THE GENETIC BASIS OF OBESITY

USING A HIGH-THROUGHPUT CANDIDATE GENE APPROACH TO IDENTIFY NOVEL VARIANTS ASSOCIATED WITH OBESITY IN MULTI-ETHNIC COHORTS

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ABSTRACT

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The prevalence of obesity has been mainly be attributed to the rise in an obesogenic environment, in which individuals are more prone to high-dense energy foods and live a sedimentary lifestyle. Familial aggregation of obesity however, has led to numerous studies focused on understanding the genetic basis of this complex disorder. To this effect, this thesis summarizes the current knowledge of obesity genetics, including the monogenic, polygenic and epigenetics field. Given the tremendous contribution of animal models, especially mouse models, to our current knowledge of obesity genetics, this thesis summarizes the methodology of genetic studies in mice, and focuses on how the synergy between human and mouse studies has led to not only the discovery of obesity causal genes, but also their biological contribution to obesity. Lastly, this thesis summarizes a candidate gene approach based on the information from mouse models that have led to identification of a novel variant associated with body mass index (BMI), hip circumference and body adiposity index (BAI) in a multi-ethnic cohort.

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LIST OF ABBREVIATIONS AND SYMBOLS

AHO – Albright Hereditary Osteodysrrophy
AS – Angelman Syndrome
BAI – Body Adiposity Index
BBS – Bardet-Biedl Syndrome
BFL – Borjeson-Forssman-Lehmann Syndrome
BMI – Body Mass Index
CAB – Chlorambucil
CGL – Congential Generalized Lipodystrophy
ChLS – Chudley Lowry Syndorme
CNV – Copy Number Variant
CSS – Chromosome Substitution Strain
DIO – Diet Induced Obesity
ENU – Ethylnitrosourea
EpiDREAM – epidemiological arm of the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study
EWAS – Epigenome Wide Association Study
FDR – False Discovery Rate
FLP – Familial Partial Lipodystrophy
GIANT – The Genetic Investigation of ANthropometric Traits
GWAS – Genome Wide Association Study
HC – Hip Circumference
HDLc – High-density Lipoprotein Cholestrol
HMPD – Hybrid Mouse Diversity Panel
HS – Heterogeneous Stock
MAF – Minor Allele Frequency
PCA – Principle Component Analysis
PTLS – Potlocki-Lupski Syndrome
PVN – Paraventicular Nuclues
PWS – Prader Willi Syndrome
QTL – Quantitative Trait Locus
ShRNA – Short hair pin RNA
SiRNA – Small interfering RNA
SMS – Smith-Magenis Syndrome
SNP – Single Nucleotide Polymorphism
SNV – Single Nucleotide Variant
T2D – Type 2 Diabetes
VAT/SAT – Visceral Adipose Tissue/Subcutaneous Adipose Tissue
VNTR – Variable Number Tandem Repeat
WAGR – Wilms Tumor Aniridia Genitourinary Anomilies & Mental Retardation
WC – Waist Circumference
WHR – Waist to Hip Ratio

DECLARATION OF ACADEMIC ACHIEVEMENT

The development of analysis plans and outlines of each work presented in this thesis was completed with Dr. Meyre's supervision and guidance. The literature review on mouse models of obesity was completed and published in 2014 and set the stage for completion of an association study in the EpiDREAM cohort based on feedback provided from committee members Dr. Schertzer and Dr. Sloboda. The data analysis completed on EpiDREAM and GIANT were completed with all ethical considerations and approvals required for access to the data.

PREFACE

The first literature review presented in this thesis were in collaboration with Dr. Meyre's post-doctoral fellow Marie Pigeyre and undergraduate student Yuvreet Kaur. All authors contributed equally to data collection and writing of the manuscript. The extensive review on mouse models of obesity was completed with consultation from Dr. Susan Clee from University of British Columbia. Dr. Meyre and I completed the data analysis and writing of the manuscript. Lastly, the data analysis on the EpiDREAM and GIANT cohorts was completed by Dr. Meyre and I, with the help from the lab's biostatistician Akram Alyass.

1.0 Introduction

Obesity is a public health problem and can be classified as a worldwide epidemic affecting over 400 million adults (WHO, 2013). It is defined as the accumulation of excess body fat to the extent that is sufficient to have adverse health outcomes and reduce life expectancy (Fontaine, Redden, Wang, Westfall, & Allison, 2003). The presence of obese individuals has been reported across all human history both through art and science (Enzi, Busetto, Inelmen, Coin, & Sergi, 2003; Józsa, 2011). However, the rising prevalence of obesity has become a health concern only in the last thirty years (M. Ng et al., 2014). Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings (M. Ng et al., 2014).

Co-morbidities associated with obesity include type 2 diabetes mellitus, hypertension, hyperlipidemia, cardiovascular disease, sleep disturbance, respiratory difficulties, joint and mobility issues, psychological distress and certain types of cancer (Switzer, Mangat, & Karmali, 2013). Obesity is associated with disability, mortality, and substantial health costs in higher income countries (Puhl & Brownell, 2001). Obesity has been associated with more frequent health complications and increased healthcare costs. Currently, direct healthcare costs attributed to obesity in Canada are approximately 2-12% while indirect costs associated with co-morbidities account for 37-55% of total healthcare expenditure (Tran, Nair, Kuhle, Ohinmaa, & Veugelers, 2013). Similarly in the United States, direct medical costs associated with obesity are 42% higher in comparison to lean adults (Finkelstein, Graham, & Malhotra, 2014). From a global perspective, obesity accounts for 2 to 6% of total healthcare costs in many developed countries, with the figure increasing significantly if obesity related comorbidities are included in the calculations (WHO, 2000) . Such data needs to be interpreted with caution, as obesity is not

considered a public health priority in some developing countries. Developing countries are home to 84% of the world's population, yet account for only 12% of the global spending on health (Gottret & Schieber, 2006). The limited budgets dedicated to healthcare in these countries may prevent most of newly diagnosed obese patients from receiving appropriate care (Alyass, Turcotte, & Meyre, 2015). From a social perspective, severe obesity is often associated with considerable social stigma, which can affect quality of life as well as educational attainment and job opportunities (Puhl & Brownell, 2001). As a result, the significant costs associated with obesity and its co-morbidities urgently call for interventions aimed at preventing and treating obesity.

Unfortunately, attempts to prevent obesity have had limited success thus far. The individualistic lifestyle approach of "eat less, move more" has been ineffective as a preventive measure for obesity (S. Kirk, Penney, McHugh, & Sharma, 2012). Effective obesity prevention and management is dependent on the acknowledgement that obesity goes beyond the individual behavior and is influenced by genetics, psychology, society and public policy (S. F. Kirk, Penney, & McHugh, 2010). An example of an implemented prevention program is Obesity Canada. Obesity Canada is a not-for-profit organization founded in 1999 that employs an evidence-based approach to develop guidelines to manage and prevent obesity in both adults and children at a population level (Lau et al., 2007). Despite the fact that prevention guidelines have been published, the rise in prevalence of obesity and its related health complications indicate that obesity prevention remains ineffective and no sensible decrease in obesity rates has been evident (M. Ng et al., 2014). This may be due to the fact that public health prevention usually involves policies and programs oriented towards the population at large and employs social marketing that reaches a diverse population (Swinburn, Gill, & Kumanyika, 2005). The impact of such policies

may be visible only indirectly or gradually, whereas the effects of therapeutic interventions on individuals can be assessed more readily (Heller & Page, 2002). However, not all prevention programs have been unsuccessful. An example of effective obesity prevention is evident in the EPODE study (Mantziki et al., 2014). Originating from France, EPODE is a community-based intervention program aimed at reducing childhood obesity by involving local community, childhood settings and family norms to encourage and facilitate adaptation of a healthy lifestyle, tailored to the needs of all socioeconomic groups (Borys et al., 2012). This approach was integral in reducing childhood obesity rates and is now implemented in more than 500 communities across six different countries (Borys et al., 2012).

Shifting the focus to treatment, there are three approaches to treating obesity. Lifestyle changes remain the foundation of obesity treatment, even though adherence to weight loss programs is poor and generally result in weight regain in the long run (Lau et al., 2007). Despite this ineffective strategy against obesity, millions of dollars are spent on fad diets and unproven agents each year (Makris & Foster, 2011). The second approach is pharmacotherapy, which is associated with greater weight loss, but adherence to these medications are generally low (Norris et al., 2005). The third approach is bariatric surgery. Patients who underwent bariatric surgery had greater weight-loss, improvement in the control of co-morbidities, and better quality of life in comparison to non-surgical treatments (Colquitt, Picot, Loveman, & Clegg, 2009). However, the capacity of current health infrastructures limits the number of patients undergoing bariatric surgery, not to mention further post-operative health complications (Frood, Johnston, Matteson, & Finegood, 2013; John G Kral, Kava, Catalano, & Moore, 2012).

In order to improve the current prevention and treatment options, researchers are conducting a number of studies on obesity. Several methods have been used to measure adiposity and body

weight. For purposes of fat quantification, underwater weighing and dual-energy X-ray absorption (DXA) have proven reliable, while computer tomography and magnetic resonance imaging are used for accurate measurements of body fat distribution (Bergman et al., 2011). Despite the high accuracy of such techniques, they are too costly and time consuming for routine clinical applications (Bergman et al., 2011). As a result alternative measures such as waist-to-hip ratio or skin-fold thickness are used in clinical settings, despite their relative inaccuracy in estimating adiposity (Goran & Nagy, 1996; Piers, Soares, Frandsen, & Dea, 2000). Other than measurements of body weight and adiposity, body mass index (BMI) is the most common method for assessing obesity in individual subjects (Bergman et al., 2011; Fesinmeyer et al., 2013; Müller, Bosy-Westphal, & Krawczak, 2010), such that an individual with a BMI value of equal or greater than 30 kg/m^2 is classified as obese (WHO, 2000). However, BMI has also received criticism in regards to precisely measuring obesity (Bergman et al., 2011). It is also argued that BMI guidelines should be ethnic dependent and using a single universal cutoff value worldwide to define obesity is not accurate (Evans, Rowe, Racette, Ross, & McAuley, 2006; Rahman & Berenson, 2010; Romero-Corral et al., 2008). Accurate BMI cutoffs with respect to different ethnicities could better assess an individual's risk for obesity and obesity related morbidity and mortality (Rahman & Berenson, 2010).

Proper investigation of obesity also requires an understanding of how the condition has reached such a high prevalence. The rise of obesity can be attributed to several major societal and environmental changes. The term 'obesogenic' encompasses these changes which include excessive consumption of energy dense foods, sedentary lifestyles, urbanization, and socioeconomic-dependent access to a healthy diet (De Henauw et al., 2011; Misra & Khurana, 2008). However, a huge variability in BMI values, from leanness to obesity, can be observed in

the presence of an obesogenic environment, suggesting that some individuals with genetic predisposition will be more prone or resistant to weight gain in comparison with others (Bouchard, 2008; van Vliet-Ostaptchouk, Snieder, & Lagou, 2012). The ethnic-dependent prevalence of obesity may be explained by specific lifestyles / environmental exposures, but admixture studies have demonstrated an important contribution of genes (Cheng et al., 2009). Additionally, considerable evidence from twin, adoption, and family studies indicate that 40 to 75% of BMI variations are due to genetic factors (Cathy E Elks et al., 2012; Jane Wardle, Susan Carnell, Claire MA Haworth, & Robert Plomin, 2008). Studies have also confirmed the contribution of age-specific or sex-specific effects on BMI and other obesity measure variations (T. W. Winkler et al., 2015). More recently, epigenetic-induced alteration in gene expression has emerged as an alternative way in which environmental compounds may influence obesity phenotype (Fleisch, Wright, & Baccarelli, 2012).

Increasing obesity rates and futile attempts in obesity prevention and treatment has prompted considerable effort to understand obesity etiology and identify causal variants that can contribute to the disease. This thesis presents two literature reviews that illustrate the current level of knowledge in obesity genetics in both human and animal models, and describes an array-wide search for novel variants in the epidemiological arm of the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study (EpiDREAM) cohort. Given the complex genetic architecture of obesity, this thesis aims to provide an extensive overview of the current knowledge in obesity genetics, and contribute to this knowledge through finding new causal variants based on candidate genes in mouse experiments.

It is noteworthy that the second chapter of this thesis is submitted for publication to the journal *Clinical Science*. The third chapter, which describes the integrated approaches in genetic studies

in mouse models and human, has been published in *PeerJ* (Yazdi, Clee, & Meyre, 2015). There are some overlapping information from chapter 2 and 3, as they both target similar topics in some sections. Lastly, the fourth chapter will be completed and submitted for publication by January 2016.

2.0 Recent progress in genetics and epigenetics unveils the pathophysiology of human obesity

In the last 20 years, the completion of the human genome sequencing, combined with tremendous technological and methodological developments, has led to the identification of an abundance of genes modulating anthropometric traits. Considerable effort is still required to identify the remaining genetic and epigenetic contributors to obesity. This chapter, currently under revision at the journal of *Clinical Science*, aims to review the current literature's stand on the molecular basis of adiposity variation and to provide a synthesis of what the genes entail in regards to pathophysiology of obesity.

2.1 Obesity as a heritable disorder

Heritability can be defined as the proportion of phenotypic variance due to additive genetic factors. The familial aggregation of body size was first published by Sir Francis Galton in 1889 (Galton, 1894). Since this observation, family history of obesity has become a well-established risk factor for childhood obesity. In studies of obese and non-obese children, which most often address children under the age of 10 years, parental BMI or overweight/obesity is associated with the BMI or the risk of overweight/obesity in the children, and it is shown to be an important predictor of obesity in adulthood (Birbilis, Moschonis, Mougios, & Manios, 2013; S Danielzik, Czerwinski-Mast, Langnäse, Dilba, & Müller, 2004; Sandra Danielzik, Langnäse, Mast, Spethmann, & Müller, 2002; Oliveira, Oliveira, Almeida, Oliveira, & Adan, 2007). Thus, the number of affected family members may represent to which extent there is a risk for a given child to develop family-prone obesity (Nielsen, Nielsen, & Holm, 2015).

Further support for heritability of obesity comes from monozygotic and dizygotic twin studies. Twin studies have shown that genetic inheritance contributes to 40% to 75% of obesity cases (Cathy E Elks et al., 2012; J. Wardle, S. Carnell, C. M. Haworth, & R. Plomin, 2008). Monozygotic twins show similar body fat acquisition in comparison to dizygotic twins, suggesting that an altered energy balance is highly influenced by the genotype (Bouchard et al., 1990; Hainer et al., 2001). Adoption studies further confirm the genetic component of obesity, as the BMI of adopted children correlate more to the BMI of their biological parents than their adopted parents (Stunkard et al., 1986).

Although family and twin/adoption studies have been influential in our understanding of the genotype contribution to development of obesity, some heritability estimates may be inflated based on the study design (Cathy E Elks et al., 2012). In family studies, it is often difficult to differentiate between the familial phenotype similarities arising from genetics as opposed to shared environmental conditions. Another main issue with family study designs is that parents and children are usually measured at different ages, often across generations and lack of consideration of age-genotype interaction could lead to lower estimates of heritability (Cathy E Elks et al., 2012). The gold standard should be a longitudinal study where parents are phenotyped at the same age as their offspring. Monozygotic twins provide a robust insight into heritability of obesity, as they share identical genetic information, but they also exposed to a common environment which feeds into overestimations of heritability (S.-W. Guo, 2001). This is partly resolved by studying twins who were separated at birth and adopted into families in different environments, but finding twin pairs in this situation is often times rare and difficult (Cathy E Elks et al., 2012). The methodological designs of family and twin studies also do not take into account the demographic factors that could influence heritability estimates. For

example, genetic factors may have been more important before the rise of obesogenic environment, and studies on children may over-represent individuals from more recent birth years. Some studies point to the fact that obesogenic environment could also amplify the effects of obesity susceptibility loci (Andreasen et al., 2008). Age could be another contributor to obesity heritability, as studies have shown that BMI heritability during the puberty stage is the highest, and that the overall contribution of genes towards the BMI increases with age. A study by Dellava *et al*. demonstrated that as the puberty stage advances, the influence of the genetic component towards the BMI increases, and that of environmental factors decreases (Dellava, Lichtenstein, & Kendler, 2012). Similarly, in a study conducted by Ortega-Alonso *et al*., the heritability of BMI in 216 twin sister pairs increased from 54% in 1975 to 72% in 2004. They concluded that the influence of genes on BMI increases consistently with age (Ortega-Alonso, Sipila, Kujala, Kaprio, & Rantanen, 2009). They also found a correlation of 0.40 between the subset of genes influencing rate of BMI change and the subset of genes influencing BMI level (Ortega-Alonso et al., 2009).Therefore, the era effect, exposure to obesogenic environment and age of study participants pose challenges on accurate estimations of heritability and longitudinal data from large twin cohort studies spanning wide eras could confirm BMI heritability (Cathy E Elks et al., 2012).

Recent heritability studies employ common SNPs in general populations. This approach is the foundation of the hypothesis that a substantial proportion of heritability can be explained by common SNPs (S. H. Lee, Wray, Goddard, & Visscher, 2011). To date, 27% of BMI variations could be attributed to common SNPs (C. Llewellyn, Trzaskowski, Plomin, & Wardle, 2013; J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk,

Snieder, Esko, et al., 2015). Nevertheless, the genetic variance accounted for by all common SNPs is still less than that expected from family-based studies (Manolio et al., 2009).

It is important to also note the genetic and environmental interactions in heritability estimates. Heritability values for BMI and obesity can be modified depending on specific environmental exposures (Hélène Choquet & David Meyre, 2011). For example, physical activity can substantially reduce the influence of genetic factors on BMI in both young adults and older adults (G. Guo, Liu, Wang, Shen, & Hu, 2015; Jeanne M McCaffery, Papandonatos, Bond, Lyons, & Wing, 2009; Mustelin, Silventoinen, Pietiläinen, Rissanen, & Kaprio, 2009). As discussed previously, ethnicity may also interact with the genetic predisposition to obesity and ethnic-specific genetic factors are likely to modulate human body weight (Cheng et al., 2009). Surprisingly, the balance of genetic and environmental effects is much the same on BMI variability when looking at data from before and after the rise of obesogenic environment. Therefore, although contemporary environments have made today's children fatter than were children 20 years ago, the primary explanation for variations within the population, then and now, is genetic differences between individual children (Jane Wardle et al., 2008).

The following sections in this chapter discuss the current consensus on the genetics of obesity.

2.2 Mendelian Obesity

Monogenic forms of obesity are the result of structural variations or mutations affecting genes that encode proteins with likely roles in appetite regulation and metabolism (Waalen, 2014). They follow a Mendelian pattern of inheritance and are autosomal or X-linked. Syndromic monogenic forms of obesity, also known as pleiotropic syndromes, are relatively rare. A syndrome is defined as a collection of signs and symptoms known to frequently appear together.

Syndromic forms of obesity can be characterized by features such as mental retardation, dysmorphic features, and organ-specific abnormalities, in addition to hyperphagic obesity (Waalen, 2014). Due to the low occurrence and the difficulty to diagnose obesity syndromes, their genetic causes have not been fully elucidated. Rapid advancements in DNA screening technology have provided an opportunity to uncover the genes associated with Mendelian obesity syndromes. More than 30 of them have been reported in literature, out of which many are unique case reports with no genetic elucidation (Garver et al., 2013). A comprehensive list of obesity syndromes which are at least partially elucidated, their clinical features and genetic abnormalities can be found in Table 1.

2.2.1 Obesity Syndromes

Prader-Willi Syndrome

Prader-Willi syndrome (PWS), first reported in 1956, is a highly researched neurodevelopmental obesity syndrome and has an incidence of 1 in 15,000 to 30,000 (S. B. Cassidy & Driscoll, 2009). Common PWS characteristics include hypotonia, feeding difficulties, poor growth and delayed development in the first year of life, followed by hyperphagia, childhood obesity, short stature and cognitive disability (S. B. Cassidy & Driscoll, 2009). Early growth hormone treatment has been shown to improve body habitus and stature in patients (Bakker, Siemensma, van Rijn, Festen, & Hokken-Koelega, 2015; Lo, Festen, Tummers-de Lind van Wijngaarden, Collin, & Hokken-Koelega, 2015). Obesity and its co-morbidities have been identified as the major cause of health complications in PWS patients (Suzanne B Cassidy, Schwartz, Miller, & Driscoll, 2011). PWS is a result of genetic abnormalities in the paternally inherited chromosome 15q11.2-q13 region. Regions on chromosome 15 are subject to genomic imprinting. Thus, areas

on various genetic areas are silenced either on the maternal or paternal chromosome and only one of the two copies is expressed (Angulo, Butler, & Cataletto, 2015). There are three types of genetic defects in this area that can lead to PWS. Paternal 15q11-q13 deletions are the most common and contribute towards 65-75% of the cases (Angulo et al., 2015). The second kind of genetic abnormality is the maternal uniparental disomy of chromosome 15, and can be found in 20-30% of PWS cases (Angulo et al., 2015). Imprinting defects are the least common types of genetic errors (1-3%) (Angulo et al., 2015). Such defects can be caused by epimutations or incomplete processing of the imprint from the father, or from microdeletions in the DNA imprinting center.

