present (n = 117). For the replication study (III), an independent study sample was collected in Finland and Sweden. The obese individuals were recruited from the Obesity Clinics in Helsinki and in Malmö, from the National FINRISK97 cohort in Finland (Vartiainen et al. 2000), and from the Botnia study (Parker et al. 2001; Lindgren et al. 2002). All the obese subjects at the Obesity Clinics in Finland and Sweden had a BMI > 30 kg/m^2. These patients had contacted the obesity clinic on their own initiative and when first contacting the clinic, the patients had a BMI > 35 kg/m^2. The obese cases selected from the National FINRISK97 cohort had a BMI > 35 kg/m^2. The obese males selected from the Botnia study had a BMI > 30 kg/m^2 and at least one additional

sibling with a BMI > 30 kg/m^2. Altogether 968 lean control subjects with a BMI ≤ 25 were collected from the same geographical regions as the obese cases. Of these lean controls, 514 were males and 195 females from Finland, 73 males and 186 females from Sweden. The age and BMI distributions of the study sample are presented in table 11.

Table 11. *Mean age and body mass index (BMI) of the Finnish and Swedish subjects in the case-control study sample for obesity.*

	All		Finnish		Swedish	
	Cases (M/F)	Controls (M/F)	Cases (M/F)	Controls (M/F)	Cases (M/F)	Controls (M/F)
n	837 (421/416)	968 (587/381)	568 (349/219)	709 (514/195)	269 (72/197)	259 (73/186)
Age (years)	48.5 ±12.3	49.1 ±11.3	51.5 ±10.7	52.1 ±9.6	41.9 ±12.2	44.8 ±14.0
BMI (kg/m^2)	39.8 ±6.6	23.0 ±1.4	38.7 ±6.4	23.4 ±1.5	41.1 ±6.9	22.3 ±1.7

** Age and BMI values are given as mean ± standard deviation. Abbreviations: M = male; F = female*

The Swedish obese and lean individuals were included in the study of the *POMC* and *MC4R* genes (I). In the study of the *LPIN1* gene (II), the Finnish obese cases and lean controls were investigated.

2 METHODS

2.1 Single-strand conformation polymorphism analyses

The exonic regions of *MC4R* and *POMC* were screened for mutations in overlapping PCR fragments using single-strand conformation polymorphism (SSCP). The *MC4R* gene was screened for mutations using radioactive SSCP as described in study I. When screening *POMC*, a real time electrophoresis system was applied with fluorescently labelled (HEX) primers (Gel Scan 2000, Corbett Research, Sydney, Australia). The amplified samples were diluted 1:1 with formamide containing 0.025% bromophenol blue and heat denatured for 3 min at 90°C. Aliquots (1 µl) of the diluted samples were loaded on two 5% polyacrylamide gels (49:1 acrylamide:polyacrylamide, with or without 2% glycerol). Runs were performed at 17°C in TBE buffer (71 mM Tris-borate pH 8.3, 1.0 mM EDTA).

2.2 SNP genotyping

The SNPs in the genes were primarily chosen in the coding regions and in the genomic regions conserved between humans and mice, see figure 8. No HapMap data were available for selecting SNPs when this study was conducted. The SNPs in the non-conserved region (crossed in figure 8) were omitted from the study at this step. The SNPs in the conserved regions (circled in figure 8) were chosen to further confirm that they were polymorphic by sequencing eight individuals. Eventually, SNPs located in the conserved regions, revealing at least one heterozygote within eight individuals and not in complete LD among others, were chosen for genotyping in the study sample. Figure 8 presents an example of a comparison between genomic sequences of human and mouse (gene ± 10 kb) performed using the Pipmaker program.

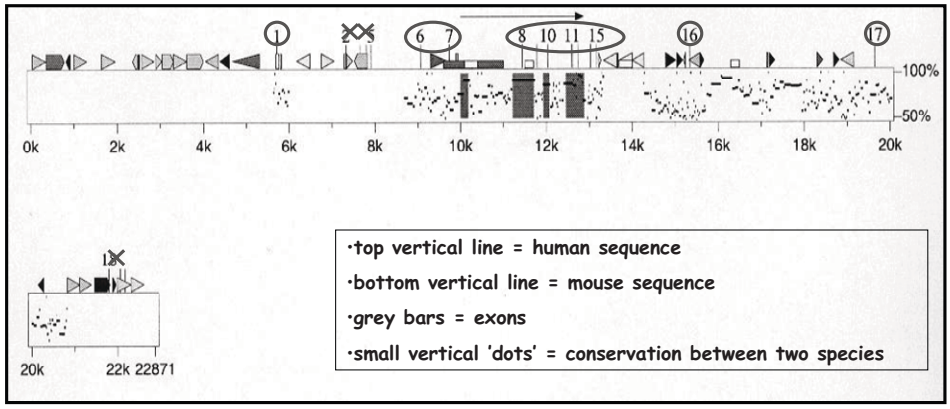

Figure 8. *An example of SNP selection procedure used in the study. A comparison of the genomic sequences between human and mouse using Pipmaker program is presented in the figure.*

A pyrosequencing technique was applied for the small scale SNP genotyping using the PSQ96 instrument and the SNP Reagent kit (Pyrosequencing AB, Uppsala, Sweden) in the finemapping stage of the study III. When the sample size exceeded 400 DNA samples homogenous Mass Extension reaction on the Mass Array System (Sequenom, San Diego, California, USA) was applied according to the manufacturer's instructions.

2.3 Sequencing

The sequencing analysis was performed in study I to determine the nucleotide variation resulting in abnormal migration patterns in SSCP, and in study III to confirm the polymorphicity of the SNPs, as well as to analyze the genomic sequence of the Solute carrier family 6 (neurotransmitter transporter) member 14 (*SLC6A14*)

gene. Sequencing was performed using the BigDye Terminator Cycle Sequencing protocol (Applied Biosystems Inc.) with minor modifications, and the samples were separated with the automated DNA sequencer ABI 377XL (Applied Biosystems Inc.). Sequence contigs were assembled through the use of Sequencher software (Gene Codes Corp., Ann Arbor, Michigan, USA). To promote sequencing of the last four GC-rich fragments 5% dimethylsulfoxide (DMSO) was added to the sequencing reaction mixture and the denaturing temperature was increased to 98°C during amplification cycles.

2.4 Microsatellite marker genotyping

The selection of the markers, as well as the order and distances of the markers, were based on the genetic maps of the Celera *(http://www.celera.com*), the Marshfield Comprehensive Human Genetic Map (*http://research.marshfieldclinic.org/genetics*), and the Human Genome Browser Gateway (*http://www.genome.ucsc.edu*). In the study III, microsatellite markers were genotyped using the ABI Prism 3700 DNA Analyzer and were analyzed with the Genotyper 3.7 software (Applied Biosystems Inc., Foster City, California, USA). In the study IV, the genotyping was performed at the Finnish Genome Center (Helsinki, Finland). The fluorescence-labeled PCR products were pooled into panels and the pooled samples were dialyzed before electrophoresis, which was performed on a MegaBace 1,000-capillary electrophoresis instrument (Molecular Dynamics). Alleles were defined by using Genetic Profiler version 1.1 software (Molecular Dynamics).