Identification of genes present in the 15q11-q13 region has helped in understanding the molecular basis of the clinical features of PWS. The genes implicated with PWS features include *MKRN3, MAGEL2, Necdin (NDN), NPAP1, SNURF-SNRPN* and 5 small nucleolar RNA (Boccaccio et al., 1999; Farber, Gross, Neesen, Buiting, & Horsthemke, 2000; Griggs, Sinnayah, & Mathai, 2015; Jong et al., 1999; MacDonald & Wevrick, 1997; Sahoo et al., 2008).

Angelman Syndrome

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by developmental delay, seizures, ataxia, speech impairment, spontaneous laughter and hypopigmentation (Larson, Shinnick, Shaaya, Thiele, & Thibert, 2015). AS patients sometimes exhibit gastroesophageal reflux, vomiting and poor weight gain during infancy and early childhood. On the contrary, clinical spectrum studies have shown that approximately 32% of the adult AS patients are overweight or obese (Larson et al., 2015). Since obesity in AS patients is not markedly more prevalent compared to the general population, it is not a prominent clinical feature of AS. Hence,

AS is not always reported as an obesity syndrome in literature (Waalen, 2014). Similar to PWS, Angelman syndrome is linked to abnormalities in chromosome 15. 70% of the cases are caused by *de novo* maternal 15q11-q13 deletions, 10-20% are due to mutations in the maternally inherited *UBE3A* gene, 2% are caused by paternal uniparental disomy of chromosome 15 and 2-3% of the cases are a result of imprinting abnormalities resulting in the lack of expression of the maternal *UBE3A* copy (Bird, 2014; Kyllerman, 2013). Patients with deletions have a more severe phenotype compared to those with uniparental disomy and imprinting defects, illustrating the phenotypic heterogeneity involved with this syndrome (Brockmann, Bohm, & Burger, 2002). *UBE3A* is an E3 ligase in the ubiquitin proteasome pathway and functions as a transcriptional coactivator (Matsuura et al., 1997).

Bardet-Biedl Syndrome

With the first case reported in 1866, Bardet-Biedl syndrome (BBS) is one of the first obesity syndromes described (Savin, 1935). A ciliopathy is a type of disorder caused by abnormalities in genes implicated in the maintenance of the primary cilium (J. M. Brown & Witman, 2014). BBS is a genetically and clinically heterogeneous pleiotropic ciliopathy with an autosomal recessive mode of inheritance. Characteristic clinical features of BBS include retinal degeneration, cognitive disability, polydactyly, and genital and renal anomalies (M'Hamdi, Ouertani, & Chaabouni-Bouhamed, 2014). Central and peripheral obesity is also a major feature, and presents itself in approximately 72-92% of BBS patients (Forsythe & Beales, 2013). Most patients acquire obesity in the early years of life, sometimes resulting in type 2 diabetes (Forsythe & Beales, 2013).

To date, 19 BBS genes have been identified (Novas, Cardenas-Rodriguez, Irigoin, & Badano, 2015). Various gene identification strategies have been utilized to identify the BBS genes. These include linkage analysis, homozygosity mapping, whole exome sequencing and comparative genomics (44, 46, 45). The BBSome complex is comprised of BBS proteins, and is known to constitute a coat complex that sorts membrane proteins to primary cilia (Jin et al., 2010). Due to the key role played by primary cilium in the differentiation of adipocytes, the pathogenesis of obesity in BBS patients can be attributed to a defect in adipogenesis (J. M. Brown & Witman, 2014). BBS proteins have also been known to mediate LepR trafficking. *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice were shown to be resistant to leptin, and displayed decreased activation of hypothalamic STAT3 by leptin (Seo et al., 2009). This resulted in a decreased *Pomc* gene expression. Seo *et al*. concluded that the energy imbalance in BBS patients can be attributed to impairments in LepR signaling (Seo et al., 2009).

Alström syndrome

Alström syndrome is a recessively inherited ciliopathy (Marshall et al., 2005). The major clinical features of Alström syndrome include retinal dystrophy, hearing impairments, early onset obesity, insulin resistance and type 2 diabetes mellitus (Marshall, Paisey, Carey, & Macdermott, 1993). With only ~300 known cases so far, the prevalence of Alström syndrome is particularly low (Marshall et al., 2005). In 1997, Alström syndrome was mapped to chromosome 2p13.1 (Collin, Marshall, Cardon, & Nishina, 1997). A few years later, Collin *et al.* performed a genetic analysis on 6 unrelated Alström syndrome families. Mutations in *ALMS1* were found, and it was identified as the gene responsible for Alström syndrome (Collin et al., 2002). In 2007, Li *et al.* used mouse models to identify the function of *ALMS1*. The lack of *ALMS1* was found to result in

stunted cilia, and it disabled mutant cells from increasing calcium influx in response to mechanical stimuli, explaining some of the clinical features of Alström syndrome (G. Li et al., 2007).

Albright hereditary osteodystrophy

Albright hereditary osteodystrophy (AHO), also known as Pseudohypoparathyroidism Ia, is associated with clinical features of hyperphagia, obesity, short stature, round facies, skeletal anomalies and mental disability (L. Wang & Shoemaker, 2014). AHO is believed to be inherited in an autosomal dominant pattern and reports have described mutations in the *GNAS* gene in AHO patients (Lemos & Thakker, 2015; Thiele et al., 2015). GNAS encodes the alpha-subunit of the stimulatory G protein (Gsα). Gsα is a signaling protein that mediates the actions of other hormones, neurotransmitters and paracrine/autocrine factors (Turan & Bastepe, 2015). This is achieved through the generation of the second messenger cAMP. AHO features are hypothesized to result from resistance to hormones such as the parathyroid hormone, thyroid stimulating hormone and gonadotropins (Levine, 2012). Mutations that lead to a decrease in Gsα expression and/or function result in AHO (Turan & Bastepe, 2015).

Borjesman-Forssman-Lehmann Syndrome

Borjeson-Forssman-Lehmann (BFL) syndrome is an X-linked disorder first described in 1962 (Borjeson, Forssman, & Lehmann, 1962). It has since been reported in numerous families and is characterized by severe mental disability, epilepsy, microcephaly, hypogonadism, obesity and gynecomastia (Turner et al., 2004). In 2002, Lower *et al.* linked BFL syndrome to the presence of mutations in the *PHF6* gene located on chromosome Xq26.2 (Lower et al., 2002). However,

not all BFL syndrome patients carry mutations in *PHF6*, indicating the presence of other unknown genetic causes. PHF6 is a key chromatin adaptor protein and plays an important role in the regulation of neurogenesis and hematopoiesis (Todd, Ivanochko, & Picketts, 2015). It has also been hypothesized to regulate transcription and ribosome biogenesis (Todd et al., 2015).

Chudley-Lowry Syndrome

The Chudley Lowry Sydnrome (ChLS) is a rare X-linked mental retardation disorder. The syndrome was first described in 1988 in a 3 year old boy and his 2 maternal uncles (Chudley, Lowry, & Hoar, 1988). Common features of ChLS include short stature, mild obesity, hypogonadism and distinctive facial features (Abidi et al., 2005). In 2005, Abidi *et al.* found that ChLS may be attributed to mutations in the 5' alternatively spliced region of the *ATR-X* gene (Abidi et al., 2005). The association between mutations in the *ATR-X* gene and ChLS is yet to be confirmed. ATR-X is a member of the SNF2 family of proteins. It plays an important role in chromatin remodelling and heterochromatin formation at centromeres and telomeres (De La Fuente, Baumann, & Viveiros, 2011; Gibbons et al., 1997).

Carpenter Syndrome

Carpenter syndrome, also known as acrocephalopolysyndactyly type II, is an autosomal recessive disorder characterized by acrocephaly, soft tissue syndactyly, brachy- or agenesis mesophalangy of the hands and feet, preaxial polydactyly, congenital heart disease, mental retardation, hypogenitalism, obesity, and umbilical hernia (Cohen, Green, Miller, Gorlin, & Reed, 1987). In 2010, Alessandri *et al.* found a mutation in the *RAB23* gene present in the homozygote state in four relatives and concluded that these mutations were responsible for

causing Carpenter syndrome (Alessandri et al., 2010). These findings were confirmed by Jenkins *et al.* after they reported on an additional 8 families with 10 Carpenter syndrome patients positive for *RAB23* mutations (Jenkins et al., 2011). The gene product is a negative regulator of the Sonic Hedgehog signalling pathway in dorsal neural cells, and has also been implicated in ciliary trafficking (Jenkins et al., 2007).

Cohen syndrome

Cohen syndrome, also known as the obesity-hypotonia syndrome, follows an autosomal recessive pattern of inheritance (El Chehadeh-Djebbar et al., 2013). Patients suffering from Cohen syndrome exhibit features such as non-progressive psychomotor retardation, motor clumsiness, characteristic facial features, microcephaly, childhood hypotonia, progressive myopia and truncal obesity (Rivera-Brugues et al., 2011). The syndrome is overrepresented in the Finnish isolated population with highly homogenous features, likely due to a population founder effect (Kivitie-Kallio & Norio, 2001). In 2003, Kolehmainen, J. *et al.* used haplotype analysis to refine the critical region on chromosome 8q22. This resulted in the discovery of *COH1* (*VPS13B*) as the gene mutated in Cohen syndrome (Kolehmainen et al., 2003). COH1 has been identified as a Golgi-enriched protein that contributes to the structural maintenance and function of the Golgi complex. Depletion of *COH1* in primary neurons negatively interferes with neurite outgrowth. The decreased neuritogenesis could likely lead to the reduced brain size in Cohen syndrome patients, explaining some of the clinical features of this syndrome (Seifert et al., 2015).

Kabuki syndrome

Kabuki syndrome was first reported in 1981 (Niikawa, Matsuura, Fukushima, Ohsawa, & Kajii, 1981). While symptoms can vary amongst patients, it is commonly characterized by mental retardation, facial gestalt, visceral and skeletal malformations and growth deficiency (Miyake, Koshimizu, et al., 2013). Although obesity is not a key feature, clinical heterogeneity results in a range of features, which include obesity and endocrinological anomalies. Using an exome sequencing strategy, Kabuki syndrome was associated with mutations in the gene *KMT2D* (*MLL2*), located on chromosome 12q13.12 (S. B. Ng et al., 2010). This genetic abnormality can be found in 56-76% of the cases, and is inherited dominantly (Lederer et al., 2012). In a recent study, Paulussen *et al.* analyzed *KMT2D* mutations in 45 Kabuki syndrome patients and identified 34 different mutations, illustrating the extent of genetic heterogeneity in Kabuki syndrome (Paulussen et al., 2011). *KMT2D* encodes an H3K4 histone methyl transferase which acts as an epigenetic transcriptional activator during development. Some cases of the Kabuki syndrome have also been linked to a mutation in the *KDM6A* gene, which is located on the X-chromosome at Xp11.3 (Miyake, Mizuno, et al., 2013). *KDM6A* encodes a histone demethylase which directly interacts with *KMT2D* (Lederer et al., 2012).

Wilms Tumor, Aniridia, Genitourinary Anomalies, and Mental Retardation (*WAGR) syndrome*

Aniridia, wilms tumor, genitourinary abnormalities, and growth and mental retardation are the major clinical features of the WAGR syndrome (Han et al., 2008). It is caused by 11p13 deletions of varying sizes. Obesity has been observed in approximately 30% of WAGR patients (Rodriguez-Lopez et al., 2013). In some cases, the *BDNF* gene, located at 11p14.1, can be a part of the deletion. Han *et al*. studied the relationship between genotype and BMI in 33 WAGR syndrome patients. Compared to patients with intact *BDNF*, it was found that those with

heterozygous *BDNF* deletions had significantly higher BMI z scores throughout childhood. Moreover, the critical region for childhood-onset obesity in the WAGR syndrome was located within 80 kb of exon 1 of *BDNF* (Han et al., 2008). This illustrates a link between *BDNF* haploinsufficiency and childhood-onset obesity in WAGR syndrome, while providing an insight into the role of *BDNF* in obesity (Han et al., 2008).

Table 1: Genetic and clinical manifestations of syndromic obesity

Name(s) of the syndrome	Clinical features	Type of inheritance	Genetic cause
Albright hereditary osteodystrophy	Brachymetaphalangism, short stature, obesity, and mental retardation (L. Wang & Shoemaker, 2014)	Autosomal dominant	*GNAS1* (Thiele et al., 2015)
Alström syndrome	Blindness, hearing impairment, childhood obesity, insulin resistance, and type 2 diabetes mellitus (Marshall et al., 1993)	Autosomal recessive	*ALMS1* (Collin et al., 2002)
Angelman syndrome	Developmental delay, speech impairment, and gait ataxia (Larson et al., 2015)	NA	Chromosome 15 abnormalities, *UBE3A* (Bird, 2014; Kyllerman, 2013)
Bardet-biedl syndrome	Retinitis pigmentosa, obesity, kidney dysfunction, polydactyly, behavioral dysfunction, and hypogonadism (J. M. Brown & Witman, 2014)	Variable	*BBS1*, *BBS2*, *BBS3/ARL6*, *BBS4*, *BBS5*, *BBS6/MKKS*, *BBS7*, *BBS8/TTC8*, *BBS9*, *BBS10*, *BBS11/TRIM32*, *BBS12*, *BBS13/MKS1*, *BBS14/CEP290*, *BBS15/C20RF86*, *BBS16/SDCCAG8*, *BBS17/LZTFL*, *BBS18/BBIP1*, *BBS19/IFT27* (Novas et al., 2015)

Borjeson-Forssman-Lehmann Syndrome	Severe intellectual disability with epilepsy, microcephaly, short stature, obesity, hypogonadism, and gynecomastia (Turner et al., 2004)	X-linked	*PHF6* (Lower et al., 2002)
Carpenter syndrome/ Acrocephalopolysyndactyly type II	Acrocephaly, soft tissue syndactyly, brachy- or agenesis mesophalangy of the hands and feet, preaxial polydactyly, congenital heart disease, mental retardation, hypogenitalism, obesity, and umbilical hernia (Cohen et al., 1987)	Autosomal recessive	*RAB23* (Alessandri et al., 2010)
Chudley-Lowry syndrome	Mental retardation, short stature, mild obesity, hypogonadism, and distinctive facial features characterized by depressed nasal bridge, anteverted nares, inverted-V-shaped upper lip, and macrostomia (Abidi et al., 2005)	X-linked	*ATR-X/XNP* (Abidi et al., 2005)
Cohen syndrome	Mental retardation, facial dysmorphism, microcephaly, retinal dystrophy, truncal obesity, joint laxity and intermittent neutropenia (Rivera-Brugues et al., 2011)	Autosomal recessive	*VPS13B/COH1* (Kolehmainen et al., 2003)
Kabuki syndrome	Facial gestalt, intellectual disability, visceral and skeletal malformations and, postnatal short stature	Autosomal dominant or X-linked dominant	*KMT2D/ MLL2/ ALR/ KABUK1, KDM6A/ UTX/ KABUK2* (S. B. Ng et al., 2010)

	with overweight (Miyake, Koshimizu, et al., 2013)		
Kallmann syndrome	Anosmia, related to defective olfactory bulb morphogenesis, and hypogonadism due to gonadotropin-releasing hormone deficiency	X-linked or autosomal dominant	*KAL1*, *FGFR1*, *PROKR2*
Prader-Willi syndrome	Low muscle tone, short stature, incomplete sexual development, cognitive disabilities, problem behaviors, and a chronic feeling of hunger that can lead to excessive eating and obesity (S. B. Cassidy & Driscoll, 2009)	NA	*MKRN3*, *MAGEL2*, *NDN*, *NPAP1*, *SNURF-SNRPN*, 5 small nucleolar RNA (Boccaccio et al., 1999; Farber et al., 2000; Griggs et al., 2015; Jong et al., 1999; MacDonald & Wevrick, 1997; Sahoo et al., 2008)
Smith-Magenis syndrome	Intellectual disability, delayed speech and language skills, distinctive facial features, sleep disturbances, and behavioral problems (MarianneJensen & Kirchhoff, 2005)	NA	*RAI1*, *MFAP4* (Carmona-Mora et al., 2012; Z. Zhao et al., 1995)
Cornelia de Lange syndrome	Facial dysmorphic features, growth and cognitive impairment, and limb malformations; obesity may develop (Pie, Gil-Rodriguez, Ciero, Lopez-Vinas, Ribate, Arnedo, Deardorff, Puisac, Legarreta, De Karam, Rubio, Bueno, Baldellou, Calvo Ma, et al., 2010)	Autosomal dominant (NIPBL, SMC3); X-linked (HDAC8, SMC1A)	*HDAC8*, *NIPBL*, *SMC1A*, *SMC3* (Deardorff et al., 2012; Pie, Gil-Rodriguez, Ciero, Lopez-Vinas, Ribate, Arnedo, Deardorff, Puisac, Legarreta, de Karam, Rubio, Bueno, Baldellou, Calvo, et al., 2010)

2.2.2 Monogenic Non-syndromic Obesity

Monogenic non-syndromic obesity refers to a single gene disorder that leads to a highly penetrant form of the disease. Studying extreme obesity in patients that exhibit single gene mutations have provided valuable information on the role of leptin-melanocortin pathway in energy balance (Supplementary Figure 1). The section below discusses the cases reported so far.

LEP/LEPR

A frameshift mutation in the leptin (*LEP*) gene resulting in truncated transcription of leptin was first discovered in two severely obese cousins within a highly consanguineous family of Pakistani origin (Montague et al., 1997). Other reports have confirmed this initial discovery in additional patients of Pakistani, Turkish and Egyptian origin (Gibson et al., 2004; Mazen, El-Gammal, Abdel-Hamid, & Amr, 2009). Other case reports involve 2 patients with severe early onset obesity with a homozygous *LEP* mutation that resulted in biologically inactive leptin. These patients exhibit detectible circulating leptin levels, which indicates the mutations impact the protein function than expression (Wabitsch, Funcke, Lennerz, et al., 2015). This finding suggests that prescreening of leptin levels as a mean to detect leptin mutations may not be reliable and leptin sequencing in severely obese children with detectible leptin levels may be considered for detection of leptin mutations (Wabitsch, Funcke, Lennerz, et al., 2015; Wabitsch, Funcke, von Schnurbein, et al., 2015).

Similarly, congenital leptin receptor (*LEPR*) deficiencies were found in severe obese siblings in 1998 (Clément et al., 1998). Other studies have reported 8 other patients with severe early onset obesity with homozygous or compound heterozygous mutations in *LEPR* were identified (Farooqi, Wangensteen, et al., 2007). These patients exhibited high serum levels of leptin and

loss of sensitivity of the receptor (Farooqi, Wangensteen, et al., 2007). In consanguineous populations such as that of Pakistan, new case reports have revealed novel homozygous mutations in *LEPR* that constitutes 3% of severely obese Pakistani children. (Saeed et al., 2014). Similarly, novel frameshift mutation in *LEPR* was identified in a French population from Reunion Island, which could suggest a founder effect in genetically isolated populations (Huvenne et al., 2015).

Clinical manifestations of patients with mutations in *LEP* or *LEPR* include rapid weight gain within the first year of life, as well as hyperphagia and aggression when food is denied (Farooqi et al., 2002). Onset of puberty is often delayed for these patients, due to hypogonadotrophic hypogonadism (Farooqi, Wangensteen, et al., 2007). Although homozygous carriers of *LEP* or *LEPR* are sterile, there has been reports of a natural pregnancy in a woman with homozygous *LEPR* mutation which calls into question the belief that leptin function is critical to reproductive function (Nizard, Dommergues, & Clément, 2012). Leptin deficient children exhibit defective T-cell mediated immunity, explaining the high rates of infection and mortality in developing countries (Farooqi, Wangensteen, et al., 2007). Furthermore, humans with loss-of-function mutations in leptin and the leptin receptor have low blood pressure, despite severe obesity (Simonds et al., 2014). Leptin deficiency clinical features are often reversed with leptin therapy. In the case of a 9 year old girl with leptin deficiency, leptin treatment for 12 months resulted in reduction in weight mainly due to loss of fat, reduced energy intake, and increase in gonadotropin concentrations (Farooqi et al., 1999). In a different study, leptin-deficient patients in a fed state gave higher ratings to food images, but these ratings were reduced after leptin treatment (Farooqi, Bullmore, et al., 2007). Studies with magnetic resonance imaging (MRI)

techniques also confirmed alteration in the functional cortical activity to food cues in key feeding and reward-related areas (Farr et al., 2014; S. Frank et al., 2013).