2.5 Statistical analyses

Comparison of allele- and genotype combination frequencies between the two groups was performed by the χ^2 test (studies I-III). The significance of differences between clinical characteristics was assessed using regression analysis in the study I. The ranks of leptin were used as dependent variable in the regression analysis where age, sex and BMI were included as covariates. The BMDP Statistical Software version 1.12 (BMDP Statistical Software Inc., Los Angeles, CA, USA) or the NCSS Statistical Package (NCSS, Kaysville, Utah, USA) was used in the analysis.

In the study II, the quantitative trait BMI was tested for association with SNPs using a measured genotype approach. Since there were differences in BMI distributions of females and males within the obese and lean groups, the measured genotype analyses were performed separately in four groups; obese males, obese females, lean males and lean females. Because BMI did not follow a normal distribution in any of these groups, the nonparametric Kruskal-Wallis test was used to evaluate differences in BMI ranks categorized by SNP genotypes. BMI values were corrected for age

before ranking the residuals. These analyses were carried out using the SPSS 12.0.1 software (SPSS Inc., Lead Technologies, Chicago, IL, USA).

To test for association with obesity with the multilocus genotypes of the two X-linked SNPs (study III), one overall analysis of males and females of Swedish and Finnish origin was performed using the option *Genetic Homogeneity* of the Mendel program (Lange et al. 2001). In this analysis, empiric p-values were calculated by permuting the case and control labels within the categories of Swedish males, Swedish females, Finnish males, and Finnish females simultaneously, and combining the data for each permutation to establish a distribution of possible genotypes for cases and controls.

In the family-based association analysis, preferential transmission of SNPs and their haplotypes to quantitative lipid traits in offspring was tested using the FBAT and HBAT (family and haplotype based association test) software (Laird et al. 2000). Options *optimize offset* (-o) and *haplotype permutation* (-p) were used to obtain information from the entire families. Permutation was done 100000 times to obtain empiric p-values for the haplotype association analyses. Prior to these analyses, BMI, TG, glucose and insulin levels, as well as LPL and HL activities were log-transformed when necessary and adjusted for age and sex by multiple regression analysis, and the residuals were used in the analyses. When analyzing males and females separately the regressions to adjust for age were conducted for each sex separately.

To test the microsatellite markers and SNPs for linkage (study III), nonparametric affected sibpair approach was applied using the MAPMAKER/SIBS 2.0 program (Terwilliger and Goring 2000). This method of analysis estimates the proportion of allele sharing on the X chromosome in three kinds of sibpairs separately: male-male, male-female and female-female. The option "all pairs weighed" was chosen to obtain information also from families having more than two obese siblings. Haplotypes were constructed using the GENEHUNTER program, version 1.3, for the male sibpairs and for male case and control subjects (Kruglyak et al. 1996). Haplotyping of X-chromosome markers in males is unambiguous, since males are hemizygous for the X chromosome.

In the study IV, qualitative analyses were performed using both parametric linkage and nonparametric affected sibpair analyses. For the parametric linkage analyses, the MLINK program of the linkage package (Lathrop et al. 1984) version FASTLINK 4.1P (Cottingham et al. 1993; Schaffer et al. 1994) was selected. The identical-by-descent status of affected sibpairs was assessed with the help of the SIBPAIR program (Kuokkanen et al. 1996) of the ANALYZE package (Terwilliger and Goring 2000). The parametric linkage analyses were performed with dominant and recessive modes of inheritance. Gene frequencies of 0.4% and 8%, reflecting an estimated population prevalence of 1%, were used for the dominant and recessive

modes of inheritance of the HDL-C trait. For TGs, gene frequencies of 0.6% and 11% were adopted for the dominant and recessive modes of inheritance.

To investigate the earlier identified regions using quantitative measures by variance component methods in extended families, QTL analyses were conducted using maximum likelihood-based approaches implemented in the computer program SOLAR, version 1.7.4 (Almasy and Blangero 1998). The significance of age, sex, and BMI as covariates was tested in our analyses of HDL-C and TGs. Individual TG and apoB levels were log transformed to reduce skewness and kurtosis. Data for the 10q11 region were also analyzed with the QTL program MERLIN (Abecasis et al. 2002) to compare the results obtained with SOLAR with another QTL-based program. A novel QTL association method included in the software package Mendel (Lange et al. 2001) was applied in the study IV.

Pairwise LD between the marker genotypes was assessed using *ldmax* option in the GOLD (graphical overview of linkage disequilibrium) program in the study II and using the Genepop v3.1b program, option 2 (*http://wbiomed.curtin.edu.au/genepop*) in study III.

RESULTS

1 Mutation screening of the *POMC* and *MC4R* genes

In the first part of this study, we estimated the prevalence of obesity caused by mutations in the *MC4R* and *POMC* genes among morbidly obese Swedish adults (n = 102). Secondly, we investigated the association of common polymorphisms in *POMC* with obesity and/or serum leptin levels in morbidly obese or lean individuals (n = 118).

In the *MC4R* gene, we detected two previously known SNPs in the coding region; val103ile (A700G) and ile251leu (A1144C). Both polymorphisms were rare with frequencies of 2.0% and 3.9%, respectively, in the obese group.

Altogether five sequence variations were detected in *POMC*, all of which have been described earlier (Hinney et al. 1998; Echwald et al. 1999; Hixson et al. 1999; Delplanque et al. 2000). Of these, two were rare SNPs; C4512T (exon 2) and C7726T (exon 3). The more common variants were a 9-bp insertion [$(AGC)_2(GGC)_1$] at codon 56 (after nucleotide 7677), an A8021G variant in the exon 3 at codon 188 and a C8246T polymorphism in the 3'-UTR region. The 9-bp insertion (ins56) is located in the coding region of the exon 3, and results in three additional amino acids in the protein product SerSerCys to the CLIP peptide. The A8021G variant leads to an amino acid change Glu188Gly in the β-lipotropin fragment, as depicted in figure 9.

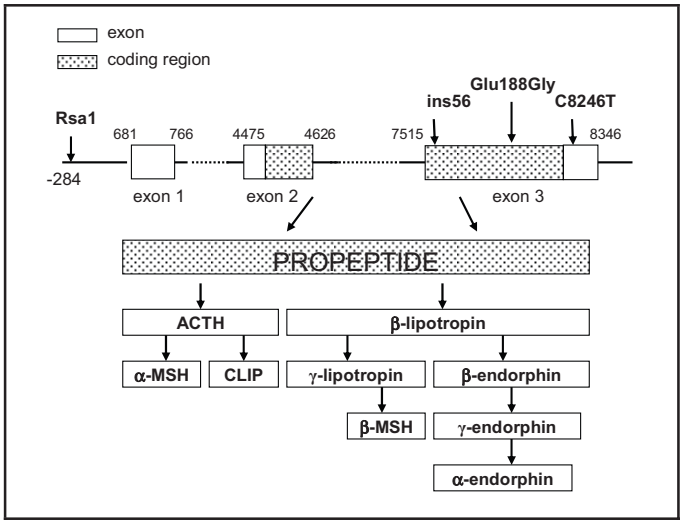

Figure 9. *Structure of the POMC gene, the peptides encoded by it and the variants detected in the study.*

The *POMC* gene variants ins56, Glu188Gly, C8246T and RsaI were genotyped in all study subjects. None of these variants in the *POMC* gene were associated with obesity. However, lean carriers of the C8246T CC-genotype had higher serum leptin levels compared to the carriers of the CT or TT genotype (9.7 ± 6.6 vs. 6.7 ± 4.4 µg/l, p = 0.003, p-values for leptin levels adjusted for age, sex and BMI in the regression analysis). Females contributed the most to the difference (13.6 ± 5.8 vs. 8.5 ± 4.4 µg/l, p = 0.004), and the difference was even more pronounced among the lean female carriers of the C8246T(CC)/RsaI(--or +-) genotype combinations (14.9 ± 4.5 vs. 10.8 ± 5.7 µg/l, p < 0.0005). In contrast, neither the C8246T variant nor the C8246T(CC)/*Rsa*I(--or +-) genotype combinations were associated with serum leptin levels in the obese subjects (32.4 ± 12.2 vs. 33.7 ± 11.9 µg/l, p > 0.05 and 32.7 ± 12.4 vs. 33.4 ± 11.9 µg/l, p > 0.05).