SH2B1

The SH2B adaptor protein 1 (*SH2B1*) is a key regulator of leptin, as it enhances leptin signaling by both stimulating JAK2 activity and assembling a JAK2/IRS1/2 signaling complex (Z. Li, Zhou, Carter-Su, Myers Jr, & Rui, 2007; Rui & Carter-Su, 1999; Rui, Gunter, Herrington, & Carter-Su, 2000). Loss-of-function mutations in *SH2B1* patients resulted in severe early onset obesity (Doche et al., 2012; Laura R Pearce et al., 2014). Clinical features included hyperphagia, childhood onset obesity, insulin resistance, reduced height and behavioral abnormalities (Doche et al., 2012). Genomic imbalances and recurrent deletions of the *SH2B1* containing region on the short arm of chromosome 16 have been associated with behavioral disorders and obesity (Bachmann-Gagescu et al., 2010). Genome wide studies have identified common variants near *SH2B1* that are associated with BMI and obesity (Berndt et al., 2013; Robiou-du-Pont et al., 2013; Gudmar Thorleifsson et al., 2009; Cristen J Willer et al., 2009).

POMC

The first recessive mutation in *POMC* was discovered in 1998 (Krude et al., 1998). In addition to obesity, patients with *POMC* mutations displayed hypocortisolism, red hair and skin hypopigmentation, neonatal hypoglycemia, seizures, cholestasis and voracious appetite (Farooqi et al., 2006; Krude, Biebermann, & Grüters, 2003; Krude et al., 1998). *POMC* deficiency may lead to increased fetal mortality and is involved in neonatal adrenal crisis, early-onset obesity, adrenal insufficiency and hypoglycemic seizures in neonatal period (Aldemir, Ozen, Sanlialp, &

Ceylaner, 2013; Dubern et al., 2008; Farooqi et al., 2006; Mendiratta et al., 2011). Other clinical features were reported in a female with *POMC* loss-of-function mutation that lead to severe motor mental retardation (Özen, Özcan, Uçar, Gökşen, & Darcan, 2015).

PCSK1

Three patients with recessive monogenic form of obesity were deficient in pro-protein convertase subtilisin/kexin type 1 (*PCSK1)* gene (Farooqi, Volders, et al., 2007; Jackson et al., 2003; Jackson et al., 1997). Complete deficiency in prohormone convertase 1 (PC 1/3), which is encoded by *PCSK1,* results in early on-set obesity, hyperphagia, hypoglycemia, and endocrine dysfunction (Farooqi, Volders, et al., 2007; Jackson et al., 2003; Jackson et al., 1997). Null mutations causing PC1/3 congenital deficiency also lead to diarrhea and diabetes insipidus in some instances (Farooqi, Volders, et al., 2007; G. R. Frank et al., 2013; Jackson et al., 1997; Martín et al., 2013; Yourshaw et al., 2013). A rare nonsense loss-of-function mutation at the heterozygous state causing a dominant form of mendelian familial obesity associated with glucose intolerance (J Philippe et al., 2014). Therefore, null mutations in *PCSK1* are associated with dominant form of monogenic obesity; depending on whether the mutation is partial or total loss-of-function (J Philippe et al., 2014). This is further supported by the PC 1/3 mutations with dominant negative effects that alter the expression of wild-type proteins, with consequential effects on PC1/3 availability (Blanco, Ramos-Molina, & Lindberg, 2015).

MC4R

The first heterozygous mutation in *MC4R* discovered in humans was in 1998 (Vaisse, Clement, Guy-Grand, & Froguel, 1998; Yeo et al., 1998). *MC4R* mutations represent the most common

form of human monogenic obesity, impacting 0.2-5.6% of individuals with severe early onset obesity (Konstantinos Rouskas et al., 2012). Majority of these mutations are heterozygous, with homozygous mutants having a fully penetrant early-onset severe form of obesity. In addition to obesity, *MC4R* deficient children display hyperinsulinemia and increased linear growth (Farooqi et al., 2000). *MC4R* mutations have been associated with an increase in bone mass in both children and adults and affected individuals have a taller stature (Farooqi et al., 2000; G. Garg et al., 2014; Timpson, Sayers, Davey-Smith, & Tobias, 2009). Also patients experience an increase in adiposity, increase in blood pressure and an increase in lean mass, which is a phenotype that is not observed in other forms of monogenic obesity (Farooqi et al., 2003). Interestingly, degree of hyperphagia in patients depends on level of receptor dysfunction, which is generally lower than that of leptin deficient patients (Farooqi et al., 2003).

TUB

Mutations in Tubby bipartite transcription factor (*TUB)* were observed in an eleven year old boy from a consanguineous Caucasian family. His symptoms included deteriorating vision, obesity, and normal glucose/cholesterol/triglycerides levels but other clinical features were not observed to classify the patient as Bardet-Biedl or Alström syndrome (Borman et al., 2014). Autozygosity mapping and whole-exome sequencing in a consanguineous UK family identified a homozygous frameshift mutation in *TUB* (c.1194_1195delAG, p.Arg398Serfs*9), which results in a truncated form of TUB. Homozygous loss-of-function of *TUB* is extremely rare in humans (Borman et al., 2014). The clinical features of TUB deficiency in humans are consistent with a new obesity syndrome in the family of ciliopathies.

BDNF/NTRK2

The neurotrophic tyrosine kinase receptor type 2 (*NTRK2)* was screened in a boy with early onset obesity, hyperphagia developmental delay, impairments in short-term memory and impaired nociception, revealing a missense mutation in *NTRK2* (Yeo et al., 2004). The impaired hypothalamic neurogenesis in patients with *NTRK2* mutations may explain the hyperphagia and obesity phenotype observed in these patients (J Gray et al., 2007). Further analysis showed an impairment in the brain-derived neurotrophic factor (*BDNF)* stimulated protein kinase phosphorylation (Yeo et al., 2004). The developmental and neurological impairments in this case is consistent with the wide spread of *TrkB* (encoded *via NTRK2*) throughout the central nervous system, where it assumes the responsibility for neuronal survival and differentiation and regulation of synaptic function (Indo et al., 1996). In another case, a girl with loss of one functional copy of *BDNF* presented with hyperphagia, severe obesity, cognitive impairment and hyperactivity (Juliette Gray et al., 2006). As mentioned previously, hyperphagia and obesity observed in a subgroup of patients with WAGR syndrome has been attributed to deletions on chromosome 11 that induce haploinsufficiency of *BDNF* (Han et al., 2008).

SIM1

Deletions on chromosome 6q16, region, have been associated with obesity and Prader-Willi like features (Bonaglia et al., 2008; El Khattabi et al., 2014; Izumi et al., 2013; Stein, Stred, Thomson, Smith, & Hoo, 1996; Villa, Urioste, Bofarull, & Martínez-Frías, 1995). *SIM1* was considered as a highly relevant candidate gene among the twelve genes present in the critical region for Prader-Willi like syndrome in the report by Bonaglia and colleagues (Bonaglia et al., 2008). *SIM1* is expressed in central nervous system and plays an essential role in formation of

paraventricular nucleus (PVN) of the hypothalamus (Michaud, DeRossi, May, Holdener, & Fan, 2000; Michaud, Rosenquist, May, & Fan, 1998). Whereas *SIM1* complete deficiency is a lethal mutation based on studies in mice, *SIM1* haploinsufficiency leads to hyperphagia, obesity and reduction of the PVN (Michaud et al., 2001). *SIM1* haploinsufficiency has been shown to inhibit the leptin-melanocortin-oxytocin pathway (Kublaoui, Holder Jr, Gemelli, & Zinn, 2006; Tolson et al., 2010). Excessive growth, severe early-onset obesity but no features suggestive of Prader-Willi were observed in a girl with a balanced translocation leading to *SIM1* haploinsufficiency (Holder, Butte, & Zinn, 2000). More recently, heterozygous deleterious mutations in *SIM1* were observed in obese children who displayed additional Prader-Willi like / neurobehavioral features (Bonnefond et al., 2013; Montagne et al., 2014; Ramachandrappa et al., 2013).

KSR2

Kinase suppressor of Ras 2 (KSR2) is a scaffolding protein involved in multiple signaling pathways through kinase cascades (Dougherty et al., 2009; Pearce et al., 2013) that are linked to regulation of food intake, body fat content and glucose homeostasis (Revelli et al., 2011). By using a whole-exome sequencing strategy, *KSR2* loss-of-function mutations were identified in humans and were associated with hyperphagia, early-onset obesity, low heart rate, reduced basal metabolic rate and severe insulin resistance (Pearce et al., 2013).

2.2.3 Oligogenic Obesity

Homozygous / compound heterozygous loss-of-function mutations in monogenic obesity genes from the leptin/melanocortin pathway lead to fully penetrant monogenic obesity but are exceptionally rare in humans (H. Choquet & D Meyre, 2011). If less extreme / not fully

penetrant forms of obesity have been observed in subjects carrying heterozygous deleterious coding mutations in these genes, their higher prevalence may explain a substantial proportion of obesity cases in humans (H. Choquet & D Meyre, 2011). For instance, based on loss-of-function mutation frequency of *MC4R* in the general population of United States (0.07%), we can expect 426,701 heterozygous *MC4R* carriers *versus* 149 homozygous carriers (N=305,000,000). Based on average penetrance of *MC4R* (60% for heterozygous and 100% for homozygous) reported in the literature (Fanny Stutzmann et al., 2008), partial *MC4R* deficiency may explain obesity in 256,021 individuals, whereas complete *MC4R* deficiency may be the cause of obesity for only 149 subjects in the US population (Hélène Choquet & David Meyre, 2011). *MC4R* heterozygous loss-of-function mutation carriers exhibit an interaction with the 'obesogenic' environment (Stanikova et al., 2014; Fanny Stutzmann et al., 2008). Heterozygous carriers of *MC4R* loss-of-function mutations consume three-times more food than their unaffected siblings during an *ad libitum* meal (Farooqi et al., 2003). Other studies have shown that these subjects also have a preference for consumption of foods with a high-fat content (van der Klaauw et al., 2015). Although heterozygous *MC4R* mutation carriers have previously been supposedly associated with binge eating (Branson et al., 2003), this association has not been confirmed by a recent meta-analysis (Meyre, personal communication, 2015).

Heterozygous loss-of-function mutations in *POMC* result in a non-fully penetrant form of obesity (Biebermann et al., 2006; Challis et al., 2002; Dubern et al., 2008; Farooqi et al., 2006; Y. S. Lee et al., 2006). Biochemical processing of *POMC* through post-translational modification enzymes results in derivation of *α-MSH* and *β-MSH* (Supplementary Figure 2). A novel heterozygous mutation in the alpha-melanocyte stimulating hormone (*α-MSH*) gene was found in a 12 year old girl with early onset obesity, which was characterized by dramatic impairment of *α*-

MSH (Dubern et al., 2008). A loss-of-function missense mutation in *β-MSH* has been associated with childhood obesity. The lack of function of *β-MSH* reduces the amount of MSH peptide in the POMC/MC4R pathway, resulting in obesity (Biebermann et al., 2006). The function of either of the POMC-derived MSH peptides can be impaired, depending on the location of the mutation (Biebermann et al., 2006; Dubern et al., 2008; Y. S. Lee et al., 2006). Furthermore, partial deficiency in *LEP* and *LEPR* has been associated with higher percentage of body fat mass (Farooqi et al., 2001; Farooqi, Wangensteen, et al., 2007). Partial loss-of-function heterozygous mutations in *PCSK1* present an non-fully penetrant form of Mendelian obesity (Creemers et al., 2012). However, heterozygous carriers of the null mutation p.Arg80* encoding a propeptide truncated to less than two exons out of 14 show a dominant form of Mendelian obesity in a large French pedigree (J Philippe et al., 2014). Heterozygous loss-of-function coding mutations in the *MC3R* gene could predispose humans to increased risk of obesity, but more evidence is needed to confirm these findings (Calton et al., 2009; Y. S. Lee, Poh, Kek, & Loke, 2007; Mencarelli et al., 2011). Melanocortin 2 receptor accessory protein 2 (*MRAP2*) is a homologue of MRAP, expressed in the brain and adrenal gland (Chan et al., 2009). *MRAP2* can interact with all melanocortin receptors, which results in MC2R surface expression and signaling. *MRAP2* can also reduce the responsiveness of *MC1R, MC3R, MC4R* and *MC5R* to *α-MSH* (Chan et al., 2009). Four rare heterozygous mutations of *MRAP2* have been identified in humans to be associated with early-onset, severe obesity (Asai et al., 2013).

Copy number variants (CNVs) also provide insight into the contribution of genes in obesity phenotype. Studies of the salivary amylase gene (*AMY1*) have depicted an association with body mass index, such that low copy numbers of the *AMY1* results in higher BMI (Falchi et al., 2014; Mejía-Benítez et al., 2015; Usher et al., 2015). Increased CNVs of *AMY1* is positively associated

with *AMY1* expression and serum enzyme levels. The odds ratio of 1.19 per copy of *AMY1* translates to an eight fold difference in the risk of obesity between subjects in the top (CNVs >9) and bottom (CNVs<4) of the top 10% of copy number distribution (Falchi et al., 2014). This finding suggests the decreased amylase levels impacts carbohydrate metabolism and predisposes individuals to obesity (Falchi et al., 2014; Mejía-Benítez et al., 2015; Usher et al., 2015).

2.2.4 Complete and Partial Lipodystrophy

Disruption in the energy storage system of the body can result in excess or in impairment of storage in adipocytes, characterized also by fat distribution abnormalities. Generalized or partial lack of adipose tissue can be inherited or acquired (Robbins & Savage, 2015). There are approximately 1000 case reports of patients with inherited forms (A. Garg, 2011).

Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder with a specific phenotype from birth in 1/1,000,000 individuals. In addition to psychological distress due to body image concerns, generalized lack of fat tissue results in hypertriglyceridemia, low high-density lipoprotein cholesterol (HDLc) and severe insulin resistance (causing refractory T2D), for which higher circulating level of insulin contributes to prominent musculature, acanthosis nigricans, and pseudoacromegaly. The severity of symptoms is heterogeneous, with some patients suffering total loss of adipose tissue while others retain some adipose depots. The most common causes of CGL are mutations in 1-acylglycerol-2-phosphate O-acyltransferase 2 (*AGPAT2*) (Agarwal et al., 2002) and Berardinelli–Seip congenital lipodystrophy 2 (*BSCL2*) (Magre et al., 2001), although mutations in other genes, such as caveolin 1 (*CAV1*) (Kim et al., 2008) and polymerase 1 and transcript release factor (*PTRF*)(Hayashi et al., 2009), have been also identified. Heterozygous mutations in *KCNJ6 (GIRK2)* can casue Keppen-Lubinsky

syndrome (KPLBS), characterized not only by a severe CGL, but also by a complex manifestation of the features including severe developmental delay, microcephaly, facial dysmorphy and aged appearance (Masotti et al., 2015). Moreover, biallelic mutations on peroxisome proliferator-activated receptor g (*PPARγ*) have been identified to cause a CGL similar as Berardinelli-Seip syndrome (Dyment et al., 2014).

Familial partial lipodystrophy (FLP) can be due to autosomal recessive or dominant mutations on several genes, resulting in a heterogeneous phenotype of fat loss and metabolic severity, with an estimated prevalence of 1/100,000 individuals. FLP is characterized by the co-occurrence of fat loss limited to the limb, gluteal and trunk regions and spared fat depots, located in the face, neck, and intra-abdominal regions (Robbins & Savage, 2015). The metabolic consequences of FLP are less severe than those seen with CGL because the severity of symptoms is proportional to the degree of fat loss; however, FLP still causes metabolic complications such as type 2 diabetes, dyslipidemia, and coronary heart disease, for which women are more severely affected than men (Robbins & Savage, 2015).

Autosomal recessive form of FPL is due to mutations in the zinc metalloproteinase (*ZMPSTE24*) gene, and is also a multisystem disease known as mandibuloacral dysplasia, characterized by an underdevelopment (hypoplasia) of the lower mandible and clavicle, and involved in the maturation of the lamin A protein (Agarwal, Fryns, Auchus, & Garg, 2003). Furthermore, a homozygous loss-of-function mutation in Cell death-Inducing Dffa-like Effector C (*CIDEC*) has also been shown to cause autosomal recessive FPL (Rubio-Cabezas et al., 2009). A strong characteristic of this case report was the presence of many multilocular white adipocytes, which are adipocytes divided into many small droplets. Moreover, a homozygous nonsense mutation on

the hormone sensitive lipase (*LIPE*) gene has been found using exome sequencing in two siblings with unusual late-onset FPL (Farhan et al., 2014).

On the other hand, mutations in the genes encoding lamin A/C (*LMNA*)(H. Cao & Hegele, 2000), peroxisome proliferator-activated receptor g (*PPARγ*)(Agarwal & Garg, 2002; Barroso et al., 1999), v-AKT murine thymoma oncogene homolog 2 (*AKT2*)(George et al., 2004), perilipin 1 (*PLIN1*)(Gandotra et al., 2011), or polymerase d1 catalytic subunit (*POLD1*) (Weedon et al., 2013) result in autosomal dominant form of FPL. The disorder caused by *POLD1* mutation is characterised not only by the metabolic features of FPL, but also by an array of other features including male hypogonadism, neurosensory deafness, and progeroid features such as resembling premature aging (Weedon et al., 2013).

In terms of pathophysiology, mutations in *AGPAT2*, *CAV1*, and *PTRF* genes may disrupt adipocyte function as a consequence of altered lipid trafficking or incorporation into triacylglycerol (Agarwal et al., 2002; Hayashi et al., 2009; Kim et al., 2008). *AGPAT2* encodes an enzyme responsible for synthesizing the precursors of phospholipids and triacylglycerol, whereas *CAV1* and *PTRF* have both been implicated in lipid metabolism through their roles in the formation of caveolae (i.e. small invagination of the adipocytes plasma membrane) (Agarwal et al., 2002; L. Liu et al., 2008; Pilch & Liu, 2011; Pol et al., 2004). Mutations in perilipin are responsible of smaller adipocytes, and in vitro experiments suggest that disruption of this lipid droplet protein increases basal lipolysis (Gandotra et al., 2011). Mutations in genes *PPARγ* and *BSCL2* may inhibit the expression of adipogenic genes and impair adipose-tissue differentiation (Agarwal & Garg, 2002; Payne et al., 2008; Rosen et al., 1999). Mutations in *LMNA* and *ZMPSTE24* appear to lead to abnormal nuclear architecture (Agarwal et al., 2003; Bergo et al., 2002; Boguslavsky, Stewart, & Worman, 2006). *CIDEC* mutations result in a multilocular

smaller lipid droplet phenotype, contributing to the elevated levels of basal lipolysis (Nishino et al., 2008; Rubio-Cabezas et al., 2009). *LIPE* mutations impair lipolysis free fatty acid flux from adipocyte (Farhan et al., 2014). *KCNJ6 (GIRK2)* mutations impair the inwardly rectifying K+ channel, for which the biological function remains unexplored (Masotti et al., 2015).

Ectopic fat accumulation (in liver, skeletal muscle, pancreas) in lipodystrophic subjects is strongly and consistently associated with insulin resistance and T2D (Perry, Samuel, Petersen, & Shulman, 2014; Samuel & Shulman, 2012). Coronary atherosclerosis could be explained by the metabolic risk factors present in these subjects, and also by the endothelial cells dysfunction and senescence as reported in some cases (Bidault et al., 2013), whereas epicardial fat is totally absent in CGL (H. S. Sacks & Fain, 2007). In terms of management of the metabolic complications, some features can be improved by recombinant leptin therapy (Oral et al., 2002; Petersen et al., 2002).

2.2.5 Mendelian form of obesity with metabolic syndrome

Gene identification efforts for metabolic syndrome have been limited so far, likely due to the lack of consensus on the diagnostic criteria. Only one study so far has highlighted the role of *DYRK1B* (Dual-Specificity Tyrosine-(Y)-Phosphorylation Regulated Kinase 1B) in the Mendelian form of metabolic syndrome (Keramati et al., 2014). The mutation R102C within *DYRK1B* was identified by linkage analysis and whole-exome sequencing in three large Iranian families with a perfect co-segregation with central obesity, T2D, hypertension, and early-onset coronary artery disease. Moreover, screening for *DYRK1B* among 300 morbidly obese white Caucasian with coronary artery disease and metabolic phenotype led to the identification of the mutation H90P in five unrelated patients, co-segregating with criteria of the metabolic syndrome

in an autosomal dominant pattern. The mutations alter two important functions of *DYR1B*, consisting of the promotion of adipogenic differentiation and the induction of key gluconeogenic enzyme, such as glucose-6-phosphatase.

2.3 Polygenic Obesity

Contrary to monogenic obesity, polygenic obesity is caused by multiple-gene defects with modest effects that interact with the environment (Cummings & Schwartz, 2003). Several approaches have been used to discover genes associated with polygenic obesity. Although linkage/positional cloning and candidate gene approaches were mostly unsuccessful, some key studies added to our knowledge of polygenic obesity.