To conclude, monogenic forms of obesity due to mutations in *POMC* or *MC4R* were rare in the Swedish obese patients. Genetic variation in *POMC* was associated with serum leptin levels within the lean individuals, but not in obese. This may emphasize the link between an adipose tissue signaling through leptin and the hypothalamic endocrine system as represented by *POMC*.

2 Cross-species analyses implicating Lipin 1 involvement in human glucose metabolism

Proper function of the *Lipin 1 (Lpin1)* gene is crucial for normal adipose tissue differentiation, as well as for maintaining glucose and lipid homeostasis in mice (Reue et al. 2000; Phan et al. 2004; Phan et al. 2005). Defects in *Lpin1* function cause lipodystrophy, hypertriglyceridemia and insulin resistance in mice (Peterfy et al. 2001), whereas overexpression of *Lpin1* results in obesity (Phan and Reue 2005). In this study, we further examined the *Lpin1* expression in mice carrying the wild-type *Lpin1* gene, as well as investigated the role of *LPIN1* in human obesity, glucose and lipid metabolism (figure 10).

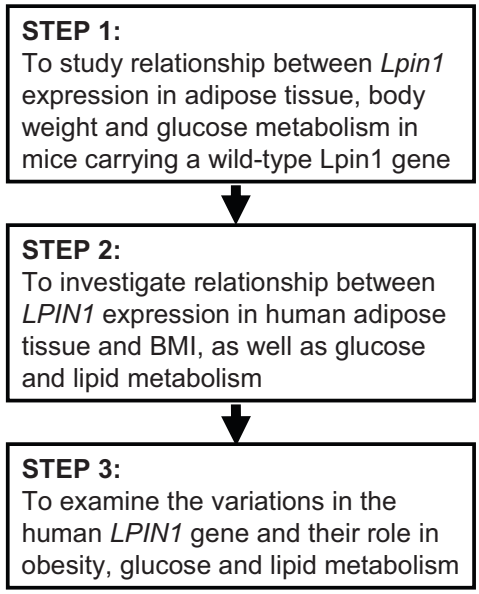

***Figure 10**. Overview of the aims in the study on the LPIN1 gene.*

The first part of this study investigated the *Lpin1* expression in mice carrying a wild-type *Lpin1* gene. Two approaches were used to generate groups of mice having individual variation in glucose metabolism parameters and/or lipin expression levels. In the first approach, individual variation was introduced by producing a mixed genetic background in F2 mice derived from a cross between two inbred strains, C57BL/6J and BALB/cByJ. In the second approach, C57BL/6J mice were fed a high fat diet to promote the development of obesity, hyperglycemia and hyperinsulinemia.

In both mice strains, a negative correlation between lipin mRNA and fasting glucose levels was observed (Spearman correlation coefficient; $r = -0.79$, $p < 0.0001$ for mixed genetic background and $r = -0.79$, $p < 0.0001$ for C57BL/6J mice). Negative correlations were also observed between lipin expression and insulin levels ($r = -0.45$, $p < 0.04$ for mixed genetic background and $r = -0.64$, $p < 0.003$ for C57BL/6J mice), and with the HOMA-IR index of insulin resistance ($r = -0.66$, $p < 0.002$ for mixed genetic background and $r = -0.69$, $p < 0.0008$ for C57BL/6J mice). The negative correlations were primarily due to effects in male mice.

To determine whether similar relationships between lipin expression and metabolic parameters occur in humans, we quantified lipin mRNA levels in 19 human fat biopsies obtained from family members of the FCHL and low HDL-C families using DNA microarray analysis. Similar to the findings using mice, negative correlations were observed between human lipin mRNA levels and glucose ($r = -0.81$, $p = 0.001$), insulin ($r = -0.74$, $p = 0.001$), HOMA-IR index of insulin resistance ($r = -0.82$; $p = 0.001$), and TG levels ($r = -0.64$, $p = 0.003$). Thus, the negative correlation initially observed in mice between lipin expression levels in fat tissue and glucose, insulin, and HOMA-IR was also observed in humans.

We next assessed whether specific alleles of the *LPIN1* gene locus on chromosome 2p25.1 were associated with human metabolic traits in dyslipidemic families, or with obesity in the study sample of obese cases and lean controls. Seven SNPs were genotyped and the LD among them was estimated using the founders in the dyslipidemic families and lean control individuals, see figure 11. None of the SNPs were redundant; however, some of them were in strong LD (D'>0.9) between each other as shown in figure 11. Altogether, the seven SNPs analyzed formed 8 different haplotypes with frequencies greater than 5% in these Finnish study samples.

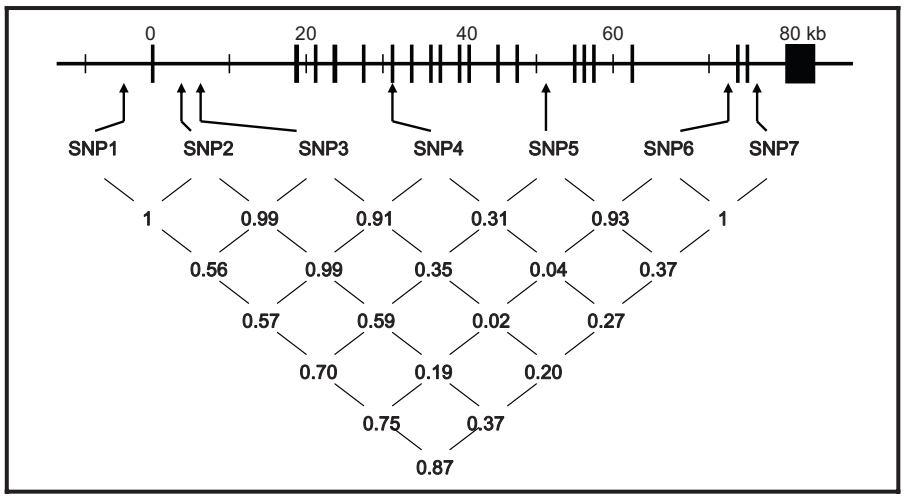

Figure 11. *Genomic structure of the LPIN1 gene and locations of the SNPs genotyped in the study. There are 20 exons in the LPIN1 gene (black boxes). The arrows on the bottom represent SNPs. Pairwise LD between each pair of 2 SNPs in the LPIN1 gene were obtained using GOLD program (Abecasis and Cookson 2000). D'-values are presented in the figure. SNP1 = rs893346, SNP2 = rs11693809, SNP3 = rs10192566, SNP4 = rs2278513, SNP5 = rs2577262, SNP6 = rs2716610, SNP7 = rs1050800.*

The set of quantitative traits to analyze in dyslipidemic families were derived from the phenotype of the *fld* mouse (Langner et al. 1989; Reue et al. 2000). These traits included BMI, fasting TG, glucose and insulin levels, as well as lipoprotein lipase and hepatic lipase activities in all the family members (n = 1109). Females (n = 571) and males (n = 538) were also analyzed separately because these traits showed significant differences between genders in our study sample ($p < 0.05$). An association with serum insulin levels was observed with SNP2 ($p = 0.008$). The sex-specific analyses showed that primarily males contributed to the association ($p = 0.01$). In females, hepatic lipase activity was associated with SNP3 ($p = 0.02$). We also performed haplotype analysis of seven SNPs using FBAT program and observed evidence of association with insulin levels in males ($p = 0.03$).

To characterize more thoroughly the alleles associated with insulin levels and hepatic lipase activity, we performed analyses using the FBAT program and monitored the transmission of different alleles and allelic haplotypes. The T-allele of SNP2 (with a frequency of 40%) was preferentially transmitted to those with elevated insulin levels and the G-allele of SNP3 (with a frequency of 55%) to those with elevated hepatic lipase activity. The most common haplotype 1 (*A-T-C-T-G-C-C*) with a frequency of 20%, as well as haplotype 10 (*A-T-C-T-G-T-C*) with a frequency of 4%, were preferentially transmitted to those with elevated insulin levels. In contrast, haplotype 8 (*A-T-C-T-A-C-T*) with a frequency of 5% was

undertransmitted to those with higher insulin values. To obtain empiric p-values for the allelic haplotype analysis with SNPs in considerable LD, we permuted the analysis 100 000 times using the haplotype permutation option in the FBAT program. The association with insulin levels remained significant in males for the overall analysis of the 7 SNP haplotype (p = 0.04), as well as for haplotype 1 (*A-T-C-T-G-C-C*, p = 0.05), haplotype 8 (*A-T-C-T-A-C-T*, p = 0.002) and haplotype 10 (*A-T-C-T-G-T-C*, p = 0.008).

We examined the association between *LPIN1* genotypes and obesity in lean (BMI 20-25 kg/m^2) and obese (BMI > 30 kg/m^2) Finnish subjects by testing the seven *LPIN1* SNPs for differences in allele frequency between obese (n = 493) and lean individuals (n = 821). A difference in allele frequency was observed for SNP 6 (*C/T*) (0.11 vs. 0.08 for the obese and lean, respectively, p = 0.02; odds ratio of 1.4; 95% confidence interval 1.04-1.86). We also analyzed the *LPIN1* SNPs for differences in mean BMI levels between different genotype groups. SNP1, SNP5 and SNP6 were associated with BMI in lean males (p = 0.01, 0.008 and 0.03, respectively) and SNP2, SNP3, SNP4 and SNP5 in obese males (p = 0.04, 0.008, 0.008 and 0.002, respectively). Since no association was observed in lean or obese females, the association of *LPIN1* alleles with BMI may be sex specific.

In summary, we found evidence of a negative correlation between the expression levels of Lipin-1 and glucose, as well as insulin levels both in mice and humans. In humans, genetic variations of the *LPIN1* gene were also associated with insulin levels and BMI in dyslipidemic families, as well as in obese case and lean control individuals, with males contributing most to the differences. This is the first study suggesting the involvement of the *LPIN1* gene in glucose metabolism and BMI in humans.

3 Fine mapping a locus linked to obesity on chromosome Xq24

The chromosome Xq24 region was previously linked to obesity in a genome-wide scan performed in 166 Finnish obese families including 367 affected individuals (Ohman et al. 2000). Other genome-wide scans in Finnish families have implicated this region in premature CHD (Pajukanta et al. 2000), and low HDL-C (Soro et al. 2002), as well as in TC and apolipoprotein B traits (Pajukanta et al. 1999). In this study, the Xq24 locus was fine mapped in Finnish nuclear families in four steps to identify the gene predisposing to obesity (figure 12).

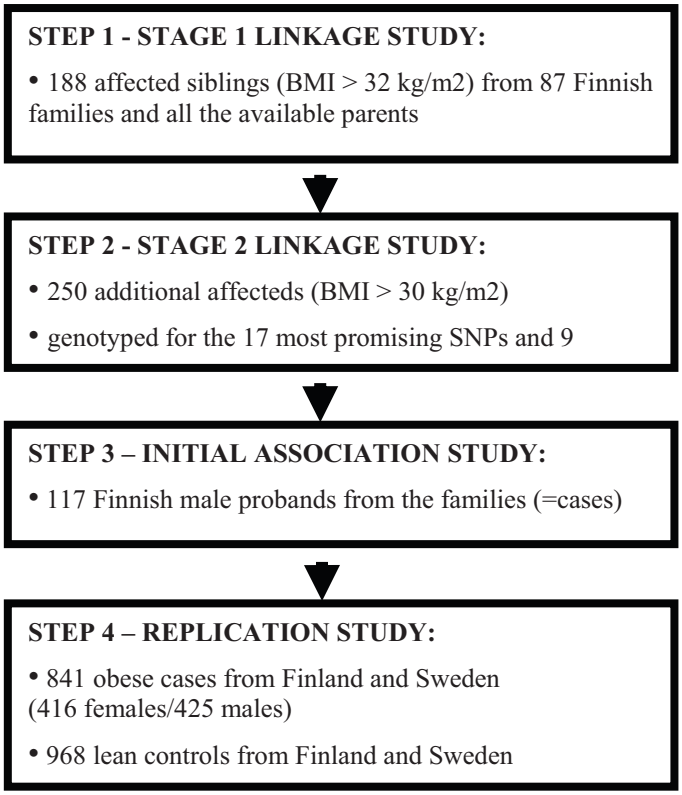

Figure 12. *Overview of the 4-step strategy used in the fine mapping of the chromosome Xq24 region.*

In step 1, we first investigated the 15-Mb region by genotyping additional microsatellite markers and SNPs for regional candidate genes, and by testing them for linkage and association. The aim was not to cover all the genes located in the 15-Mb region, but to target the relevant functional candidate genes for obesity (figure 13). Altogether 36 SNPs in nine genes and nine microsatellite markers (figure 13) were genotyped in 100 obese sibpairs and in all available parents (figure 12). In step 2, the genotyping was further extended to 118 additional sibpairs for the 9 microsatellite markers and 17 SNPs in the *AGTR2*, *SLC6A14*, *SLC25A5*, *NDUFA1* and *HTR2C* genes, representing the most promising findings in stage 1.

In the linkage analysis of steps 1-2, most of the allele sharing among the obese sibs occurred over an 8-Mb region between the marker DXS6804 and the *HTR2C* gene. Interestingly, the obese male sibpairs contributed the most to the linkage signal in this region.