In conjunction with positional cloning, genetic linkage analysis has been used to detect the chromosomal location of a disease gene. The concept is based on the observation that genes located close to each other remain linked during meiosis (Pulst, 1999). Meyre *et al.* used linkage analysis to identify K121Q, a 3 SNP haplotype in *ENPP1* that contributes to childhood and adult obesity under a recessive mode of inheritance in European populations (Meyre et al., 2005). Follow-up meta-analyses of the gain-of-function coding variant K121Q (rs1044498) confirmed the association with adult obesity in populations of European descent (R. Wang et al., 2011). Transgenic/knock-out mouse models have demonstrated that overexpression of *ENPP1* can result in adipocyte insulin resistance and defective adipocyte maturation (Dong et al., 2005; Liang, Fu, Ciociola, Chandalia, & Abate, 2007).

Congenic female mice lacking *TBC1D1* presented with a reduction in body weight, suggesting that *TBC1D1* induced a high-fat diet-induced obesity by increasing lipid use in skeletal muscle (Chadt et al., 2008; Hargett, Walker, Hussain, Hoehn, & Keller, 2015). Additionally, *TBC1D1* is

involved in the regulation of GLUT4 protein levels and in exercise mediated glucose uptake in non-oxidative muscle fibers (Stockli et al., 2015). The variant R125W (rs35859249) in *TBC1D1* was identified as a candidate for severe obesity in females through a linkage study in US and French populations (Stone et al., 2006). Although not associated with BMI or obesity, R125W has been known to play a role in predisposition to familial obesity (Meyre et al., 2008). This mutation impairs skeletal muscle glucose transport and results in complete loss of insulin-responsiveness acquisition (Hatakeyama & Kanzaki, 2013; Scherag, Dina, et al., 2010).

Mutations in *PCSK1* are a frequent cause of monogenic obesity in human and mouse models (Lloyd, Bohan, & Gekakis, 2006; J. Philippe et al., 2015). The *PCSK1* gene encodes an enzyme expressed in neuroendocrine cells that converts prohormones into functional hormones (Benzinou et al., 2008). These hormones are known to regulate central (e.g. proopiomelanocortin) and/or peripheral (e.g. insulin) energy metabolism (Benzinou et al., 2008). Following the identification of a linkage region for adult obesity on chromosome 5q14-21 in the French population, Benzinou *et al.* were prompted to use *PCSK1* as a positional candidate gene for polygenic obesity (Bell et al., 2004; Benzinou et al., 2008). SNPs rs6232 and rs6234-rs6235 were found to be consistently associated with severe obesity in both adults and children (Benzinou et al., 2008). Recently, a systematic review in more than 330,000 individuals demonstrated an association of the same variants with moderate obesity and BMI variation (Nead et al., 2015). Impairments in the N221D-mutant PC1/3 protein's catalytic activity were also observed (Benzinou et al., 2008). Through transfected rat models, Mbikay *et al*. illustrated that the presence of the triple variants (rs6232, rs6234 and rs6235) can result in a subtle deficit of PC1/3 enzymatic activity in endocrine and neuroendocrine cells (Mbikay, Sirois, Nkongolo, Basak, & Chretien, 2011). This can result in impairments in converting prohormones and

proneuropeptides to their bioactive forms (Mbikay et al., 2011). Another functional *PCSK1* variant, R80Q (rs1799904), negatively impacts the maturation and in-vitro catalytic activity of PC1/3 (Pickett et al., 2013). This provides insights into how polymorphisms in the *PCSK1* gene could lead to an increase in BMI and obesity.

Another approach in polygenic obesity studies is the candidate gene approach. This approach involves the identification and analysis of a gene with a likely role in the pathogenesis of the disease or due to the gene's chromosomal location (Apalasamy & Mohamed, 2015). *BDNF* has been associated with monogenic obesity in humans and mice, and is involved in weight regulation downstream of *MC4R* (Xu et al., 2003). Using a candidate gene approach, Gunstad *et al*. discovered that the Val66Met polymorphism in *BDNF* is related to BMI in healthy adults (Gunstad et al., 2006). This association was further confirmed in two large populations comprising of British women (Shugart et al., 2009). The Val66Met variant decreases BDNF secretion and leads to memory impairment and increased susceptibility to neuropsychiatric disorders such as anxiety and depression (Bath & Lee, 2006; Egan et al., 2003). The candidate gene approach was also used on *GPR120* (*O3FAR1*), a receptor for unsaturated long-chain free fatty acids (Ichimura et al., 2012). It was found that a deleterious non-synonymous mutation, variant p.R270H, resulted in the dysfunction of *GPR120*. Lack of functioning GPR120 led to obesity in both humans and mice (Ichimura et al., 2012).

More recently, genome-wide association studies (GWAS) have been extensively and successfully used to identify obesity-associated genetic loci. GWAS involve the rapid and dense screening of markers across the genome to identify genetic variations associated with a trait (Hinney, Vogel, & Hebebrand, 2010). Common variants in the fat mass and obesity associated (*FTO*) gene that increase susceptibility to early-onset and severe obesity in children and adults

were the first to be successfully identified through GWAS and other methods (Dina et al., 2007). The variants were simultaneously discovered in four independent studies using different methods. Frayling *et al*. conducted a GWAS for type 2 diabetes susceptibility genes and identified an *FTO* variant (rs9939609) that predisposes children and adults to diabetes through an increase in BMI (Frayling et al., 2007). Homozygosity for the risk allele was associated with a 3 kg body weight increase and 1.67 fold higher chance of developing obesity (Frayling et al., 2007). Scuteri *et al*. conducted a GWAS to identify genetic variants associated with obesity related quantitative traits. They found that the *FTO* SNP rs9930506 was positively associated with BMI, hip circumference, and weight (Scuteri et al., 2007). Hinney *et al*. conducted a GWAS for early onset extreme obesity in 487 obese German children and found SNP rs1121980 in the *FTO* gene to be strongly associated with childhood obesity (Hinney et al., 2007). Concurrently, Dina *et al.* used a population stratification approach to identify an association between the *FTO* SNP rs1421085 and human obesity (Dina et al., 2007). Although all four of the initial studies were conducted in European populations, associations between *FTO* variants and obesity in other ethnicities have also confirmed a positive relationship between the two (Al-Attar et al., 2008; Apalasamy, Ming, Rampal, Bulgiba, & Mohamed, 2012; Hotta et al., 2008). An approximate of 1% of BMI variance can be explained by these variants (Frayling et al., 2007).

In 2009, Fischer *et al.* found that the loss-of-functional *Fto* gene in mice led to postnatal growth retardation and a reduction in adipose tissue and lean body mass (Fischer et al., 2009). In the heterozygous state, the knockout mice are similar to the wild-type mice (Church et al., 2009). However, in the homozygous state, the knockout mice exhibit significantly lower body weight starting at the age of 2 days (Church et al., 2009). *Fto* knockout mice are smaller than the wild-type, and fat mass is reduced more significantly in males than in and females (Church et al.,

2009). *Fto* knockout mice also demonstrate increased postnatal lethality, hyperphagia and decreased spontaneous locomotor activity (Church et al., 2009). Humans homozygous for a catalytically inactive *FTO* and $Fto^{-/-}$ mice show similarities such as severe growth retardation (Boissel et al., 2009). However, only humans are reported to show developmental abnormalities in the central nervous or cardiovascular system (Boissel et al., 2009).

The leanness in *Fto* deficient mice developed due to an increase in energy expenditure and systemic sympathetic activation, implicating *FTO* with energy homeostasis (Fischer et al., 2009). Correspondingly, Church *et al*. later demonstrated that overexpression of *Fto* in mice results in obesity (Church et al., 2010). These findings illustrate that the common *FTO* risk variants in intron 1 are associated with an increased expression or function of *FTO*. *FTO* is also essential for the normal development of the central nervous and cardiovascular systems, and functions as a transcriptional coactivator (Boissel et al., 2009). Additionally, *FTO* demethylates N6-methyladenosine residue in nuclear RNA (Waalen, 2014).

Studies have also shown that *FTO* variants can have an impact on the expression of *FTO* and other genes. For instance, homozygosity for the *FTO* SNP rs8050136 results in an increase in DNA methylation on the *FTO* obesity susceptibility haplotype (Bell et al., 2010). Recent studies have shown that the obesity associated *FTO* regions directly interact with the homeobox gene *IRX3* (Ragvin et al., 2010). SNP rs1421085 leads to a doubling of *IRX3* and *IRX5* expression during early adipocyte differentiation (M. Claussnitzer et al., 2015). Over expression of *IRX3* and *IRX5* result in an obesity phenotype (M. Claussnitzer et al., 2015). Thus, *IRX3* is a long range target of variants in *FTO* (Ragvin et al., 2010).

RPGRIP1L is a ciliary gene located near *FTO*. Stratigopoulos *et al*. showed that $Rpgrip1l^{+/-}$ mice tend to be hyperphagic and fatter, and displayed a decreased suppression of food intake in

response to leptin (Stratigopoulos et al., 2014). They concluded that *RPGRIP1L* may be partly or wholly responsible for the obesity susceptibility signal observed at the *FTO* locus (Stratigopoulos et al., 2014). *FTO* and *RPGRIP1L* are regulated by isoforms P200 and P110 of *CUX1*, a transcription factor (Stratigopoulos, LeDuc, Cremona, Chung, & Leibel, 2011). Presence of *FTO* SNP rs8050136 reduces the affinity for P110, resulting in decreased *FTO* and *RPGRIP1L* mRNA levels, decreased LepR trafficking to the cilium and subsequently, a diminished cellular response to leptin (Stratigopoulos et al., 2011). Considering the impact of *FTO* SNPs on the regulation of other genes, the strong effect of *FTO* intron 1 variation on human obesity may be explained by a pleiotropic effect on genes involved in energy homeostasis.

Since the discovery of *FTO*, many other loci that contribute to BMI, adult obesity, childhood obesity and WHR have been identified using GWAS (A. E. Locke et al., 2015). This highlights the benefits of large international consortiums in identifying important disease genes contributing to polygenic obesity.

In a GWAS meta-analysis conducted by Berndt *et al.* to identify new loci for anthropometric traits, a SNP near *HNF4G* was found to be associated with the overweight class, as well as obesity class I (35 kg/m^2 > BMI ≥ 30 kg/m^2) (Berndt et al., 2013). The *FTO* gene also has a binary obesity status as SNPs in the *FTO* gene can lead to adult and childhood obesity, young-onset extreme overweight and adult overweight (Warrington et al., 2015). Meyre *et al*. conducted a GWAS for early onset and morbid adult obesity in European populations. Due to the binary nature of the study, the researchers identified new susceptibility loci in *NCP1*, near *MAF* and near *PTER* (D. Meyre et al., 2009). Similarly, Scherag *et al*. conducted a GWAS for obesity in French and German study groups with adults and children. They detected a new locus in *SDCCAG8* and one between *TNKS* and *MSRA* (Scherag, Dina, et al., 2010). The *TNKS/MSRA*

locus was strongly associated with childhood and adult obesity and with adult waist circumference, adding to the list of genes with a binary status for obesity predisposition (Scherag, Dina, et al., 2010). Other genes with an obesity binary status for adult and childhood obesity due to SNPs located in or near them include *TMEM18, POMC, SEC16B, OLFM4* and *NEGR1* (Bradfield et al., 2012; Warrington et al., 2015; Wheeler et al., 2013).

GWAS have identified major overlaps between variants associated with BMI and with obesity. However, genes responsible for body weight in the general population and in cases of extreme obesity differ slightly. Several studies have shown that pre-existent obesity can further amplify the effect of certain genes and their genetic variants and lead to an increase in body weight (Beyerlein, von Kries, Ness, & Ong, 2011). For instance, obesity risk alleles of *FTO, MC4R, TMEM18, BDNF, TNNI3K, NRXN3, SEC16B AND GNPDA2* were found to be more strongly associated with increases in BMI z-scores in children with higher BMI (Mitchell, Hakonarson, Rebbeck, & Grant, 2013).

GWAS is only the first step toward the identification of causal genes or functional variants. Recently, fine-mapping is being used for the identification of loci. Fine-mapping refers to the process of searching a region previously identified by GWAS for possible causal alleles (Spain & Barrett, 2015). Gong *et al*. used the Metabochip to genotype and evaluate 21 BMI loci in African Americans. They concluded that due to a lower linkage disequilibrium in African Americans, fine-mapping is a powerful tool for identifying underlying causal variants in known loci and discovering new loci (Gong et al., 2013). In another fine-mapping study, non-synonymous variants associated with increased BMI were detected in *APOBR, SULT1A1* and *SULT1A2* (Volckmar et al., 2015). Thus, genetic fine-mapping of variants can provide insights into the functional and regulatory mechanisms through which their effects on obesity-related

traits are mediated (Horikoshi et al., 2015). Fine-mapping has also successfully identified variants in the *FTO* gene that are related to obesity-related traits (Peters et al., 2013). Moreover, Akiyama *et al*. showed that causal variants from those in strong linkage disequilibrium can be resolved better when distant ethnic populations are included in the follow-up study (Akiyama et al., 2014). Thus, although not all fine-mapping experiments are successful, an integrative approach based on trans-ethnic fine mapping, functional and evolutionary basis can be efficiently used to identify the causal SNPs.

SNPs in most monogenic and some Mendelian syndromic genes have been shown to contribute towards polygenic obesity as well. Dickson *et al.* proposed that uncommon or rare variants may create "synthetic associations" by occurring more often in association with one of the alleles at the common site versus the other allele (Dickson, Wang, Krantz, Hakonarson, & Goldstein, 2010). They also concluded that these synthetic associations account for part of the association signals identified through GWAS (Dickson et al., 2010). These findings call for interpreting GWAS signals with great caution and consideration. However, subsequent studies demonstrated that synthetic associations due to rare variants do not successfully explain most of the GWAS signals (Anderson, Soranzo, Zeggini, & Barrett, 2011; Wray, Purcell, & Visscher, 2011). Scherag *et al*. used GWAS data to analyse a genomic region encompassing *MC4R* and found that a haplotype extending from 5' to 3' of *MC4R* showed a stronger association to obesity than single SNPs (Scherag, Jarick, et al., 2010). After analyzing synthetic associations, they concluded that *MC4R* coding variants had a negligible impact on the association signal (Scherag, Jarick, et al., 2010).

Altogether, SNPs identified by GWAS contribute ~3% towards the BMI variance. This motivated researchers to search for the causes of the 'missing' heritability (A. E. Locke et al.,

2015). As discussed previously, a source of heritability often missed by GWAS studies is the contribution of copy number variations (CNVs). CNVs are chromosomal segments encompassing large replications or deletions in genes (Waalen, 2014). CNV studies have identified candidate regions near the *NEGR1* locus and on chromosomes 11q11 and 10q26.3 associated with obesity (Wheeler et al., 2013). In 2009, a GWAS conducted in the Chinese population found a CNV associated with BMI (Sha et al., 2009). The CNV, located at 10q11.22, was shown to contribute 1.6% to BMI variation (Sha et al., 2009). The pancreatic polypeptide receptor 1 (*PPYR1*) gene, a key regulator of energy homeostasis, is located within this region, providing an insight into the CNV's association with BMI (Sha et al., 2009). Using a novel variable number tandem repeats (VNTR) association method, El-Sayed Moustafa *et al*. showed significant associations between *DOCK5* VNTRs and childhood and adult severe obesity. They also estimated that these VNTRs explain approximately 0.8% of variance in BMI (El-Sayed Moustafa et al., 2012). Wheeler *et al*. also identified rare single CNVs to be significantly associated with severe obesity. Analysis of those rare deletions indicated that an enrichment of genes affecting G protein-coupled receptors (GPCR) involved in energy homeostasis through neuronal regulation (Wheeler et al., 2013). Interestingly, CNVs can also have ethnicity-specific impacts. For instance, CNV 16p12.3 was confirmed to be significantly associated with obesity in European populations, but not in Chinese populations (T. L. Yang et al., 2013). Large international consortia can be used to discover other single nucleotide variants (SNVs) associated with obesity. For instance, the HumanExome BeadChip was recently used to identify novel associations between SNVs and T2D susceptibility (Wessel et al., 2015). If such experimental designs are extended to study obesity, additional SNVs associated with obesity can be identified.

Although there is a big overlap between both childhood and adult polygenic obesity, there are variants whose effect on obesity varies with age and development. In a meta-analysis of pre-existing GWAS, Nead *et al.* collected phenotypic and genotypic data from more than 330,000 individuals. They found significant associations between *PCSK1* variants and childhood and adult obesity (Nead et al., 2015). However, the SNPs, rs6232 and rs6234/rs6235, had a stronger effect in children/adolescents than in adults. Similarly, SNP rs6232 is more strongly associated with BMI in younger versus older adult Europeans (Nead et al., 2015). Longitudinal and cross-sectional studies have shown that SNP rs9939609 in the *FTO* gene increases BMI from birth up to the age of 20-30 years (Hertel et al., 2011). After the youth years, the difference in BMI due to the *FTO* genotype remains at the same level throughout life (Hertel et al., 2011). On the other hand, a variant in *ADCY3* has a relatively constant impact on BMI throughout childhood (Warrington et al., 2015). A longitudinal study in children from birth to age 11 was conducted by Elks *et al*. They used data from 7146 children to study the impact of eight genetic variants that were known to be associated with childhood BMI (C. E. Elks et al., 2010). They found that the risk alleles were not significantly associated with birth weight, but had a large impact on increased weight gain during early infancy than on subsequent childhood weight gain (C. E. Elks et al., 2010).

Winkler *et al*. conducted a genome wide search in more than 300,000 adults of European ancestry. They found that 15 loci were associated with BMI in an age dependent manner, with a more prominent effect in younger adults (T. W. Winkler et al., 2015). In doing so, they also identified four novel loci associated with BMI (T. W. Winkler et al., 2015). In a study conducted by Warrington *et al.,* a *OLFM4* variant was found to be associated with increased BMI at 8 years and increased BMI change through childhood (Warrington et al., 2015). The complex etiology of

BMI and BMI change was studied by North *et al.* in more than 4000 siblings and twins by following them from adolescence to young adulthood. They found shared genetic effects between BMI and BMI change, with a subsequent weakening in adulthood (North et al., 2010). Household effects, or unmeasured non-genetic factors, were mostly noticed during young adulthood, and not adolescence (North et al., 2010).

Several multi-ethnic GWAS have also been conducted to study the differences between the effects of variants in specific ethnicities. For instance, the *PCSK1* SNP rs6234/rs6235 was found to be associated with obesity in white Caucasian, African and Hispanic ethnic groups, but a similar association was not found in the East Asian populations (Nead et al., 2015). Ethnic-specific epistasis was hypothesized to be the cause of the differential associations (Nead et al., 2015). Similarly, GWAS for obesity susceptibility in different ethnic groups have found different loci. Bollepalli et al. conducted a GWAS in African-American adolescents to identify *FTO* variants associated with adiposity and found a novel SNP rs8057044 (Bollepalli, Dolan, Deka, & Martin, 2010). This *FTO* SNP had not been identified in European populations, illustrating the complex relationship between ethnicity and the genetics of obesity.

To date, numerous gene-gene interaction studies have been conducted. Okada *et al.* conducted a GWAS in 62,245 East Asian subjects and identified two novel loci associated with BMI (Okada et al., 2012). In the process, they also discovered gene-gene interactions between *KLF9* SNP rs11142387 and *GDF8* SNP rs13034723 (Okada et al., 2012). Ochoa *et al.* studied gene-gene interactions in a Spanish population comprising of children and adolescents. They found a synergistic effect between SNP Pro12Ala of the *PPARγ2* gene and Trp64Arg in the *ADRβ3* gene on obesity risk (Ochoa et al., 2004). Similarly, Liu *et al*. demonstrated the presence of gene-gene interactions amongst variants in three different genes (F. H. Liu et al., 2014). They conducted a

case-control study in Chinese children and discovered a 3-locus interaction on obesity involving genetic variants of *INSIG2, SCAP*, and *SREBP2* (F. H. Liu et al., 2014). A study in a Greek adult population found 24 SNPs in *FTO, MC4R, TMEM18, PRL, AIF1* and *PCSK1* to be associated with obesity (K. Rouskas, A. Kouvatsi, et al., 2012). They also observed gene-gene interactions, however, no significant pair-wise interactions between SNPs were found (K. Rouskas, A. Kouvatsi, et al., 2012). Despite the extent and importance of gene-gene and gene-environment interactions, a simulation study demonstrated that the inclusion of such effects in risk-prediction models does not significantly improve the discrimination ability of these models (Aschard et al., 2012).