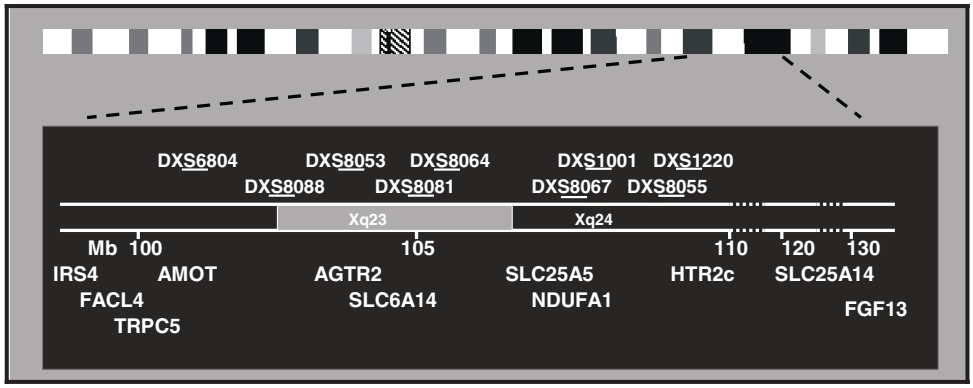

Figure 13. *The genes and microsatellite markers on chromosome Xq selected for the study. Distances and order of the markers and genes are based on the public NCBI and the commercial Celera Genomics databases.*

Because most of the linkage evidence of the SNPs and microsatellite markers in the Xq24 region emerged from the obese male sibpairs, haplotypes of all male sibpairs were analyzed. In the *SLC6A14* gene, we detected putative haplotypes that were shared between the sibpairs in different families. These shared haplotypes of the *SLC6A14* gene partially extended to the neighboring markers (DXS8053 and DXS8081) and genes (*AGTR2*, *SLC25A5* and *NDUFA1*). Subsequently, for the initial association analysis, we selected 11 informative SNPs for the *SLC6A14* gene and for its two neighboring genes (*AGTR2* and *SLC25A5*) and also three microsatellite markers (DXS8081, DXS8053 and DXS8064) located in this region.

In step 3, for the initial association analysis, the first genotyped obese male from each of the families was selected to represent cases (n = 117) and 182 lean unrelated males were selected as controls. The allele frequencies differed significantly between cases and controls for the SNP3 (p = 0.0002) and marginally for SNP2 (p = 0.07) in the *SLC6A14* gene. When combining these two intragenic SNPs into haplotypes, the difference in allele frequencies remained significant (p = 0.0007), as occurred when combining all three SNPs of the *SLC6A14* gene (p = 0.006). The structure of the *SLC6A14* gene and the locations of the SNP1, SNP2 and SNP3 are presented in figure 14.

In step 4, the SNP2 and SNP3 of the *SLC6A14* gene were genotyped in an independent replication sample of 837 obese cases (416 females / 421 males, table 11 on page 48) and 968 lean controls (381 females/ 587 males, table 11 on page 48) from Finland and Sweden. Significant differences in allele frequencies were detected for SNP3 between the lean and obese groups (p = 0.003). Surprisingly, the opposite allele in comparison wih the initial association analysis (SNP3C) was more common among obese subjects in the replication study (allele frequencies of 0.49 in cases and 0.43 in controls). For SNP2, the *T*-allele was more frequent in the cases than in the

controls, but the difference was not statistically significant (allele frequencies for *T*-allele 0.38 vs. 0.35 for the cases and the controls, respectively, $p = 0.08$).

One overall analysis combining the information of SNP2 and SNP3 was performed using *Genetic Homogeneity* option of the Mendel program (Lange et al. 2001). In this analysis, empiric p-values were calculated by permuting the case and control labels within the categories of Swedish males, Swedish females, Finnish males and Finnish females simultaneously, and combining the data for each permutation to establish a distribution of possible genotypes for cases and controls. The association with obesity was observed ($p < 0.05$), suggesting that a combined analysis of both SNPs provided evidence of association in the replication study sample.

When investigating the SNPs separately in both sexes, the females were found to contribute most to the observed association ($p = 0.006$) for SNP3 with the allele frequencies of 0.48 in cases ($n = 416$) and 0.41 in controls ($n = 381$) for the *C*-allele. The C-allele was more common among both the Swedish and Finnish obese groups ($p = 0.08$ and 0.03, respectively).

The entire *SLC6A14* gene, 800 bp of its proximal promoter, and 2 kb downstream from the 3'-end of *SLC6A14* (including an adjacent predicted gene, *LOC203413*, Genebank accession no. XM_117548) were sequenced in 20 obese male individuals. The sequence analysis of the *SLC6A14* gene and its proximal 5'-promoter revealed two variants in the promoter, two in the coding region, four in the 3'-UTR and 41 in the intronic regions, in addition to the three SNPs genotyped earlier. None of the variants changed an amino acid. In the predicted *LOC203413* gene, we found one SNP in the 3'-UTR of the gene and an insertion of four bases in intron 1. In total, 30 of these variants were novel, not found previously in the public (NCBI, LocusLink) or commercial (Celera) SNP databases.

Five additional variants (*–715C/G* and *–298A/G* in the putative *SLC6A14* promoter, *3401C/T* in intron 2, *23041C/T* and *24447A/G* in exon 14) were genotyped for LD analysis in a subset of obese ($n = 180$) and lean ($n = 180$) Finnish males. The location of these five variants in the *SLC6A14* gene is presented in figure 14. SNP2 and SNP3 were in tight, but not in complete LD ($p < 0.00001$). Both of these SNPs were also in LD with the two SNPs in exon 14 ($p < 0.00001$). All four SNPs were located within a 4-kb region. The LD breaks down with the three SNPs in the promoter and in the intron 2 ($p > 0.003$), located 17-21 kb from SNP2. No difference was observed between the male cases and controls in the extent of LD.

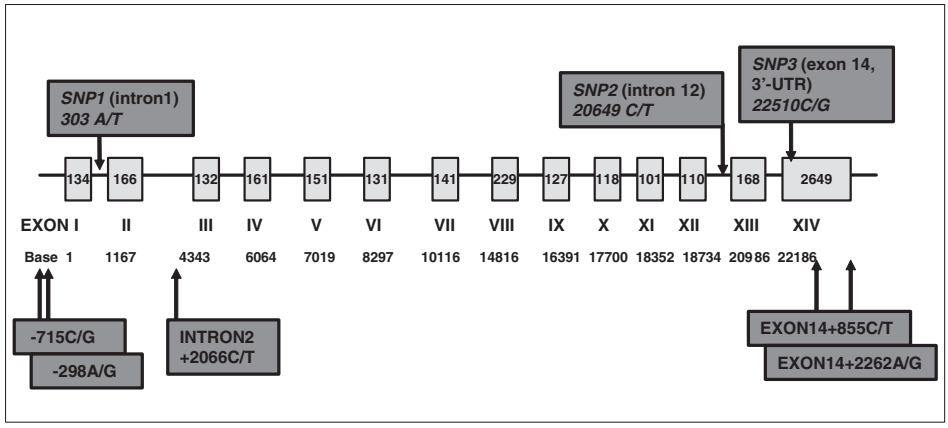

Figure 14. *The structure of the SLC6A14 gene and locations of the genotyped SNPs.*

To summarize, a novel candidate gene for obesity *SLC6A14* was identified after fine mapping the Xq24 region previously linked to obesity, premature CHD and dyslipidemias (Pajukanta et al. 1999; Ohman et al. 2000; Pajukanta et al. 2000; Soro et al. 2002). Initially, linkage and shared haplotypes were identified in obese male sibpairs for this region. Subsequently, significant evidence of association between variations in the *SLC6A14* gene and obesity was observed in two different study populations. The *SLC6A14* gene represents the second novel candidate gene for obesity that was identified using positional cloning approach in humans.