It remains that SNPs are the more common source of genetic variation and explain a substantial fraction of heritability for complex traits such as obesity (A. Li & Meyre, 2014b). Recently, Yang *et al.* proposed a new method to estimate heritability for complex human traits (J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk, Snieder, LifeLines Cohort, et al., 2015). They used simulations based on whole-genome sequencing data to identify that a majority of the variation at common and rare variants can be captured by imputation (J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk, Snieder, Esko, et al., 2015). Using the new method, GREML-LDMS, they estimated that variants explained 27% of the variance for BMI, which is 10% more than what had previously been reported (J. Yang, Lee, Goddard, & Visscher, 2011). They also concluded that the actual heritability estimate for BMI is between 30% and 40% (J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk, Snieder, Esko, et al., 2015). This further exemplifies the importance of elucidating the

mechanisms of polygenic obesity in order to improve our understanding of obesity and the treatment and care of patients.

2.4 Body fat distribution related SNPs

General obesity is generally assessed by BMI as a surrogate measure, but another trait of interest is fat distribution. Fat distribution is an important parameter, as abdominal obesity has a stronger association with cardiometabolic complications in comparison to BMI (de Koning et al., 2010). Different measures of fat distribution have been used so far, including body fat percentage, VAT/SAT ratio, waist circumference (WC), hip circumference (HC), waist to hip ratio (WHR) and WC-, HC- or WHR- adjusted for BMI (WCadjBMI, HCadjBMI and WHRadjBMI).

Independent GWAS approaches (Chambers et al., 2008; Cho et al., 2009; Fox et al., 2012; Heard-Costa et al., 2009; C. T. Liu et al., 2013; Randall et al., 2013; K. Wang et al., 2011) and GWAS meta-analysis studies (Heid et al., 2010; Kilpelainen et al., 2011; Lindgren et al., 2009; Pei et al., 2014; Dmitry Shungin et al., 2015; T. W. Winkler et al., 2015; Yoneyama et al., 2014) have identified over 97 loci related to body fat distribution in all ancestries, but mostly in Europeans. 70 loci were associated with WHRadjBMI (Heid et al., 2010; C. T. Liu et al., 2013; Randall et al., 2013; Dmitry Shungin et al., 2015; T. W. Winkler et al., 2015; Yoneyama et al., 2014), 7 with unadjusted WHR (Cho et al., 2009; Heid et al., 2010; Lindgren et al., 2009; D. Shungin et al., 2015; K. Wang et al., 2011), 14 with WC (Chambers et al., 2008; Heard-Costa et al., 2009; Kilpelainen et al., 2011; Lindgren et al., 2009; C. T. Liu et al., 2013; D. Shungin et al., 2015; K. Wang et al., 2011), 9 with HC (D. Shungin et al., 2015), 3 with body fat percentage (Heard-Costa et al., 2009; Kilpelainen et al., 2011; Pei et al., 2014), 3 with VAT or SAT (Fox et al., 2012), 3 with fat body mass (Kilpelainen et al., 2011; Pei et al., 2014).

Collectively, only ten loci were associated both with WHRadjBMI and other measures of fat distribution. Besides the BMI association, *IRS1*, which is a contributor to insulin receptor signalling and insulin resistance has also been associated with body fat percentage, *MC4R* with WC and *FTO* with fat mass, body fat percentage and WC. Interestingly, only *FTO* and *MC4R* have been both associated with fat distribution, BMI and obesity status, and only *PPARγ* overlaps with fat distribution (WHR) and monogenic form of lipodystrophy.

Considering the multiethnic aspect of body fat distribution, Pei *et al.* has reinforced the association between *FTO* and *MC4R* with fat mass in a multiethnic population and found a new locus within CTSS (Pie, Gil-Rodriguez, Ciero, Lopez-Vinas, Ribate, Arnedo, Deardorff, Puisac, Legarreta, de Karam, Rubio, Bueno, Baldellou, Calvo, et al., 2010). Liu *et al*. found two new loci within *LHX2* for WC and *RREB1* for WHRadjBMI in African ancestries and confirmed 6 loci previously identified in European ancestries (C. T. Liu et al., 2013). Cho *et al.* identified a new locus within *HECTD4* associated with WHR in Asian ancestries (Cho et al., 2009).

Interestingly, sex- and/or age-specific effect has been screened in two meta-analyses, conducted recently in up to 225,000 and 320,000 individuals (Dmitry Shungin et al., 2015; T. W. Winkler et al., 2015). Shungin *et al.* showed a sexual dimorphism for half of the WHRadjBMI loci, showing higher effect sizes in women compared to men (D. Shungin et al., 2015). The cumulative effect of the WHRadjBMI-related alleles was estimated at 1.36 % (0.82 % in men and 2.40 % in women) of the phenotypic variance (D. Shungin et al., 2015). Winkler *et al.* found no age dependent-effects for WHRadjBMI, by stratifying at the threshold of 50 y-old (T. W. Winkler et al., 2015).

Furthermore, using in silico tools and algorithms, the identified loci were enriched for genes expressed in adipose tissue and for putative regulatory elements in adipocytes. Pathway analysis

implicated adipogenesis, angiogenesis, skeletal growth, transcriptional regulation and insulin resistance as processes affecting fat distribution and providing insight into potential pathophysiological mechanisms (Dmitry Shungin et al., 2015).

Very few studies investigated SNPs x lifestyle factors interaction on WHR, because of the difficulties to reach the required power (Velez Edwards et al., 2013). Other anthropometric trait, such as body adiposity index (BAI) calculated from the height and the hip circumference, could also be used in GWAS meta-analysis.

2.5 Gut Microbiota in Obesity

Recently, the gut microbiota and the genes that contribute to their diversity and population has been discovered to play a role in developing obesity. The human gut microbiota is composed of up to 100 trillion microbes and carry more than 5 million genes (microbiome) (Ursell et al., 2014). The dominant phyla in the gut are Bacteroidetes (20–25%), Firmicutes (60–65%), Proteobacteria (5–10%), and Actinobacteria (3%), which together constitute over 97% of the gut microbe population (Ursell et al., 2014). This composition not only presents variability among individuals, but also longitudinal variability within individuals. Both the abundance and the composition of microbial population are influenced by diet, medication, weight, and metabolic state of the host (Rosenbaum, Knight, & Leibel, 2015).

In rodents, obesity is associated with an increase in the relative population of the Firmicutes versus a decrease of Bacteroidetes population in the gut, and a decrease in the diversity of the microbiota (Ravussin et al., 2012). Studies of germ-free mice, or previously germ-free mice colonized with a defined microbial community (gnotobiotic mice), showed that the diversity, as well as the presence and relative proportion of different microbes in the gut, play a role in energy

homeostasis. Inoculation of germ-free mice with conventional microbes results in weight gain to similar levels of fatness to the donor mice (Backhed et al., 2004). The amount of weight gained by gnotobiotic rodents differs depending on which microbes are inoculated (Ridaura et al., 2013; Turnbaugh et al., 2006) and are also dependent on the environment in which animals are studied (Duca et al., 2014; Ericsson et al., 2015; Ridaura et al., 2013). Significant heritability has been also reported of weight gain and of gut microbial composition and plasticity in response to a high-fat/high-sugar diet (Parks et al., 2013).

In humans, in most (Turnbaugh et al., 2009; Turnbaugh et al., 2006), but not all (W. A. Walters, Xu, & Knight, 2014), studies, both the diversity of the microbiota and the fractional proportion of Bacteroidetes species relative to Firmicutes are decreased in obese *versus* lean individuals (Kocelak et al., 2013; Turnbaugh et al., 2009; Turnbaugh et al., 2006; W. A. Walters et al., 2014). These proportions are extremely sensitive to energy balance (Faith et al., 2013). Weight loss increased the relative proportion of Bacteroidetes species and microbial diversity, thereby 'reversing' some of the microbial characteristics of obese versus lean individuals (Ley, Turnbaugh, Klein, & Gordon, 2006). Similarly, studies of the microbiome have shown that gene richness is diminished in obese versus lean individuals (Turnbaugh et al., 2009) and is increased by dietary weight loss (Cotillard et al., 2013; Jumpertz et al., 2011). A key question is whether the positive association of the abundance of Firmicutes and negative association of the of Bacteroidetes population with changes in body weight represent a direct effect of the degree and duration of negative or positive energy balance, or of changes in energy stores as fat mass. The predominance of the environment in determining the composition of the gut microbiome is also evident (Wu et al., 2011). By examining the effects of underfeeding and overfeeding, differences between caloric intake and weight maintenance calories were positively correlated with the

relative abundance of Firmicutes species and negatively correlated with the relative abundance of Bacteroidetes species in lean and obese humans (Jumpertz et al., 2011). Moreover, the population of Firmicutes was negatively correlated with resting energy expenditure, but not in multiple regression analysis including fat mass (Kocelak et al., 2013).

Few studies in humans examined the effects of alterations in the gut microbiome on energy expenditure and food intake, whereas many hypotheses regarding the mechanisms by which the human gut microbiota might affect these phenotypes, are largely based on mice studies. Administration of conventional mouse bacteria to germ-free mice results in decreased energy intake and increased energy expenditure and suggests a role of the microbiome in appetite regulation and the efficiency of energy harvest from food (Backhed et al., 2004; Hartstra, Bouter, Backhed, & Nieuwdorp, 2015; Ley et al., 2006; Turnbaugh et al., 2006).

Administration of probiotics (live microorganisms which when administered in adequate amounts confer a health benefit on the host) – such as Lactobacillus species– reduce weight and body fat in DIO mice without changing energy intake (H. Y. Lee et al., 2006).

On the other hand, there are prebiotics that are substances specifically support the growth and/or activity of health-promoting bacteria that colonize the gastro-intestinal tract. Examples of prebiotics include dietary inulin-type fructo-oligosaccharides, which stimulate the growth of health-promoting Bifidobacterium, Lactobacillus, Roseburia, and Faecalibacterium species in humans (Macfarlane, Macfarlane, & Cummings, 2006). Administration of prebiotic was associated with a significant decrease in hunger and post-prandial glucose excursions, and a significantly greater satiation after a meal versus a similar-tasting placebo (Moran & Dailey, 2011).

Introduction of conventional gut bacteria into gnotobiotic mice increases fatness but does not appear to affect the anticipated increases in *LEP, POMC* and *CART*, and decreased expression of *AGRP* and *NPY* (Everard & Cani, 2014; Schele et al., 2013). This suggests that these changes are induced by increases in adipose tissue mass rather than by a primary centrally mediated effect. However, the colonization of germ-free mice with conventional microbiota significantly blunts both the weight loss and the decline in *Agrp* and *Npy* expression following leptin administration (Everard & Cani, 2014; Schele et al., 2013), and prebiotic manipulation of the microbiota to decrease Firmicutes and increase Bacteroidetes phyla in leptin-deficient mice resulted in increased leptin-sensitivity (Everard et al., 2011). This suggests that the gut microbiota centrally affect leptin signaling. Reductions are also evident in the expression of the anorexigenic *Bdnf* and leptin resistance-associated suppressor of cytokine signaling 3 (*Socs3*) in both the brainstem and hypothalamus, compared to conventionally raised mice.

Specific alterations of the gut microbiota might constitute a potential therapeutic intervention to prevent obesity and/or to promote and sustain weight loss in humans. Before implementation of such applications, further studies that involve manipulations in prebiotics, probiotics, and diet composition is necessary. Manipulation of the microbiota by transplantation, diet or prebiotics in different metabolic states (obese, formerly-obese, and never-obese) could be used to isolate the possible roles of the microbiota in the regulation of energy homeostasis. As there is large inter-individual variation in human responses to diet, exercise, pharmacological or other weight-loss interventions, there are likely to be differences between individuals in the salience of the roles of microbiota in energy homeostasis.

2.5 Gene-environment interactions

Environmental factors have also been known to interact with the genetics of polygenic obesity. Rosenquist *et al*. studied longitudinal data from the Framingham Heart Study (Rosenquist et al., 2015). They found a variation in the effect of the *FTO* risk allele on BMI over time, hypothetically due to global environmental changes that influence allelic penetrance (Rosenquist et al., 2015). Despite the increase in an obesogenic environment, studies have shown that the genetic influence on BMI and adiposity is significantly high. Moreover, children born since the onset of the pediatric obesity epidemic have demonstrated an increase in the additive genetic variance of BMI (J. Wardle et al., 2008). Between 1951 and 1983, the BMI heritability increased from 75% to 78.8%, suggesting that the obesogenic environment enhanced the influence of adiposity related genes (Rokholm et al., 2011). Foraita *et al*. showed that compared to children with the protective *FTO* genotype, those heterozygous or homozygous for SNP rs9939609 were found to be less protected from the effects of an obesity-promoting socioeconomic status (Foraita et al., 2015). Studies have also shown that the *FTO* gene variants interact with diet and physical activity (Grau et al., 2009; Muc, Padez, & Manco, 2015). Interestingly, the low penetrance of *MC4R* loss-of-function mutation in Greek population, suggests a beneficial interaction with Mediterranean diet (K. Rouskas, D. Meyre, et al., 2012). This could be due to the different fatty acids, oleic acid or omega 3 versus saturated acid, that reduce food intake and body weight, through a modulation of *POMC* and *MC4R* signalling (Schwinkendorf, Tsatsos, Gosnell, & Mashek, 2011). Analysis of gene and environment interaction with BMI heritability show that shorter sleep duration is associated with increased BMI and increased genetic influences on BMI, suggesting that shorter sleep duration increases expression of genetic risks for high body weight

(Watson et al., 2012). These findings illustrate the impact of the environment on the expression of genes involved with obesity.

2.7 Epigenetics of obesity

Epigenetics is defined as changes in gene transcription and expression that occur without altering the DNA sequence and result in long-term changes in cellular and biological functions. Epigenetic mechanisms were initially used to explain the fetal origins of disease theory, also known as the Barker hypothesis (Barker, 1990). David Barker observed that infants with a low birth weight tend to have an increased risk of coronary artery disease, stroke and type 2 diabetes later in life. He hypothesized that nutritional and other environmental exposures in early life can lead to changes in predisposition to T2D and cardiovascular disorders in the long term (Barker, 1990). His hypothesis was validated by strong epidemiological data from the Dutch famine cohort (Drong, Lindgren, & McCarthy, 2012).

Some of the currently known epigenetic mechanisms include modifications in: 1. Cell differentiation through DNA methylation and histone modification; 2. Dosage compensation through inactivation of redundant genes; 3. Genome structure maintenance (DNA damage repair); 4. Genomic/parental imprinting; 5. Repetitive element repression resulting in heterochromatin formation (Bays & Scinta, 2015).

Numerous epigenetic heritability studies have been conducted to date. DNA methylation and histone acetylation patterns were analyzed in monozygotic twin pairs. They found that twins were epigenetically similar during the early years of life, and differed considerably later in life. Apart from illustrating the high heritability of epigenetic patterns, this study also shows how significantly the environment can modify the genome through epigenetics (Fraga et al., 2005).

Factors such as diet, physical activity, pharmacological agents and environmental toxicants have the potential to influence epigenetic mechanisms (McAllister et al., 2009). The percentage of phenotypic variance that can be explained by epigenetics still remains unclear and new methodologies are currently being developed to better understand complex traits. For instance, Varona *et al.* recently developed a Bayesian mixed model method to estimate epigenetic variance (Varona et al., 2015).

As DNA methylation remains the major way to assess epigenetic patterns, various approaches have been developed to study the associations between methylation and obesity. Two of these include the candidate gene approaches and epigenome wide association studies (EWAS). Candidate gene approaches have often proven to be unsuccessful due to replication difficulties and small sample sizes. EWAS is a relatively new approach. It is still being debated whether it is best to look for rare or common variants, and a consensus on which cell types to explore has not been established. Through a systematic review of 46 epigenetics and obesity studies, it was observed that a majority of them used DNA from peripheral blood cells to analyze global methylation patterns (van Dijk et al., 2015). This can be attributed to the ease of access of obtaining blood samples. However, this can be problematic as different blood cells have varying methylation profiles. The potential negative consequences of this cellular heterogeneity can be prevented by correcting the data based on the number of each cell type in the sample (van Dijk et al., 2015). Moreover, blood cell methylation patterns may not be an accurate representation of epigenetic patterns in other tissues. During early life, methylation patterns are reflected in all germ layers. However, in later life, environmentally induced epigenetic influences may be more tissue-specific and not accurately represented in blood cells (van Dijk et al., 2015). Thus, caution should be applied when using blood cells to analyze epigenetic patterns.

The 1944 Dutch famine cohort was the first cohort used to establish the presence of epigenetic influences. It was observed that depending on the gestation period, exposure to the food shortage resulted in varying health complications for the offspring (Roseboom, Painter, van Abeelen, Veenendaal, & de Rooij, 2011). Higher levels of obesity, coronary heart disease, lipids and altered clotting were observed if exposed during early gestation, illustrating the effect of maternal nutrition on the offspring's health in later life (Roseboom et al., 2011). Heijmans *et al.* demonstrated that periconceptional exposure to the famine was associated with a lower methylation of the *IGF2* DMR. However, exposure later in the gestation period was not associated with *IGF2* methylation (Heijmans et al., 2008). This illustrates that the early development period is crucial for establishing and maintaining epigenetic marks (Heijmans et al., 2008).

Studies have shown that dietary effects on the epigenome can increase the risk of developing an altered metabolic profile, including obesity, in the offspring for up to two generations (Milagro, Mansego, De Miguel, & Martinez, 2013) Moreover, intrauterine environment of an obese woman can induce developmental adaptations in her developing fetus that predispose the offspring to obesity (Levin, 2000). Compared to children born before maternal weight loss, those born after maternal weight loss by bariatric surgery have been shown to have a lower risk for obesity (J. G. Kral et al., 2006). This indicates the presence of a strong epigenetic influence early in life on the development of obesity later in life. Another study analyzed DNA methylation patterns in adipose tissue in obese women. They showed that more CpGs are hypermethylated before weight loss through gastric bypass (Benton et al., 2015). Moreover, relative to promoter regions, increased methylation was observed in the 3' untranslated region and gene bodies before weight loss. These changes in methylation patterns were observed in genes associated with

obesity, epigenetic regulation and development (Benton et al., 2015). Huang *et al*. studied methylation patterns in individuals who went from being obese to normal weight and concluded that peripheral blood mononuclear cell methylation is associated with weight status (Y. T. Huang et al., 2015).

DNA of 479 individuals of European origin and two replication cohorts for a total of 2128 participants was used to analyze whole blood DNA CpG-sites in relation to BMI (Dick et al., 2014). Increased BMI in adults was found to be associated with increased methylation at the *HIF3A* locus in blood cells and adipose tissue (Dick et al., 2014). Successful replication studies have also been conducted using leukocyte DNA, in a different ethnic group (African Americans), and in neonates (Demerath et al., 2015). Rönn et al. analyzed DNA methylation of ~480,000 sites in human adipose tissue from 96 males and 94 females to study the impact of age, BMI and HbA1c levels on DNA methylation (Ronn et al., 2015). Age was found to be significantly associated with an altered DNA methylation and expression of 1050 genes. A correlation between BMI and DNA methylation and expression was identified in 2825 genes. HbA1c also significantly correlated with methylation at 711 sites (Ronn et al., 2015). Similarly, in obese cases, another study found higher methylation levels in a CpG site in the *UBASH2A* gene, and lower methylation levels in a CpG site in the *TRIM3* gene (X. Wang et al., 2010). These studies have provided us with additional evidence and knowledge about the link between epigenetics, DNA methylation patterns and obesity.

Studies have also suggested an interaction between genetic and epigenetic factors. The *FTO* gene has been shown to influence the methylation level of other genes and of itself. The obesity risk allele of *FTO* has been implicated with higher methylation of sites within intron 1 of the *FTO* gene and methylation of other genes (Bell et al., 2010). Voisin *et al*. studied 52 known obesity-

associated SNPs and found that alleles at 28 of them are associated with methylation levels at 107 proximal CpG sites (Voisin et al., 2015). This suggests that in addition to *FTO,* several obesity-associated SNPs are also associated with proximal gene regulation (Voisin et al., 2015). Although the study was conducted using DNA methylation levels in blood cells, four of the associations were replicated in skin fibroblasts (Voisin et al., 2015). Thus, recent research has provided evidence for the significant role played by epigenetics in predisposing offspring to obesity and other medical complications.

2.8 Inherited obesity and underlying biological mechanisms

One of the main goals of genetic studies in metabolic diseases is to not only identify novel genetic variants, but to also decipher the novel variant's functional contribution to the phenotype (Hirschhorn, 2009). Unfortunately, the majority of variants identified to be associated with obesity do not possess a clear connection to the biology of body weight regulation (Speliotes et al., 2010), which speaks to our limited understanding of the biological pathways involved in obesity. Gene targeting experiments in mice have been instrumental in confirming the role of many genes in the aetiology of obesity and paving the way towards finding fundamental physiological pathways in energy homeostasis (Challis & Yeo, 2002; Yazdi et al., 2015). Irrespective of their importance contribution in obesity studies, mouse models represent the tip of the iceberg. Other methodological studies such as functional genomics, crystallography, endophenotype studies and molecular biology experiments have provided additional insight into underlying mechanisms of obesity (Bureau et al., 2015; Calvani et al., 2014; Homuth et al., 2015; D. Yang, Jiang, & He, 2009). In terms of syndromic obesity, positional cloning, homozygosity mapping, candidate gene and whole exome sequencing approaches have led to the

discovery of 19 genes that are linked to syndromic Bardet-Biedl Syndrome so far (Novas et al., 2015). Genetic elucidations in syndromic obesity indicate the proximity of syndromic obesity genes to the BBsome complex (J. M. Brown & Witman, 2014). Follow-up studies in animal models resulted in development of BBS knockout mice (*Bbs2, Bbs4* and *Bbs6*) that developed hyperphagia, reduced energy expenditure and increased circulating leptin levels, which suggests that leptin deficiency is not the mechanism of obesity in BBS (Rahmouni et al., 2008). As leptin administration did not reverse the phenotype, this experiment highlighted the possibility of leptin resistance in BBS mice. On the other hand, expression of *Pomc* in BBS knock-out mice were significantly lower than that of controls, while agouti-related peptide (*Agrp*) and neuropeptide Y (*Npy*) levels were comparable to controls, suggesting that *Pomc* is the main player in obesity in BBS mice (Rahmouni et al., 2008). This phenomenon is compatible with the role of BBS in cilia function, as abrogating cilia in POMC neurons increases food intake and causes obesity in mice (Davenport et al., 2007).

Aside from syndromic obesity studies, defects in the monogenic candidate genes have led us to understand the important role of leptin-melanocortin pathway in the hypothalamus in controlling food intake and energy balance, as patients harboring these defects show similar clinical features such as obesity and hyperphagia (S. O'Rahilly & I. Farooqi, 2008). Further analysis of these genes have also highlighted their important role in hypothalamic development. For example, *SIM1* and *BDNF* are essential in development and differentiation of the paraventricular hypothalamic nucleus (Michaud et al., 2000; Tolson et al., 2010), which is an important integrator of sensory information influencing coordination of visceromotor and neuroendocrine responses(Swanson & Sawchenko, 1983). The discovery of hypothalamic involvement in energy balance suggested that traits related to appetite and satiety might represent one behavioral

mechanism in the genetic susceptibility to obesity. In relation to this hypothesis, Carnell *et al.* developed the behavioral susceptibility model, which emphasizes that genetic predisposition to obesity may act through appetitive traits reflecting lack of control over eating or eating in response to negative emotions (Carnell & Wardle, 2008; Konttinen et al., 2015).

In continuum with monogenic obesity, studies on polygenic obesity mechanism has also highlighted the importance of the central nervous system in body weight regulation, and attest further to the complexity of obesity biology (Walley, Asher, & Froguel, 2009). In polygenic obesity, satiety responsiveness also mediated the association between predisposition to obesity and adiposity, in parallel to what has been observed in monogenic obesity studies (C. H. Llewellyn, Trzaskowski, van Jaarsveld, Plomin, & Wardle, 2014). With progress in the methodology of genetic studies, the role of other regions of the brain as well as other organs in energy homeostasis have been discovered. For example, in a recent study, the role of hippocampus and the limbic system that have been previously associated with learning, cognition, memory and emotion, has been discovered in regulation of BMI (Adam E Locke et al., 2015). Furthermore, current literature on genes that are associated with BMI show the involvement if these genes in pathways such as in neural transmission, adipocytes, development, glucose homeostasis, lipid homeostasis and limb development (Adam E Locke et al., 2015).

Polygenic obesity paints a more complex picture of obesity mechanisms. BMI risk variants such as *GIPR*, which is a receptor for gastric inhibitory polypeptide (GIP) that is expressed in the intestine, mediate insulin secretion in response to oral glucose intake (Saxena et al., 2010; Speliotes et al., 2010). Although no human reports of *GIPR* mutation has been reported, mouse models of *Gipr* are resistant to diet induced obesity (Miyawaki et al., 2002). Furthermore, G-protein coupled receptor 120 (*GPR120*) is a receptor for unsaturated long chain fatty acids, and

plays a role in adipogenesis, regulation of appetite and food preference, and has been associated with higher BMI (Hirasawa et al., 2005; Ichimura et al., 2012). Interestingly, some T2D risk variants such as *CDKAL1, KCNQ1, TCF7L2* play a protective role against increased BMI (Speliotes et al., 2010; J. Zhao et al., 2010), which could be due to deficient insulin secretion that could impact BMI, as insulin is necessary for adipose storage (J. Zhao et al., 2010). Studies on the *FTO* and *ADRB3* genes have highlighted biological pathways that are related to browning of white adipocytes, lipid storage gene expression, repression of basal mitochondrial respiration, a decrease in thermogenesis in response to stimulus, and an increase in adipocyte size (Melina Claussnitzer et al., 2015; Loos, 2012). Adiponectin levels, which is a hormone produced predominantly by adipocytes, have been associated with increased BMI (Dastani et al., 2012). Environmental impacts on polygenic obesity add another layer of complexity to studies of obesity mechanisms. For example, individuals who are able to digest lactose (carriers of variants on the lactose gene) show higher BMI levels (Kettunen et al., 2010). As mentioned previously, CNVs on *AMY1* gene also impact obesity, as reduced copy number of *AMY1* is associated with higher BMI, most likely due to reduction in proper carbohydrate metabolism(Falchi et al., 2014). As evident, polygenic obesity encompass a diverse number of biological pathways and portrays a complicated framework of underlying mechanisms in obesity etiology. It is important to note that although thermogenesis or alterations in adipocytes or impaired insulin signaling could contribute to increase in weight gain, what separates monogenic from polygenic obesity is the hyperphagic clinical feature. Mechanisms involved in polygenic obesity could have an additive effect, contribute to excessive weight gain and result in increased BMI levels, but genetically driven hyperphagic behavior in monogenic obesity is powerful enough to single-handedly raise

BMI levels significantly. This suggests that excessive food intake is the main culprit in severe obesity and controlling energy intake is the first defense line against obesity.

In light of the complexity of the biology of polygenic obesity, novel ways of identifying mechanisms have surfaced in the literature in an attempt to develop a deeper understanding of obesity. One approach is the Mendelian randomization study, which infers causality from associations between genetic variants that mimic the influence of a modifiable environmental exposure and the outcome of interest (Smith & Ebrahim, 2003). This approach has been utilized to conclude that higher BMI levels lead to vitamin D deficiency, while vitamin D deficiency does not contribute to high BMI levels (Vimaleswaran et al., 2013). This approach was also used to support a direct genetic association between *FTO* and deep venous thrombosis (Klovaite, Benn, & Nordestgaard, 2015). In another study of similar caliber, the role of circulating fetuin-A and BMI was investigated. Fetuin-A is a multifunctional protein of hepatic origin and inhibits insulin receptor autophosphorylation and tyrosine kinase activity (Kalabay et al., 1998). This study highlighted the causal relationship between high fetuin-A levels and BMI, but the reverse causality was not significant (Thakkinstian et al., 2014). Such discoveries depict that obesity biology goes beyond the central nervous system and is impacted by several organs and cell signaling pathways.

Relative to monogenic and polygenic obesity studies, epigenetic studies are new and further investigation is required for analysis of the biological pathways behind epigenetics of obesity. As discussed, epigenetics impact the expression of genes that may be involved in obesity progression. An example of a mechanistic pathway that epigenetics contribute to obesity is the study of hypoxia-inducible factor-3α (HIF-3α). HIF-2α, which is one of the HIF-α subunits, induces HIF-3α, which then acts as an accelerator of adipogenesis through the induction of

adipocytes-related genes (Hatanaka et al., 2009). It has also been reported that insulin and 2-deoxy-D-glucose increased HIF-3α expression in vivo, which supports HIF-3α expression may play a role in the pathologies of metabolic diseases (Hatanaka et al., 2009; Heidbreder et al., 2007).

Despite the heterogeneity in phenotype expression in different types of lipodystrophies, most patients suffer from the same metabolic complications that obese patients suffer from, such as insulin resistance, diabetes, hepatic steatosis and dyslipidemia (Simha & Garg, 2006). This raises the possibility of a 'final common pathway' that may result in defective adipocyte differentiation, development and function. Normally, the adipose tissue sequesters free fatty acids (FFAs) and other lipids in form of inert triglycerides, but in conditions such as metabolic syndrome, the neutral lipid storage of the adipose tissue is exceeded after the saturation of normal depots (Krahmer, Farese, & Walther, 2013). In lipodystrophy, impairment in triglyceride synthesis, impaired adipocyte differentiation or adipocyte apoptosis could also lead to excess FFAs. In both conditions, the excess FFAs could lead to ectopic triglyceride depositions in the muscle, liver or pancreas that could consequentially lead to insulin resistance and dyslipidemia (Simha & Garg, 2006).

2.8 Genetics of Leanness

Severe obesity and being underweight could in part have mirroring aetiologies, possibly through opposing effects on energy balance. A complementary approach based on studies of genes that are associated with lean phenotype or resistance to weight gain in obesogenic environment could lead not only to a better understanding of the molecular basis of bodyweight regulation, but also to the identification of new pharmacological targets against obesity (Harosh, 2014).

Leanness is presented in clinical synopsis for a dozen of syndromes (http://omim.org), which most of them are neurodegenerative, causing poor swallowing abilities and consequently low food intake, such as the case in *NPC1* (Vanier, 2015). Among the syndromes with leanness that possibly result in metabolic alterations, a region within chromosome 16p11.2 is particularly well studied. A large deletion in this chromosome region is associated with severe obesity (R. Walters et al., 2010) and duplication in this region is associated with an underweight phenotype (Jacquemont et al., 2011). Deletion of 16p11.2 spanned 28 genes (R. Walters et al., 2010), including *SH2B1* that enhances leptin and insulin signalling (L. R. Pearce et al., 2014). Jacquemont *et al.* identified 138 duplication carriers, from over 95,000 individuals with developmental and intellectual disabilities, psychiatric disorders, or from European population-based cohorts (Jacquemont et al., 2011). The phenotypes correlated with changes in transcription levels for genes mapping within the duplication. Moreover, these features were associated with a notable frequency of selective food and restrictive eating behaviours (Jacquemont et al., 2011).

Correlated anthropometric phenotype with copy number variants of genes has been suggested in the region within 17p11.2. Smith-Magenis syndrome (SMS), manifesting intellectual disabilities, early-onset obesity with hyperphagia and abnormal fat distribution, is caused by a haploinsufficiency of *RAI1*, downregulating the hypothalamic expression of *BDNF* (Burns et al., 2010). The reciprocal duplication in 17p11.2 causes Potocki-Lupski syndrome (PTLS) (Lacaria et al., 2012; Soler-Alfonso et al., 2011) which demonstrates the opposite metabolic phenotype as SMS. PTLS is characterized by leanness, hyperactivity and resistance to diet-induced obesity (Lacaria et al., 2012), due to a overexpression of *RAI1* (L. Cao et al., 2014).

A third syndrome of interest is the LEOPARD syndrome, also called Noonan syndrome with multiple lentigines, a rare autosomal dominant disorder associating various developmental

defects, cardiopathies, dysmorphism, short stature and lower-than-average BMI (E. E. Zhang, Chapeau, Hagihara, & Feng, 2004). It is mainly caused by mutations of the *PTPN11* gene that inactivate the tyrosine phosphatase SHP2 (Src-homology 2 domain-containing phosphatase 2), a component of leptin receptor signalling (E. E. Zhang et al., 2004). LEOPARD syndrome in mouse experiments reveal that besides expected symptoms, animals display a strong reduction of adiposity and resistance to diet-induced obesity, associated with overall better metabolic profile. This phenotype results from impaired adipogenesis, increased energy expenditure, and enhanced insulin signalling (E. E. Zhang et al., 2004).

Whereas identification of obesity predisposing genes has received a lot of attention, only few studies investigated the polygenic determinants of leanness. Although, genetic factors have been shown to influence BMI across the entire BMI range, the contribution of genetic factors to the low BMI phenotype has been supported by genealogy data analysis (Yates, Johnson, McKee, & Cannon-Albright, 2013).

Several studies have showed that some variants in *MC4R* could protect against obesity. Negative association of V103I and I251L variants with obesity have been initially found in two studies led in Europeans (Geller et al., 2004; F. Stutzmann et al., 2007) and conclusively confirmed by meta-analysis (Young et al., 2007) and GWAS (Speliotes et al., 2010). The gain-of-function mutations I251L and V103I (Xiang et al., 2006) are also in opposition to the loss-of-function mutations in the same gene associated with hyperphagic obesity.

There are some additional reports for additional genes: individuals homozygous for the 67Thr allele of Agouti-related peptide gene (*AGRP*) had a BMI slightly below of the ideal range for their age (Marks et al., 2004), a rare variant in the visfatin gene (*NAMPT/PBEF1*) was associated with protection from obesity (Blakemore et al., 2009), and common variant in *GPR74* was

associated with leanness and increased lipolysis (Dahlman et al., 2007). However, these results have not been replicated and require more confirmation at this stage.

Genes that predispose to anorexia nervosa-induced underweight are thought to be different from the ones predisposing to inherited leanness because of the psychological component of anorexia nervosa. However, an inverse genetic correlation between BMI, childhood obesity and anorexia has been recently demonstrated using a cross-trait LD Score regression realized from GWAS summary statistics (Bulik-Sullivan et al., 2015). No anorexia nervosa predisposing genes have been identified by GWAS so far (Boraska et al., 2014).

2.9 From genomics to clinical practice

There is a growing enthusiasm for personalized medicine, an approach of care tailored for each patient based on their clinical characteristics and their genomic signatures, to optimize health outcomes (Feero & Guttmacher, 2014). The recent enthusiasm is partially due to the quick progress in technology, especially the development of high-throughput next generation sequencing and customized microarrays (Alyass et al., 2015) . Even if technological advancements facilitate the routine screening for known pathogenic mutations in cases of severe and/or early-onset obesity in clinical practice, functional studies are still required to determine the biological significance of obesity-associated variants and to fill the gap between the discovery of genetic susceptibility loci and its translation to preventive and therapeutic applications.

One aim of personalized medicine for obesity consists of designing health risk assessments to quantify the individual risk to develop (or aggravate) obesity and its related complications (Snyderman & Dinan, 2010). Given the diversity of factors that influence obesity and its

comorbidities, the risk assessments should be as most precise and exhaustive as possible. Such assessments would have to include personal and family history, psychological and social context, lifestyle behaviours (including eating and exercise habits), detailed medical examination, as well as radiological, histological, biological and omics data. Extending the analysis beyond genomics, the integration of other omics, such as transcriptomics, proteomics, epigenomics, metagenomics, metabolomics and nutriomics would increase the phenotypic prediction and will lead to better categorization of individuals based on risk profiles for BMI and comorbidities progression (Shah et al., 2015).

The first predictor tool that took into account the 32 common BMI-associated SNPs was a little disappointing, revealing a poor predictive value of these SNPs for obesity (Loos, 2012). By contrast, a predictive equation for childhood and adolescent obesity, taking into account clinical risk factors (parental BMI, birth weight, maternal gestational weight gain, behaviour and social indicators) and genetic score of 39 BMI/obesity-associated polymorphisms showed a correct accuracy in Italian, American and Finnish newborns (Morandi et al., 2012).

However, using GWAS significant SNPs for prediction of obesity may not be correct. Stringent thresholds of GWAS results in missing some true positive association signals. Moreover, using GWAS derived gene scores in personalized medicine could be questionable in terms of benefits for the patient; as they may change their health-related behaviours on the basis of weak predictor score (A. Li & Meyre, 2014a). Finally, SNPs can have more or less predictive impact depending on the accumulation of environmental and biological risk factors such as family history of cardio-metabolic diseases, age, sex, ethnicity, lack of physical activity, and diet habits. Instead, using new methods that take into account the whole genetic and environmental information to predict the disease, such as learning machine algorithms (Kruppa, Ziegler, & Konig, 2012; Wei

et al., 2009; Wei et al., 2013) and Bayesian approaches (J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk, Snieder, Esko, et al., 2015) would be the best approach towards personalized medicine.

Even if the technological progress in genomics are able to produce these algorithms, some limitations still exist. The supreme court of United States ruled that genomes cannot be patented, focusing the application of this rule on genetic tests for breast cancer, colon cancer, Alzheimer's disease, or muscular dystrophy. Cost-effectiveness of this high-throughput technology limits their access and contributes to aggravate the gap in medical practice in low and high-income countries (A. Li & Meyre, 2014a).

Another aim of personalized medicine consists in the prediction of response to therapy, in order to tailor treatment strategies. Currently, patients presenting with "obesity plus" phenotype (obesity plus growth abnormalities, mental retardation, dysmorphism…), suggesting a syndromic form of obesity, can be screened depending on the diagnosis hypothesis, with conventional cytogenetic, molecular tests, fluorescence in situ hybridization (FISH), or chromosomal microarray analysis technique, capable of detecting copy number variants (CNVs)(El-Sayed Moustafa & Froguel, 2013). Identification of causative chromosomal structural variation, or mutations in patients might be beneficial in providing an informed prognosis for the patient and early opportunities for intervention and adapted treatment of any commonly associated pathologies, such as specific behavior management for Prader Willi patients (Griggs et al., 2015).

Genetic screening for known mutations in monogenic obesity could be performed in patients presenting with early, rapid-onset or severe obesity, severe hyperphagia, hypopigmentation of hair and skin, and co-existence of lean and obese siblings in the family. Indeed, patients with

leptin deficiency resulting from loss-of-function mutations in *LEP* can be successfully treated by administration of leptin (Farooqi et al., 2002). In cases in which a monogenic obesity variant is identified in a proband, the patients' families should be offered genetic counselling as they might wish to consider genetic screening of other family members. Early genetic testing could in turn permit the identification of additional at-risk individuals and offer opportunities for early intervention in such patients, focusing in education and guidance on healthy eating and lifestyle choices (El-Sayed Moustafa & Froguel, 2013). This step is of utter importance, as children with *MC4R* functional mutations were able to lose weight in a lifestyle intervention but had much greater difficulties to maintain this weight loss (Reinehr et al., 2009).The same weight loss observation is evident in children carrying *POMC* variant (Santoro et al., 2006).

In the Pounds Lost trial, significant interactions were found between *IRS1* and carbohydrate intake in relation to changes in weight loss and insulin resistance at 6 years, but attenuated at 2 years with the weight regain (Qi, 2014; F. M. Sacks et al., 2009). Interaction between *FTO* and dietary protein was also found with body composition at 2 years (X. Zhang et al., 2012). DIOGENES showed interactions with variants especially in *GHRL*, *CCK* and *LEPR* and dietary protein on weight regain, but none variants remains associated after correction for multiple testing (Larsen et al., 2012). In Diabetes Prevention Program, a *MC4R* variant was associated with less short-term and long-term weight loss in the lifestyle intervention group, but not in placebo group (Pan et al., 2013). In the 2-year Dietary Intervention Randomized Controlled Trial, variant in *LEP* were found to be associated with weight regain; and addition of *LEP* genotype to the other variables in the prediction model increased its predictive value of weight regain (Erez et al., 2011). In the Look Action For Health in Diabetes trial, variant in *FTO* showed significant prediction for weigh regain, in diabetes support and education group, but not

within intensive lifestyle intervention group (J. M. McCaffery et al., 2013). A randomized trial of diabetes genetic risk counselling among overweight patients showed that genetic counselling did not significantly alter self-reported motivation or prevention program adherence (Grant et al., 2013). In children, no evidence was found for effects of 12 GWAS-based obesity marker alleles on weight regain in the course of 1 year after an intervention (Hinney et al., 2013), and only the obesity risk allele at a variant in intron 1 of *FTO* was associated with weight regain (Reinehr, Wolters, Roth, & Hinney, 2014).

However, these findings are rarely replicated, and they highlight the importance and challenges in replication of gene–diet interactions in randomized clinical trials. Replication of these interactions could make it easier to predict which lifestyle components (diet or exercise or both) interacted with the genotype (Qi, 2014).