4 Locus for quantitative HDL-C on chromosome 10q11 in Finnish families with dyslipidemia

The aim of this study was to better address the phenotype linked to chromosome Xq24. This region was previously linked to obesity and premature CHD, low HDL-C, as well as TC and apolipoprotein B traits in Finnish families (Pajukanta et al. 1999; Ohman et al. 2000; Pajukanta et al. 2000; Soro et al. 2002). Suggestive linkage for this region was also observed for, low-HDL-C, TC and apoB traits in dyslipidemic Finnish families (Pajukanta et al. 1999; Soro et al. 2002). In addition, five other chromosomal regions, including 2q31, 8q23, 10q11, 16q24.1-24.2 and 20q13.11, were investigated. These five regions were previously linked to premature CHD, FCHL and low HDL-C trait in Finnish study samples (table 12).

In previous genome scans for premature CHD, FCHL, low HDL-C and obesity, several chromosomal loci were identified that showed evidence of linkage to these traits in Finnish families (Pajukanta et al. 1999; Ohman et al. 2000; Pajukanta et al.

2000; Soro et al. 2002; Pajukanta et al. 2003), table 12). The aim of this study was to further investigate six of these chromosomal regions in an extended study sample of 1109 genotyped individuals from 39 low HDL-C and 53 FCHL families. The regions included: loci for low HDL-C on 8q23, 16q24.1-24.2 and 20q13.11 (Soro et al. 2002); a locus for premature coronary on 2q31 (Pajukanta et al. 2000); a locus for serum TGs on 10q11 (Pajukanta et al. 1999) and a locus for premature CHD and obesity on Xq24 (Ohman et al. 2000; Pajukanta et al. 2000). A total of 67 microsatellite markers in these regions were genotyped and analyzed for qualitative and quantitative lipid traits.

***Table 12**. Previous findings from genome wide scans performed in Finnish families.*

STUDY SAMPLE	TRAIT	LOCUS	REFERENCE
FCHL	High TGs	10q11	(Pajukanta et al. 1999)
Low HDL-C	Low HDL-C	8q23.1 16q23.3 20q13.32	(Soro et al. 2002; Pajukanta et al. 2003)
Premature CHD	CHD	2q31 Xq24	(Pajukanta et al. 2000)
Obesity	Obesity	Xq24	(Ohman et al. 2000)

The 10q11 region was originally identified for high TGs in a qualitative linkage analysis (lod = 3.2) (Pajukanta et al. 1999). In the present study, this region provided significant evidence of the quantitative HDL-C (lod = 3.2) with the marker D10S1772 in the present variance component analyses. Since serum TG and HDL-C levels are highly inversely correlated, they may at least partially share the same genetic background. To better address this, a novel QTL association analysis was performed using quantitative HDL-C and TG together, in addition to HDL-C and TG traits separately. In these analyses, evidence of association with quantitative TGs was observed with D10S546, about 7 cM from D10S1772 ($p = 0.0006$), see table 13. For HDL-C the association analysis produced a p-value of 0.02 with marker D10S1790 located in the close vicinity of D10S546 (0.0 cM, 889 kb). Furthermore, a combined analysis of quantitative HDL-C and TGs produced a p-value of 0.008, again with marker D10S546 in the association analysis. No other regions were observed with lod scores of over 3.0.

Table 13. *Results of the quantitative association analyses of low HDL-C and FCHL families on chromosome 10q11.*

Locus	Distance (cM) [a]	p-values		
		HDL-C trait	TG trait	Combined HDL-C and TG trait
D10S1233	0	ns.	ns.	ns.
D10S604	0.10	ns.	ns.	ns.
D10S1772	2.28	ns.	0.06	ns.
D10S1793	2.38	ns.	ns.	ns.
D10S1724	3.80	ns.	ns.	ns.
D10S196	3.90	ns.	ns.	ns.
D10S220	4.00	ns.	0.06	ns.
D10S1220	4.10	ns.	ns.	ns.
D10S568	5.73	ns.	ns.	ns.
D10S1790	9.31	0.02	ns.	0.05
D10S546	9.41	ns.	0.0006	0.008

[a)] *The distance from the first marker. ns. indicates not significant*

In summary, a novel QTL for HDL-C was identified on chromosome 10q11. The markers in this region produced significant evidence of linkage with serum HDL-C levels, as well as provided evidence of association with serum HDL-C and TG levels. The data suggests that the locus on chromosome 10q11 harbors variations influencing the serum HDL-C and TG levels.

DISCUSSION

The aim of this thesis was to identify genes predisposing to obesity and related metabolic traits. Two types of approaches were applied: testing functional candidate genes for obesity and fine mapping chromosomal regions that have been previously linked to obesity, premature CHD or familial dyslipidemias.

In the beginning of this thesis, five monogenic forms of obesity (defects in Leptin, Leptin receptor, *POMC*, *ENPP1* and *MC4R*) were described (Jackson et al. 1997; Montague et al. 1997; Clement et al. 1998; Krude et al. 1998; Vaisse et al. 1998; Yeo et al. 1998). However, the proportion of morbid obesity that they explained in the population was unclear. As observed in the first part of this thesis, the mutations in the *MC4R* and *POMC* genes do not explain a substantial part of obesity in obese Swedish adults (I). Of these five genes, mutations in the *MC4R* are currently the most common known genetic cause of obesity, as up to four percent of obese individuals carry mutations in the *MC4R* gene (Farooqi et al. 2000; Vaisse et al. 2000; Perusse et al. 2005). However, the studies reporting such high frequencies have focused on obese children or adults with early-onset obesity instead of obesity later in life. The mutations identified in these genes causing monogenic forms of obesity have been nonsense or missense mutations, resulting in a more or less non-functional protein product.

During the course of this thesis it became increasingly evident that the common forms of obesity are not caused by single gene mutations (Bell et al. 2005). The general focus of the research in obesity genetics has shifted from screening rare coding mutations in functional candidate genes towards searching for more common predisposing alleles or haplotypes in functional or positional candidate genes. Variants involved in the common forms of obesity are likely to result in minor changes in protein product or may reside in promoters or other regulatory regions (Mackay 2001; Korstanje and Paigen 2002; Thomas and Kejariwal 2004). The variants may cause small changes in the binding affinity of a transcription factor or slightly alter gene expression levels, timing and tissue specificity. Nutrients or other environmental factors may potentially also modulate differences introduced by genetic variation. Each of the individual variants is likely to cause subtle changes in the disease phenotype. However, both multiple predisposing genetic variants and environmental factors are suggested to contribute together to manifestation of symptoms, ultimately leading to the disease.

The *POMC* gene is an interesting candidate gene also for the common forms of obesity. The chromosome 2p23.3 region, where *POMC* resides, was previously linked to BMI and serum leptin levels (Comuzzie et al. 1997; Hager et al. 1998;

Rotimi et al. 1999; Delplanque et al. 2000; Moslehi et al. 2003; Palmer et al. 2003). Variations in *POMC* were associated with serum leptin levels in Mexican-American families (Hixson et al. 1999). In our study sample of adult individuals from Sweden, the common polymorphisms in the promoter and in the 3'-UTR were associated with serum leptin levels in lean individuals. No such association was observed in obese individuals. Higher serum leptin levels are known to activate POMC containing neurons (Cowley et al. 2001) and induce *POMC* expression in the arcuate nucleus in the hypothalamus (Thornton et al. 1997). Functional variations in the *POMC* gene may possibly result in differential responses to leptin levels. A potential feedback signaling could then further enhance the leptin production to compensate for the reduced effect of the serum leptin on the function of *POMC*. The association observed only in lean individuals may suggest that the regulation of the leptin-POMC system is important in normal weight individuals. However, other signals may override this regulation when body weight increases substantially.

Recently, three studies with relatively large sample sizes have investigated the variations in the *POMC* gene (Baker et al. 2005; Chen et al. 2005; Sutton et al. 2005). All three studies detected associations with *POMC* polymorphisms and traits related to body composition (BMI, WHR, waist circumference, total fat mass and serum leptin levels). The 8246C/T variant in the 3'-UTR showed evidence of association in these three recent studies (Baker et al. 2005; Chen et al. 2005; Sutton et al. 2005), as well as in the previous study by Hixson et al. (1999) and our present study. However, opposite alleles were associated with obesity-related traits in different studies. This may reflect a different haplotype background of the study populations (Hixson et al. 1999; Baker et al. 2005; Chen et al. 2005; Sutton et al. 2005).

LPIN1, residing in the 2p25.1, is another interesting candidate gene for obesity. The mouse homologue, *Lpin1*, was originally identified to cause lipodystrophy, insulin resistance and hypertriglyceridemia in the *fld* mice (Peterfy et al. 2001). Recently, overexpression of the *Lpin1* gene was reported to promote obesity in mice (Phan and Reue 2005). No study has previously investigated the role of the human *LPIN1* gene in obesity and related metabolic disorders. In the present study, a negative correlation was observed between *LPIN1* mRNA levels in adipose tissue of the dyslipidemic individuals and fasting glucose levels, insulin levels, as well as HOMA-IR, an indicator of insulin resistance. The study was extended to investigate the variations of the *LPIN1* gene. Evidence of association was observed between insulin levels and *LPIN1* SNPs, and their haplotypes in the dyslipidemic families. The *LPIN1* variants were also associated with BMI in the obese male cases and lean male controls. It would have been interesting to test whether the association with parameters of glucose metabolism is also present in obese and lean individuals; however, these parameters were unfortunately not available for the obese and lean individuals. In conclusion, the study provides evidence that the *Lpin1* gene, identified in a spontaneous mutant mouse

strain with an aberrant metabolic phenotype, may also have relevance to human dyslipidemias and body weight regulation.

Obese individuals represent a heterogenous group of individuals in regard to related diseases they exhibit (such as cardiovascular diseases, T2DM, dyslipidemias etc.), age-of-onset, feeding behaviour, body composition and physical activity. By studying specific subgroups or individual traits related to obesity may make it easier to identify the underlying genetic variation(s). Obesity, metabolic syndrome, T2DM, disturbances in lipid and glucose metabolism often co-occur on same individuals. However, it is still not clear which is the underlying dysfunction. Genetic factors may predispose to any of these individual components and further result in obesity and related disorders. For this reason we also studied metabolic traits related to obesity.

In this thesis, we fine mapped six chromosomal regions that were previously linked to obesity, premature CHD or familial dyslipidemias in Finnish families. Of them, chromosome 10q11 showed evidence of linkage (lod = 3.2) and association (p = 0.0006) with quantitative HDL-C and TG levels in extended study sample of Finnish dyslipidemic families. Further studies are warranted to identify the underlying gene likely to confer susceptibility to high serum TG and low HDL-C levels in this region.

The other regions including 2q31, 8q23, 16q24.1-24.2, 20q13.11 and Xq24 provided stronger evidence of linkage in the original study samples than in the extended families. This may reflect the risk of introducing genetic heterogeneity while extending the study sample to more distant relatives or additional families. This phenomenom has also been observed in other studies (Watanabe et al. 2000; Naoumova et al. 2003). However, the finding on chromosome 10q11 region may suggest that the underlying gene has a broader effect on HDL-C and/or TG levels. HDL-C and TG levels are highly inversely correlated and they may exhibit at leat partially shared genetic background.

Obesity and related metabolic traits show sex-specific features (Harris et al. 1995; Pietilainen et al. 1999; Wauters and Van Gaal 1999; Lahti-Koski et al. 2000; Blaak 2001), making X-chromosomal genes potential candidates for these phenotypes. The region on chromosome Xq24 was previously linked to obesity (Ohman et al. 2000), premature CHD (Pajukanta et al. 2000), as well as TC and ApoB traits in Finnish families (Pajukanta et al. 1999). Overweight predisposes to CHD and patients with CHD in turn are often obese (Hubert et al. 1983; Willett et al. 1995; Hu et al. 2005) and exhibit elevated serum TC and ApoB levels (Genest et al. 1992). It is thus tempting to hypothesize that allelic variants of the same gene may predispose to both of these traits. In the second part of this thesis, the region on chromosome Xq24 was fine mapped in the Finnish obese families and in the two study samples of obese cases and lean controls collected from Finland and Sweden. Variants in a novel

candidate gene for obesity, *SLC6A14,* showed evidence of association with obesity in the original and in the replication study samples.

Very recently the association of obesity with *SLC6A14* was replicated in a large French obese study sample (Durand et al. 2004). Interestingly, the *SLC6A14* variants were also found to modulate differences in hunger and satiety scores among these French obese subjects. However, there were differences in the obesity-associated allele of the same variant, as well as in gender-specificity in the different study samples. Several explanations may account for these inconsistencies. The variants studied here so far may not be the functional ones, but instead they may reflect the causative variant(s) in close proximity. In a different ethnic and haplotype background the underlying LD pattern may be different. Different study samples may also be exposed to different environmental factors or display interactions with other genes. These potential gene-gene or gene-environment interactions and phenotypic heterogeneity may thus complicate the analysis. In addition, refining the phenotype associated with this gene is important when dealing with such a complex disorder as obesity. Considering that our original and replication study samples were recruited solely based on the BMI, these groups of patients may be phenotypically very heterogeneous. It is also possible that *SLC6A14* harbors multiple variants, predisposing to different degrees of obesity. These variants may be unevenly distributed in the two groups of patients. The X-chromosomal inactivation in females may further complicate or add noise to the analyses of *SLC6A14* located on chromosome Xq24.

Based on its function, *SLC6A14* is an interesting candidate gene for obesity. It encodes a sodium- and chloride-dependent transporter for neutral and cationic amino acids with high affinity for the non-polar amino acids such as tryptophan (Sloan and Mager 1999). Tryptophan is a precursor to serotonin, which is a neurotransmitter suggested to be involved in the signaling of satiety (Halford and Blundell 2000). Therefore, factors affecting serotonin concentrations, such as its precursor availability, could potentially have an effect on the appetite regulation and, thus, on the development of obesity. *SLC6A14* is expressed in multiple tissues, including stomach, intestine, colon, salivary tract, pituitary gland, blood, liver, lung, mammary gland, muscle, pancreas, prostate and uterus. The gene could potentially modulate the amino acid absorption from the stomach or amino acid transport through the cell membranes and, thus, modulate the precursor availability for the serotonin synthesis. Figure 15 shows the most important regulators of appetite control together with proposed hypothetical sites of the *SLC6A14* action. Interestingly, expression of the tryptophan hydroxylase, the rate-limiting enzyme of the serotonin synthesis, is detected in the pituitary gland in addition to other parts of the brain (Zill et al. 2005).