Several studies have also explored the response of bariatric surgery, trying to draw the phenotypic and genotypic profile of best respondents (Neff, Olbers, & le Roux, 2013). The heritability of weight loss following bariatric surgery has been demonstrated (Hatoum et al., 2011), but the current interpretation of studies are heterogeneous depending on the study design, duration of follow up and type of surgery. Case reports on carriers of homozygous *LEPR* (Le Beyec et al., 2013) and *MC4R* (Aslan, Ranadive, et al., 2011) mutations showed a lower weight loss and poorer out-comes after bariatric surgery. A more complex relationship has been reported for heterozygous *MC4R* mutations, showing no significant effects on bariatric surgery outcomes (Aslan, Campos, et al., 2011; Hatoum et al., 2012). Among 648 bariatric surgery patients, 9 carriers of functional *MC4R* mutations, 10 carriers of *MC4R* V103L and I251L SNPs, 7 carriers of the rs17792313 variant and 22 carriers of the A-178C SNP were matched with two randomly paired controls without mutation. Weight loss until 12 months after surgery was not different

between cases and controls (Valette et al., 2012), but design of functional characterization of mutations and variants was questionable (Meyre, Froguel, Horber, & Kral, 2014). Among 1433 gastric bypass subjects, including fifteen carriers of rare variants of *MC4R* matched with the *MC4R* reference alleles carriers, comparable weight-loss were observed between groups, but subjects carrying three of these variants, V95I, I137T or L250Q lost less weight after surgery (Moore et al., 2014).

In 1443 subjects of the Swedish Obesity Study, *FTO* was associated with maximum weight loss, in gastric banding surgery subjects but not in gastric bypass subjects. The 10 other SNPs tested were not associated with this trait and no SNPs were associated with weight regain over 6 years of follow-up (Sarzynski et al., 2011). In 119 subjects undergoing a biliopancreatic diversion surgery showed that *FTO* has been shown to be associated with improved early weight loss post-biliopancreatic diversion, but this association was not significant at 1 year of follow-up (de Luis et al., 2012). In 200 gastric bypass subjects no difference was found in weight excess loss and amelioration of comorbidities according to the genotyping of *INSIG2* and *MC4R* (Goergen et al., 2011). In 1,001 gastric bypass surgery subjects, genotyped for 4 SNPs (*FTO, INSIG2, MC4R*, and *PCSK1*), weight loss trajectories was different across groups with low, intermediate, and high numbers of obesity risk alleles, with an interaction with the pre-operative BMI (Still et al., 2011).

A 2-stage GWAS of 693 and 327 gastric bypass subjects found that a 15q26.1 locus (rs17702901) was significantly associated with weight loss (Hatoum et al., 2013). However, larger studies, longitudinal analyses, and subsequent meta-analyses are required to definitively establish the role of genetic variants on postsurgical outcomes in individuals with obesity and determining whether treatment outcomes can be improved through assignment of patients to

personalized intervention strategies on the basis of their genetic profiles.

Another challenge in pursuit of personalized medicine is the response to medication against obesity. All new centrally active anti-obesity drugs (Fujioka, 2015) should be carefully assessed by using genetics, to ensure optimal prescribing in order to avoid potential side effects (Lazary, Juhasz, Hunyady, & Bagdy, 2011). It is interesting to note the reluctance of pharmacology industry to be involved in pharmacogenomics studies to avoid fragmenting the market.

Conclusively, for personalized medicine to become part of routine care, a new healthcare system approach that is predictive, preventive, personalized and participatory must emerge (Flores, Glusman, Brogaard, Price, & Hood, 2013). There also needs to be innovative and integrated to lead the way towards a cost-effective health system (Bousquet et al., 2011; N. Yang, Ginsburg, & Simmons, 2013).

2.11 Conclusion

Great progress in genetics and epigenetics studies have contributed to the unveiling of the pathophysiological architecture of obesity, even though more genes for syndromic, monogenic, oligogenic, and polygenic obesity remain to be discovered. The notable heterogeneity regarding the genetic elucidation of traits in different ethnic groups also encourage further investigation on an ethnic-specific basis and invite scientific collaborations through implementation of recruitment strategies in multiethnic societies. Our attempts in understanding obesity contribute to a fraction of obesity heritability explained so far. We count on technological advancements and new methodologies that may lead to an immediate and exhaustive gene harvest. The reality remains that obesity is a complex disorder and understanding its roots require more time and investigation. In an attempt to understand the methodological approaches employed so far in this

pursuit, the next chapter of this thesis will focus on the progress in understanding obesity genetics from studies based on rodent models.

3.0 Obesity genetics in mouse and human

Understanding the molecular roots of obesity is an important prerequisite to improve both prevention and management of this condition (Bessesen, 2008). This has prompted considerable effort to identify the genes predisposing to obesity by conducting studies in rodents and humans. This chapter, currently published in *PeerJ,* summarizes the progress in the elucidation of obesity genes focusing on the synergies developed between mouse and human obesity genetic fields.

3.1 What have mouse models taught us about human obesity?

Mouse models are the most common experimental animals in obesity genetics. General advantages of mouse models include: low maintenance cost, small size, ease of breeding and short gestation period. They reach sexual maturity faster than other mammals and have a shorter life span (A. W. Lee & Cox, 2011; Rosenthal & Brown, 2007). The availability of genetically defined strains, the complete genome sequence of numerous strains, dense single nucleotide polymorphism (SNP) characterization of many others, and well-developed genetic manipulation tools facilitate sophisticated genetic analyses. Genetic manipulation techniques employed in mouse models, such as knock-out/knock-in, overexpression or tissue-specific expression methods can be used to test the functionality of genes associated with obesity (Cox & Church, 2011). Additionally, since homozygote null mutations are exceptionally rare in humans, knock-out mouse models are an attractive alternative to study such rare occurrences (Rees & Alcolado, 2005). Mice also exhibit obesity and metabolic phenotypes that are comparable to humans and can be measured with standardized diagnostic tests (Toye et al., 2004). More detailed phenotyping, such as direct metabolic measurements and assessment of body fat content, that are difficult and costly in large numbers of humans are also possible in mice (Butler & Kozak, 2010)

(Ellacott, Morton, Woods, Tso, & Schwartz, 2010; Kaiyala et al., 2010). Importantly, environmental factors can be carefully controlled and specifically manipulated in mouse models (Ayala et al., 2010). Reducing environmental heterogeneity results in an increased power to link genetic variation to the phenotypic differences observed. Specific environmental manipulations allow direct testing of hypotheses to inform about gene-environment interactions. Mice also provide obesity-related tissues such as brain tissue that are otherwise difficult to obtain in humans (Tung et al., 2010).

However, it should be noted that mouse models of obesity are not without their limitations. Different conclusions may arise in mouse and human because of the use of different phenotypes in the study. For instance, BMI measurements are typical in human studies, whereas the direct measurement of percent body fat or body fat mass is more common in mouse models. Unlike humans, there is no defined threshold for obesity based on BMI in mice. Also, in comparison to humans, the secondary complications of obesity substantially depend upon the background strain (Clee & Attie, 2007). Moreover, some physiological differences between humans and mice make studying certain important genes or pathways difficult. For example, the role of *β-MSH* in control of energy balance was overlooked in humans mainly due to the fact that mouse models lack *β-MSH* (Y. S. Lee et al., 2006).

3.2 Tools and approaches available in mouse and human

The advancement in methodological techniques have led to many novel gene discoveries and intrigued further investigation into each discovery. The following section summarizes some of the techniques used in both mouse and human studies.

3.2.1 Human approaches

Linkage analysis

This approach aims to map the location of a disease causing loci by looking for genetic markers that co-segregate with the disease within pedigrees (Teare, Barrett, Road, & Sheffield, 2005). Different linkage approaches are applied depending on the type of the disease or trait. For example, parametric analysis is used if the disease is a Mendelian disease (Aihua Li & Meyre, 2014).

Homozygosity mapping

This is a powerful method to map genes responsible for recessive Mendelian disorders in consanguineous pedigrees. This approach requires less than a dozen of affected individuals, and no additional family members are required to identify the disease causing locus (Lander & Botstein, 1987).

Candidate gene studies

Candidate gene approach is hypothesis-driven and has been widely used before the rise of GWAS. Candidate genes have a known biological function that directly or indirectly influence the trait being investigated (M. Zhu & Zhao, 2007). The main disadvantage of this approach that it is heavily reliant on the current level of knowledge of a specific gene (Hirschhorn, Lohmueller, Byrne, & Hirschhorn, 2002). Candidate genes also have a low success rate overall, as consistent associations have been reported only for a selected few candidate genes (Vimaleswaran et al., 2012).

Genome-wide association studies

This approach exhaustively tests the genotype / phenotype associations across up to 4.8 million genetic markers and to date represents the most efficient way to identify common variants (MAF> 1%) associated with complex diseases (Visscher, Brown, McCarthy, & Yang, 2012).

Whole exome /whole genome sequencing

This relatively new approach is efficiently applied to identify rare variants associated with Mendelian or complex traits for a reasonable cost in comparison to classical approaches such as Sanger sequencing. It is powerful because it detects mutations in novel genes not previously detected by candidate gene approaches. The main challenge is to identify a causal gene analyzing the large sequencing dataset (Aihua Li & Meyre, 2014). With advances in sequencing technology, it is now possible to sequence approximately 95% of all protein-coding bases of all known genes (the "exome") at a cost that is comparable to sequencing a single gene by the Sanger method (Shendure, 2011; Singleton, 2011). Despite the fact that whole-genome sequencing experiments are more expensive than whole-exome sequencing experiments, they are more and more used to identify genetic variants associated with Mendelian and complex traits (Morrison et al., 2013; Styrkarsdottir et al., 2014).

3.2.2 Mouse genetic approaches

Natural mutations

Naturally occurring mutations are spontaneous mutations in mice that could be linked to the trait of interest. Natural mutations can range from simple single nucleotide substitution to complex rearrangements (Justice, 2000). They occur by chance and transmission from parent to offspring

results in fixation of these mutations within a population (Justice, 2000). These mutations are often studied by quantitative trait loci (QTLs), which link a chromosomal region to the trait of interest (Chiu, Diament, Fisler, & Warden, 2006; Diament, Fisler, & Warden, 2003).

Although studying natural variants may be appealing, regrettably the spontaneity of their appearance is often matched by their impromptu disappearance (Stanford, Cohn, & Cordes, 2001). Furthermore, studying obesity genes in mouse models with natural mutations may be a more time consuming approach as spontaneous natural mutations compared to chemically induced mutations.

Chemically Induced Mutations

Chemical mutagenesis increases frequency and variety of mutations for functional genetic studies. Furthermore, with the use of inbreeding techniques, chemical mutagenesis can create a genetic variant that is identical to parent strain except for the induced mutation that may be responsible for phenotypic diversity compared to parent strain (Svenson, Bogue, & Peters, 2003). This approach creates a set of mutants that differ minimally in genotype from parental strain, but differ robustly in phenotype, making it a promising approach in functional genetic studies (Svenson et al., 2003).

Successful genetic manipulation requires DNA modifications of germ-line cells so that the modification is heritable (Strachan & Read, 1999). Target cells usually can differentiate into different cells or give rise to germ-line cells, which makes embryonic stem cells (ES) ideal because they can differentiate to somatic and germ line cells (Strachan & Read, 1999).

X-Ray mutagenesis

This method induces mutations 20-100 times greater than spontaneous mutations. It causes chromosomal rearrangements which can range from simple deletions, inversions and translocations to complex rearrangements (Silver, 1995). In this approach, several genes are affected by chromosomal rearrangements, therefore this method adds complexity to genetic studies and makes it difficult to dissect individual gene function (Stanford et al., 2001).

Chlorambucil

Chlorambucil (CAB) induces similar chromosomal translocations and multigene deletions to X-ray (Russell et al., 1989). CAB is an alkalyting agent that impacts cell division and results in aneugenic activity (Efthimiou, Andrianopoulos, Stephanou, Demopoulos, & Nikolaropoulos, 2007). CAB causes smaller deletions and translocations in comparison to X-ray mutagenesis, but it does not lead to single-gene identification and therefore, is not used in high-throughput approaches (Stanford et al., 2001).

Ethylnitrosourea

Ethylnitrosourea (ENU) is an alkylating agent that induces point mutations in the DNA of spermatogonial stem cells via single-base mismatching to the unrepaired alkylated base (Svenson et al., 2003). ENU is advantageous since it is easy to administer, results in higher mutagenesis rate and is amendable to high-throughput screening (Justice, 2000). Through international collaborative efforts several archives of DNA, embryonic stem (ES) cells or typically sperm, from mutagenized mice have been created and catalogued along with some standardized phenotypic data and the listing of mutations they contain. The corresponding ES cells/sperm can

be ordered and used to regenerate mice harboring the mutation of interest (Acevedo-Arozena et al., 2008).

3.2.3 Insertional mutagenesis

Pronuclear injection

This approach involves microinjection of DNA into fertilized oocytes to affect the function of an endogenous gene. This approach requires the cloning of cDNA (coding sequence of a selected gene). The sequence is then inserted in frame with a constitutively active promoter that drives transcription (Gordon & Ruddle, 1981; Jaenisch et al., 1981). This method disturbs endogenous gene expression and can generate chromosomal rearrangements and deletions (Belizário, Akamini, Wolf, Strauss, & Xavier-Neto, 2012). Pronuclear injections are labor intensive and highly technical, thus they are not used in high-throughput screening (Stanford et al., 2001).

Gene targeting

Targeted mutagenesis by homologous recombination in embryonic stem cells is used to efficiently target a single gene (Sun, Abil, & Zhao, 2012). Gene targeting was first pioneered by K.R. Thomas and M.R. Capecchi, who were able to exchange the endogenous gene with a mutated copy in cultured mammalian cells by using a homologous sequence of the gene (Thomas & Capecchi, 1987). This approach is able to produce specific alterations to the mouse genome to analyze targeted gene function (Menke, 2013).

Gene trapping

Gene trapping is a vector insertion that disrupts the regular transcription of endogenous genes (O'Kane & Gehring, 1987). Gene trapping includes enhancer, promoter and exon traps. The enhancer trap is used for gene identification, and it involves the introduction of a reporter construct that requires a cis-acting DNA to activate gene expression. Genes are then identified depending on the expression information (Yamamura & Araki, 2008). The promoter and the exon traps are mainly used for mutagenesis. The promoter trap contains the coding sequence of the reporter gene and can interfere with normal coding capacity of endogenous genes and create a mutation. The exon trap is designed to create spliced fusion transcripts between the reporter and the endogenous gene (Yamamura & Araki, 2008). Gene trapping is an efficient system for simultaneous studies of gene function, sequence and expression (Stanford et al., 2001). Many of the targeted ES cells produced by the international consortia include gene-trap vectors.

Lentivirus vectors

Viral vectors are a stable, long-term gene delivery system of genetic information to host cells. The system depends on replicating viruses that have the genetic information for the targeted gene instead of their own coding regions (Kootstra & Verma, 2003). Lentiviral vectors are often preferred over other viral vectors because they are more efficient at delivering complex gene expression cassettes (May et al., 2000), they can mediate long term gene expression (Seppen, Barry, Harder, & Osborne, 2001) and they are relatively safe (C. Y. Brown et al., 2010). They provide high control over the manipulated gene, and are ideal for studying gene function in small populations (Osten, Dittgen, & Licznerski, 2006). Other viral vectors that are commonly used, particularly adeno-associated virus (AAV) vectors, have different tissue tropisms that can be used for the over-expression of genes of interest even within the central nervous system, which

may be of particular relevance for obesity (Lentz, Gray, & Samulski, 2012). The development of novel viral vectors is ongoing (S. Huang & Kamihira, 2013). Recent advances in materials and nanotechnologies may also facilitate non-viral methods of direct gene delivery (Yin et al., 2014), although these are not yet routinely used.

3.2.4 Inbreeding methods

Once a mutation is successfully induced, a mutant model is obtained and the next step is to dissect the genetic roots of the phenotype. Supplementary Figure 3 is a graphical representation of different inbreeding techniques (Rogner & Avner, 2003). This section provides an overview of the different inbreeding approaches used to reach this objective.

Genetic cross

Genetic cross is a classical cross where two inbred strains are mated and their offspring are either mated to each other (an intercross F2 design) or to a progenitor strain (a backcross design) (Flint & Eskin, 2013). Second-generation offspring are then phenotyped and genotyped, and linkage analysis is carried out to identify a region that is associated with the trait of interest (Silver, 1995).

The classical inbred strains

This method provides a higher mapping resolution than the genetic cross, since the inbred mouse strains are separated from their founders by more generations, thus increasing the recombination events between the genomes of the founding strains (McClurg et al., 2007). These strains are

commercially available for purchase from vendors, and no breeding steps are required in this approach.

The recombinant inbred lines

Recombinant inbred lines are created through cross breeding of inbred mice, amplifying the genotypic / phenotypic diversity found across inbred mice. The power of recombinant inbred lines are in that they represent a fixed polygenic model that can be phenotyped deeply, in multiple environments (Zou et al., 2005). This fixed model is established *via* crossing two different inbred parental strains to produce F1 offspring, and then generating a series of brother-sister mating for at least 20 generations. This produces fully inbred strains which are homozygous at all loci for a unique combination of the original parental genomes (Pomp, 2007). An example of recombinant inbred lines is the Hybrid Mouse Diversity Panel (HMDP). This is a panel of approximately 100 strains that are phenotyped and association is carried out after correcting for population structure using efficient mixed-model association and made available to the scientific community. The combined populations in the HMDP provide a high statistical power and a high resolution (Flint & Eskin, 2013), which makes this model ideal for systems-level analysis of gene by environment interactions (Parks et al., 2013).

Another illustration of recombinant lines is the Collaborative Cross, which is a large-scale effort to create a set of recombinant inbred strains that are specifically designed for mapping traits (Aylor et al., 2011). By using wild derived strains, a substantial amount of genetic diversity is introduced, giving the collaborative cross the advantage of covering more genetic variations compared to other approaches (Philip et al., 2011).

Chromosome substitution strains or consomic strains

Chromosome substitution occurs when a single, full-length chromosome from one inbred strain has been transferred onto the genetic background of a second strain by repeated backcrossing (Nadeau, Singer, Matin, & Lander, 2000). The method involves construction of chromosome substitution strains (CSS) between a donor and a host strain, which partitions the variation between two strains and becomes a resource for studying genetic control of phenotype variation (Singer et al., 2004).

Long-term selection lines

Long-term selection lines are developed through selective breeding for a wide variety of phenotypes (Paigen, 2003). Several of these lines can be joint and inbred to characterize different metabolic traits together and develop models for gene mapping. For example, the body fat phenotype in mice were developed by long-term selection of fat (F) and lean (L) mice in over 60 generations (Horvat et al., 2000). A genome-wide quantitative trait locus (QTL) analysis of a cross between F and L lines revealed QTLs that mapped to regions that were previously described as obesity QTLs (Horvat et al., 2000).

The heterogeneous stock

The heterogeneous stock (HS) uses outbred mice to increase statistical power compared to recombinant inbred strains. The outbred mice are similar to F2 animals from a cross, but they have ancestry from eight founder strains instead of only two, and the population is bred for more generations (Valdar et al., 2006). Commercial outbred stock animals have been maintained for many generations, and they provide high-resolution mapping (Flint & Eskin, 2013).

3.2.5 Genetic manipulations in mice

Gene manipulation techniques allow for definitive alterations of specific genes at the systemic level. They also enable gene alterations in a time or tissue specific manner (Speakman, Hambly, Mitchell, & Król, 2007).

Over-expression of target genes

This method involves cloning of a full-length coding sequence downstream of a promoter, which may provide a global or tissue-specific overexpression of a target gene in the transgenic offspring (Speakman et al., 2007). This technique is straightforward and inexpensive, but the extent of changes in gene and protein expression is not always predictable. It can also disturb endogenous gene expression (Monica J Justice et al., 2011; Stanford et al., 2001).

Knock-out models

Knock-out models involve the total ablation of the target gene in all tissues, but the ablation of target genes could reveal unpredicted effects (Davey & MacLean, 2006). For example, homozygous knock-outs may result in embryonic death (eg: *Sim1* $^{-/-}$) (Michaud et al., 2001), or developmental compensation (eg: *Npy)* (Erickson, Hollopeter, & Palmiter, 1996; Erikson, Clegg, & Palmiter, 1996) in which no particular phenotype will be observed. In the example of *Sim1*, heterozygous knock outs of this gene survive, but develop severe obesity associated with increase in food intake without measurable energy expenditure. This is indicative of how *Sim1* plays a role in energy homeostasis (Ramachandrappa et al., 2013).

SiRNA/shRNA

Conditional gene knockdown is a powerful tool for studying gene function, and methods of gene knockdowns are in constant evolution (Brown et al., 2010). The discovery of small interfering RNA (siRNA) as a viable mechanism for eliciting RNA interference (RNAi) expanded opportunities for functional genetic studies (Elbashir et al., 2001). Vector directed siRNA technology allows for rapid generation of large number of knockdown mice in any strain of interest (De Souza et al., 2006), which produces strong and specific suppression of gene expression with no cytotoxicity (Elbashir et al., 2001).