Another possible hypothesis of the role of *SLC6A14* in obesity is related to the thrifty phenotype hypothesis (Hales et al. 1991; Hales and Barker 1992; Barker et al.

1993) According to the thrifty phenotype hypothesis, unfavorable nutrition in utero affects the development of the body organs and leaves them susceptible to certain diseases related to nutrition and environment later in life, such as obesity, diabetes, metabolic syndrome and cardiovascular diseases (Barker et al. 1993). Since *SLC6A14* is expressed in blastocysts in mice (Martin et al. 2003; Van Winkle et al. 2005), and it is an important transporter of amino acids at this developmental stage of the fetus, it can be speculated that if the efficiency of the amino acid supply via *SLC6A14* is affected by variations in the gene, it could further affect the availability of amino acids for the fetus in its early stage of development.

The phenomenon where opposite alleles associate with the phenotype in different study samples, as observed here for *POMC* and *SLC6A14*, has also been observed in previous studies (Meirhaeghe and Amouyel 2004). The role of common variants in the *PPARG* gene has been studied thoroughly in context with T2DM. The rare ala-allele of the pro12ala variant in the *PPARG* gene was originally reported by Deeb et al. (1998) to be associated with a lower BMI, improved insulin sensitivity and T2DM. Later studies have detected both a protective effect and a deleterious effect of the ala-allele in T2DM (Meirhaeghe and Amouyel 2004). In 2001, Altshuler et al. performed a meta-analysis on the pro12ala variant and T2DM and found a significant protective effect of the ala-allele for T2DM (Altshuler et al. 2000). To detect small effects that common variants may present, meta-analysis might be the way to proceed, in order to increase the power and decrease the chance of false positive and negative findings. Nevertheless, if the effects of certain variants are population or environment specific to a high degree, it may not be helpful to combine results from different populations.

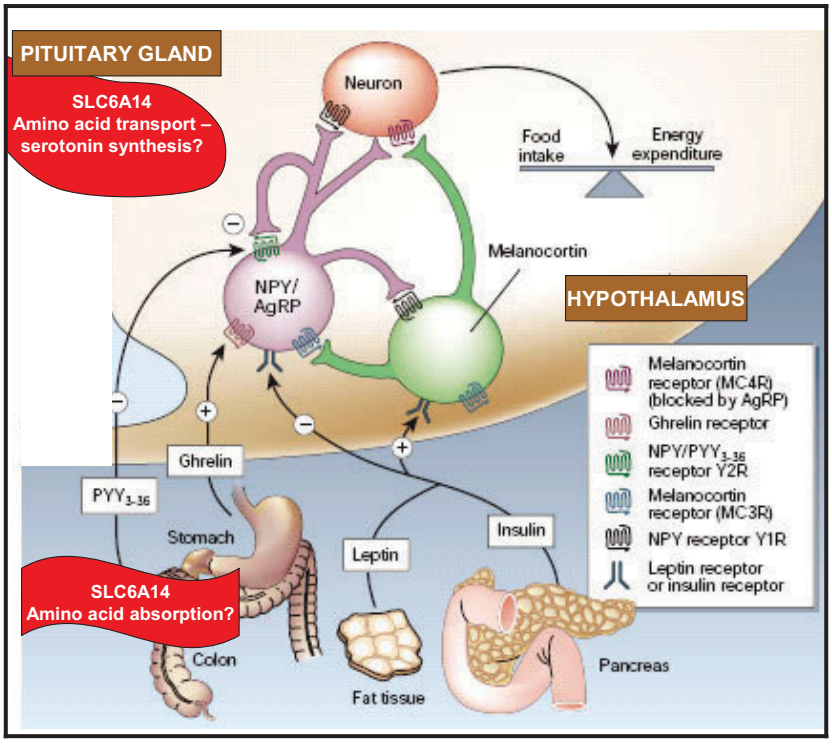

Figure 15. *Hormones controlling appetite. Leptin excreted from adipose tissue and insulin from pancreas decrease appetite. In the arcuate nucleus of hypothalamus, they inhibit neurons that produce Neuropeptide Y (NPY) and Agouti-related protein (AgRP) that stimulate melanocortin-producing neurons. Neuropeptide Y and Agouti-related protein are peptides that stimulate eating. The α-melanocyte-stimulating hormone, produced by post-translational processing from pro-opiomelanocortin, is an endogenous ligand for melanocortin receptors MC4R and MC3R that signal for satiety. Ghrelin released from stomach stimulates appetite by activating NPY/AgRP-expressing neurons. PYY_{3-36} produced from colon acts through the Neuropeptide Y2 receptor and inhibits the NPY/AgRP neurons and, thus, decreases appetite. SLC6A14 is an amino acid transporter, but its potential role in the body weight regulation is not known. The potential sites of action include the intestinal track where it could affect the amino acid absorption. SLC6A14 is expressed in pituitary gland and might affect the amino acid transport and, thus, have an effect on the precursor availability for synthesis of certain neurotransmitters such as serotonin. Modified from (Schwartz and Morton 2002).*

CONCLUDING REMARKS

In the course of this thesis, important advances have been made in the tools and methods applied in searching genes predisposing to complex diseases. The first draft of the human genome sequence became available in 2001 (Lander et al. 2001; Venter et al. 2001) and the sequence was completed in 2003 by the HGP (Collins et al. 2003). The project provided initial information of the variations in the genome between individuals, and by now information of millions of SNPs (approximately 9 million by now) has been deposited into public databases. Rapid improvements in the SNP genotyping technologies have made it possible to genotype vast numbers of SNPs in extensive study samples. The International HapMap project, an extension of the HGP, has provided detailed information of the variation in the genome, LD structure and haplotype blocks, further facilitating the research aiming to identify genes associated with complex diseases (The International HapMap Consortium 2003).

Obesity and related metabolic disorders are important diseases world wide, as they increase the risk of several other common diseases such as T2DM, CHD, hypertension, osteoarthritis and certain forms of cancer. Mapping the genes for complex disorders has turned out to be more challenging than initially expected. However, recent progress in obesity genetics, for example mapping the novel candidate genes through positional cloning approach, has shown the task to be possible (Boutin et al. 2003; Meyre et al. 2005a). The finding of the *SLC6A14* gene in the present study presents the identification of the second novel candidate gene for obesity using positional cloning strategy. The recent replication of the initial results in an independent study population strongly suggests the involvement of the gene in obesity (Durand et al. 2004). A future task is to define the haplotype background in different populations and to identify the underlying causative variant(s) in the *SLC6A14* gene. Ultimately, discovering the mechanisms by which the gene and the underlying variants are involved in body weight regulation may reveal novel molecular mechanisms underlying obesity, and ultimately provide new possibilities for treatment and prevention of obesity and related metabolic diseases.

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