Short hair pin RNAs (shRNA) provide a more straightforward approach for down regulation of gene expression, because unlike siRNAs, they can be stably expressed within the cell and are not lost with cell division (Brown et al., 2010). Lentivirus vectors containing polymerase III promoters for shRNA expression and polymerase II promoter for fluorescent protein expression (for labeling the cell) can be used in order to knock-down endogenous genes (Dittgen et al., 2004). An example of this application is silencing of the leptin receptor gene related protein (*OB-RGPR*) that was accomplished *via* lentiviral vector encoding a shRNA directed against *OB-RGPR* in the hypothalamus (Couturier et al., 2007). Silencing of *OB-RGPR* in hypothalamus prevents the development of diet-induced obesity in mice fed high-fat diet (HFD) (Couturier et al., 2007). Expression of microRNAs (miRNA) can also be used to modulate gene expression (Casola, 2010).

Knock-in models

These models involve the replacement of the endogenous gene with the mutated form and are used to study more specific roles of the changes in protein function (Speakman et al., 2007). This

technique could also be used to confirm the impact of target mutations on phenotype of the disease. For example, humans with dominant negative *PPAR$_\gamma$* L466A mutation display severe insulin resistance, dyslipidemia and hypertension (Freedman, Lee, Park, & Jameson, 2005).. Further studies of the mutation in mice revealed that mice with *Ppar$_\gamma$* knock-in L466A mutation exhibit lipodystrophy, decrease in adipogenic genes, high circulating free fatty acids (FFAs), and low adiponectin. Human studies of this mutation, coupled with the animal experiments confirm the importance of *PPAR$_\gamma$* in adipose tissue maintenance (Freedman et al., 2005).

The Cre/loxP system

This is a tool for tissue-specific and time specific knock out genes. When Cre is expressed in mice with a loxP containing gene, the desired gene is excised (Kühn & Torres, 2002). The expression of Cre can be driven either through a transgenic under a tissue specific and/or temporally regulated promoter, or by direct delivery to cells. Depending on the specific tissue or time of Cre expression, modifications can be restricted to a certain cell type or a development stage (Kühn & Torres, 2002). Delivery of Cre *via* viral vectors provides a more specific gene delivery in the nervous system (Kaspar et al., 2002), particularly if performed by targeted injection.

Newer gene expression manipulation techniques such as zinc fingers and TALENs are also used in obesity genetics field, albeit less frequently than Cre/loxP. Zinc fingers are a versatile DNA recognition domain that have been combined in a modular fashion to generate fusion proteins that recognize unique DNA sites in complex eukaryotic genomes (Urnov, Rebar, Holmes, Zhang, & Gregory, 2010). Similarly, transcription activator-like effector nuclease (TALENs) from pathogenic bacterium *Xanthomonas* can be engineered to virtually bind to any DNA sequence

(Boch, 2011). Each TALEN can travel to the nucleus, bind to the promoters of target genes and induce transcription based on their specific DNA-binding site (Boch, 2011).

3.3 The golden age of mouse obesity genetics

3.3.1 Monogenic obesity mouse models and candidate gene studies in human

The identification of genes underlying monogenic obesity relied heavily on mouse genetic studies. By searching the available literature on mouse models of obesity, we collected 221 genes that have been linked to obesity or weight gain *via* knock-out or transgenic mice, or by utilizing techniques such as over-expression or Cre/loxP (Supplementary Table 1). We conducted this literature review by searching key terms in PubMed and OMIM databases. We have focused on the obesity and weight gain phenotype and did not detail genes responsible for leanness phenotypes. Leanness may truly result from the manipulation of a gene important in energy balance (e.g. FTO inactivation leads to leanness, FTO overexpression leads to obesity). However, leanness may also be linked to toxicity and sickness of the animal due to genetic manipulations (Reed, Lawler, & Tordoff, 2008). The study of monogenic obesity in mice pioneered our understanding of the mechanisms underlying the regulation of body weight in humans. The genes underlying monogenic forms of obesity in humans all encode members of these highly conserved pathways, which are essential in regulation of body weight and energy homeostasis (Figure 1) (Hélène Choquet & David Meyre, 2011; S. O'Rahilly & I. S. Farooqi, 2008). Since a detailed discussion of all the genes listed in Table 1 is not feasible for one review paper, we will focus on a subset of the genes that are all part of the leptin / melanocortin or paraventricular nucleus development pathways in the section below.

Leptin (LEP) and Leptin Receptor (LEPR)

The *obese* (*ob*) mutation was first described in 1949 by a team from Jackson Laboratory (D L Coleman & Hummel, 1973; Kanasaki & Koya, 2011). The *ob* mutation originated in non-inbred heterogeneous stock, but was subsequently transferred onto various inbred strains for further analysis (Clee & Attie, 2007; D L Coleman & Hummel, 1973; D L Coleman, 1982). This model exhibits morbid obesity associated with hyperphagia and hyperglycemia along with other neuroendocrine abnormalities (D L Coleman & Hummel, 1973; D L Coleman, 1982). Elegant parabiosis studies demonstrated that the *obese* gene encodes a circulating factor, while the *diabetes* gene encodes its receptor (D. L. Coleman, 1973). Through ground-breaking positional cloning studies, the *ob* mutation was characterized as a single-base deletion which results in a premature stop codon in the previously unknown leptin gene (Y. Zhang et al., 1994). This landmark study was the first to identify this hormone and largely initiated research efforts on adipokines.

The *diabetes* (*db*) mutation was identified in 1966 in the C57BL/KsJ inbred mouse strain (D L Coleman & Hummel, 1967; Hummel, Dickie, & Coleman, 1966). This mouse model exhibits persistent hyperphagia and obesity, resulting in hyperleptinemia, insulin resistance and increased leptin levels (D L Coleman & Hummel, 1967). Positional cloning and related studies identified the *diabetes* (*db*) mutation in the leptin receptor gene (H. Chen et al., 1996; Tartaglia et al., 1995).

After the discovery of leptin , a mutation in the leptin gene (*LEP*) was discovered in two severely obese cousins within a highly consanguineous family of Pakistani origin (Montague et al., 1997). The mutation was characterized as a frameshift mutation resulting in truncated transcription of leptin (Montague et al., 1997). Other reports have confirmed this initial discovery in additional

homozygous patients of Pakistani, Turkish and Egyptian origin (Gibson et al., 2004; Mazen et al., 2009). In a study in Pakistan, where consanguineous marriages are preferred, 16.1% of the probands from 62 unrelated children with early onset obesity exhibited mutations in *LEP*. Of these probands, 9 carried a homozygous frameshift mutation. Given the high prevalence of monogenic obesity in this consanguineous population, early detection of this mutation for counselling and management of obesity could be beneficial (Saeed, Butt, Anwer, Arslan, & Froguel, 2012).

Similarly, shortly after its identification in mice, congenital leptin receptor (LEPR) deficiencies were found in severe obese siblings in 1998 (K Clément et al., 1998). In a more recent study, 8 other patients with severe early onset obesity with homozygous or compound heterozygous mutations in *LEPR* were identified (S. Farooqi et al., 2007). These patients exhibited high serum levels of leptin and loss of sensitivity of the receptor (S. Farooqi et al., 2007).

Patients with mutations in *LEP* or *LEPR* experience rapid weight gain within the first year of life (I Sadaf Farooqi et al., 2002). Patients all experience hyperphagia and display aggression when food is denied (I Sadaf Farooqi et al., 2002). Onset of puberty is often delayed for these patients, due to hypogonadotrophic hypogonadism (S. Farooqi et al., 2007). Leptin deficient children exhibit defective T-cell mediated immunity, explaining the high rates of infection and mortality in developing countries (S. Farooqi et al., 2007).

The role of leptin in energy homeostasis has also been demonstrated in studies employing novel tools for genetic studies, such as whole exome sequencing. For example, whole exome sequencing was conducted in extreme obese individuals from four consanguineous families to determine the role of rare coding variants in pathogenesis of obesity (Gill et al., 2014). The study found two novel frameshift mutations (p.C186AfsX27 and p.H160LfsX9) that truncate the LEPR

protein, resulting in protein products that lack the necessary binding domain for leptin signaling (Gill et al., 2014).

Considering the symptoms associated with leptin deficiency, the impact of leptin deficiency in the body is reversible *via* leptin treatment. A 9 year old girl with leptin deficiency experienced reduction in weight mainly due to loss of fat, reduced energy intake, and increase in gonadotropin concentrations after treatment with recombinant human leptin for 12 months (S. Farooqi et al., 1999). In a different study, leptin-deficient patients in a fed state gave higher ratings to food images, but these ratings were reduced after leptin treatment (I Sadaf Farooqi, Bullmore, et al., 2007). As a result of these findings, leptin treatment has been deemed a promising therapeutic option for leptin deficient patients. It should be noted however that normal leptin levels do not preclude the presence of a deleterious mutation. A recent study described a 2 year old boy with a deleterious leptin mutation with normal leptin levels (Wabitsch et al., 2015). This mutation had an impact on the protein function rather than expression, which questions the reliability of leptin levels as a prescreening tool for detecting leptin mutation.

TUB

Tubby bipartite transcription factor (TUB) is a member of the tubby-like proteins, which present a highly conserved C-terminus domain (Carroll, Gomez, & Shapiro, 2004). *TUB* is a substrate for insulin receptor tyrosine kinase (IRTK) and leptin receptor Janus kinase 2 (LEPR JAK2) in the hypothalamus. TUB is translocated to the nucleus after binding to LEPR *via* JAK2. Inhibition of TUB expression in the hypothalamus results in increased food intake, fasting glucose levels, hepatic glucose output, decreased oxygen consumption, and reduced sensitivity of POMC to leptin (Prada et al., 2013). A mutation in the *tubby* gene occurred spontaneously at the Jackson

Laboratory in a C57BL/6J mouse (D L Coleman & Eicher, 1990). These mice developed milder obesity compared to the other mutant models, hyperinsulinemia and mild hypoglycemia (D L Coleman & Eicher, 1990). Positional cloning of the mutated *tubby* gene identified a single-base change in the splice donor site that results in the incorrect retention of a single intron in the mature tub mRNA transcript (Kleyn et al., 1996).

Mutations in *TUB* were observed in an eleven year old boy from a consanguineous Caucasian family. His symptoms included deteriorating vision, obesity, and normal glucose/cholesterol/triglycerides levels but other clinical features were not observed to classify the patient as Bardet-Biedl or Alström syndrome (Borman et al., 2014). The mutation was identified as a homozygous frameshift mutation in *TUB* (c.1194_1195delAG, p.Arg398Serfs∗9), which results in a truncated form of *TUB*. Homozygous loss-of-function of *TUB* is extremely rare in humans (Borman et al., 2014).

MC4R

The melanocortin-4 receptor (*Mc4r)* model was identified in 1997 through targeted gene disruption (Huszar et al., 1997). *MC4R* is a G protein couple receptor mainly expressed in the brain that is involved in both energy intake and expenditure (Gantz et al., 1993; Huszar et al., 1997). *Mc4r* $^{-/-}$ mice exhibit obesity, hyperphagia, hyperinsulinemia, hyperglycemia, and increased linear growth (Huszar et al., 1997). Comparatively, *Mc4r* $^{+/-}$ mice display milder forms of obesity, with increased weight gain in response to high-fat diet, suggesting a gene-dosage effect (Srisai et al., 2011).

The first heterozygous mutation in *MC4R* discovered in humans was in 1998 (Vaisse, Clement, Guy-grand, & Froguel, 1998; G. S. Yeo et al., 1998). *MC4R* mutations represent the most

common form of human monogenic obesity, impacting 0.2-5.6% of individuals with severe early onset obesity (Rouskas et al., 2012). Majority of these mutations are heterozygous, with homozygous mutants having a fully penetrant early-onset severe form of obesity. Not all heterozygote mutants are obese however, which is indicative of the dosage effect described previously in the mouse models (I Sadaf Farooqi, Keogh, Yeo, Lank, & Cheetham, 2003; Stutzmann et al., 2008). In addition to obesity, *MC4R* deficient children display hyperinsulinemia and increased linear growth (I S Farooqi et al., 2000). Also patients experience an increase in adiposity, as well as an increase in lean mass, which is a phenotype that is not observed in other forms of monogenic obesity (I Sadaf Farooqi et al., 2003). Interestingly, degree of hyperphagia in patients depends on level of receptor dysfunction, which is generally lower than that of leptin deficient patients (I Sadaf Farooqi et al., 2003).

MC3R

Both melanocortin receptor 3 (MC3R) and MC4R are expressed in the hypothalamus and are involved in energy homeostasis (Roselli-Rehfuss et al., 1993). *Mc3r* deficient mice exhibit 50-60% more adipose mass and 50% reduction in energy expenditure (A Butler et al., 2000). *Mc3r* deficient mice are also hyperleptinaemic and male $Mc3r^{-/-}$ mice develop mild hyperinsulinemia (A. S. Chen et al., 2000). Mice lacking both *Mc3r* and *Mc4r* become significantly heavier than either mutation alone, suggesting that *Mc3r* and *Mc4r* have non-redundant roles in energy homeostasis (A. S. Chen et al., 2000).

While the role of *MC4R* in monogenic obesity is well-defined, the role of *MC3R* mutations in human monogenic obesity is debated (Zegers et al., 2013). Although mutations in the *MC3R* gene may not be involved in autosomal dominant form of monogenic obesity, these mutations

could predispose humans to increased risk of obesity. *MC3R* mutations that result in defective receptors have been associated with obesity in French and Italian populations (Mencarelli et al., 2011). A non-significant two-fold enrichment in *MC3R* loss-of-function mutations was observed in a severe obese population from United States (Calton et al., 2009).

POMC

The pro-opiomelanocortin (*Pomc*) derived peptides have a variety of biological functions, such as pigmentation, adrenocortical function, and energy stores (Smith & Funder, 1988). Supplementary Figure 2 is a depiction of *POMC*-derived peptides, including α and β-MSH. Deleting the coding region of *Pomc* in mouse models resulted in obesity, defective adrenal development and altered pigmentation (Yaswen, Diehl, Brennan, & Hochgeschwender, 1999).

Interest in the melanocortin pathway stemmed from the studies of agouti mice. Mouse coat color was a trait studied by the mouse model experts whose stocks founded many of the commonly studied strains (Clee & Attie, 2007). The dominant lethal "yellow" mutation (A^y) was identified in 1905 (Dickies, 1962). A non-lethal, viable, allele (A^{vy}) occurred as a spontaneous mutation in 1960 in the C3H/HeJ strain at the Jackson Laboratory (Dickies, 1962). In addition to their yellow coat color, mutant mice exhibit adult-onset obesity, type 2 diabetes associated with insulin resistance, hyperleptinemia, higher benign tumor susceptibility and infertility (Klebig, Wilkinson, Geisler, & Woychik, 1995). The mutations in the *agouti* gene in carriers of the yellow alleles leads to dysregulation of its expression in multiple tissues (Bultman, Michaud, & Woychik, 1992; David M. J. Duhl, Harry Vrieling, Kimberly A. Miller, 1994; Miller et al., 1993). The agouti model displays a defect in proopiomelanocortin (*POMC*) signaling pathway and is desensitized to leptin signaling (Boston, Blaydon, Varnerin, & Cone, 1997).

The first recessive mutation in *POMC* was discovered in 1998 (H Krude et al., 1998). In addition to obesity, patients with *POMC* mutations displayed hypocortisolism, hair and skin hypopigmentation, neonatal hypoglycemia, seizures, cholestasis and voracious appetite (I Sadaf Farooqi et al., 2006; H Krude et al., 1998; Heiko Krude et al., 2003).The hypopigmentation of the hair and skin is not always observed in non-European populations and *POMC* mutations should still be considered in individuals with severe early onset obesity even if typical pigmentary phenotype is missing (Cirillo et al., 2012; Karine Clément et al., 2008; Mendiratta et al., 2011). In general, *POMC* deficiencies are extremely rare in human population (Beales, 2010) and the position of the mutation is important, as missense mutations have been reported to directly impact the melanocortin peptide-encoding regions, whereas other missense mutations have been reported to impact the peptide-receptor binding affinity (Challis et al., 2002).

A novel mutation in the alpha-melanocyte stimulating hormone (*α-MSH*) gene was found in a 12 year old girl with early onset obesity (transmitted through the father) (Dubern et al., 2008). The mutation was characterized by dramatic impairment of *α-MSH* (Dubern et al., 2008). The patient's obese father had less pronounced form of obesity in comparison to the daughter, which may be due to a gene-environment interaction. This means the younger generation is more exposed to obesogenic environment, thus more likely to develop obesity (Dubern et al., 2008). Most research has been focused on *α-MSH* since rodent models lack beta-melanocyte stimulating hormone (*β-MSH)* (Bennett, 1986) but a loss-of-function missense mutation in *β-MSH* has been associated with childhood obesity. The lack of function of *β-MSH* reduces the amount of MSH peptide in the POMC/MC4R pathway, resulting in obesity (Biebermann et al., 2006). *β-MSH* mutations may result in a non fully penetrant form of monogenic obesity, as some patients with this mutation are not obese (Y. S. Lee et al., 2006).

PCSK1

A mutation was discovered in 1973 in the HRS/J inbred mouse strain which homozygous mice exhibit a slower increase in body weight compared to *ob/ob* and *db/db* mice but ultimately develop severe obesity, with hyperinsulinemia and transient hyperglycemia in males (D L Coleman & Eicher, 1990). Coding non-synonymous mutation in the carboxypeptidase E (*Cpe*) gene was found to induce the *fat* mouse phenotype (Naggert et al., 1995). *Cpe* gene is involved in processing of prohormone convertase 1 (*PC1)* as illustrated in figure 1A, which led scientists to study the association of this gene to obesity as well (P. Li et al., 2014).

PC1/3 functions as a processing enzyme of precursor proteins in the regulated secretory pathways (J. W. Creemers, Jackson, & Hutton, 1998). *Pc1/3* knock-out mice do not exhibit obesity, but instead show growth retardation and multiple neuroendocrine disorders (X. Zhu et al., 2002). An ENU mutagenesis experiment resulted in development of a mouse model with mutation on the *Pc1/3* (N222D allele) that exhibits obesity (Lloyd, Bohan, & Gekakis, 2006) . $Pc1^{N222D/N222D}$ mice have lower *α-MSH* and display defects in *POMC* processing, affecting the melanocortin signaling (Lloyd et al., 2006). $Pc1^{N222D/N222D}$ mice exhibit abnormal proinsulin processing, multiple endocrinological defects, hyperphagia and obesity, while heterozygous mice exhibit an intermediate phenotype for weight gain and fasting insulin processing (Lloyd et al., 2006).

In human studies, three patients with recessive monogenic form of obesity were deficient in the pro-protein convertase subtilisin/kexin type 1 (*PCSK1)* gene (I Sadaf Farooqi, Volders, et al., 2007; R. S. Jackson et al., 2003; R. Jackson et al., 1997). Complete prohormone convertase 1 deficiency results in early on-set severe obesity, hyperphagia, hypoglycemia, and endocrine

dysfunction (I Sadaf Farooqi, Volders, et al., 2007; R. S. Jackson et al., 2003; R. Jackson et al., 1997). Null mutations causing prohormone convertase 1 congenital deficiency also lead to generalized malabsorptive diarrhea and diabetes insipidus in some instances (I Sadaf Farooqi, Volders, et al., 2007; Frank et al., 2013; R. Jackson et al., 1997; Martín et al., 2013; Yourshaw et al., 2013). Partial loss-of-function heterozygous mutations in *PCSK1* present a non-fully penetrant intermediate obesity phenotype (J. W. M. Creemers et al., 2012) However, heterozygous carriers of a null mutation show a dominantly inherited form of Mendelian obesity (Philippe et al., 2014).

SH2B1

The SH2B adaptor protein 1 (SH2B1) activates the JAK2 cytoplasmic tyrosine kinase to mediate cell signaling (Ren, Li, Duan, & Rui, 2005). SH2-B is a key regulator of leptin sensitivity. *Sh2b1*$^{-/-}$ mice exhibit hyperphagia, hyperlipidemia, hyperglycemia, hyperleptinemia, hyperinsulinemia and hepatic steatosis (Ren et al., 2005).

In humans, loss-of-function mutations in *SH2B1* patients resulted in severe early onset obesity (Doche et al., 2012; Pearce et al., 2014). These patients exhibit hyperphagia, childhood onset obesity, insulin resistance, and reduced height (Doche et al., 2012). Behavioral abnormalities were also noted in patients, such as social isolation and aggression (Doche et al., 2012). The severity of the phenotype may depend on the impact of mutations on the disruption of different isoforms of SH2B1 (Pearce et al., 2014). Genomic imbalances and recurrent deletions of the *SH2B1* containing region on the short arm of chromosome 16 have been associated with behavioral disorders and obesity (Bachmann-Gagescu et al., 2010). It is interesting to note that while deletion of a region on chromosome 16 that contains *SH2B1* increases the risk of obesity

significantly (Bochukova et al., 2010; Walters, Jacquemont, Valsesia, Smith, & Martinet, 2010), reciprocal duplication of this region results in an increase in gene dosage which influences BMI in the reverse manner (leanness) (Jacquemont et al., 2011). The relevance of *SH2B1* locus in human energy balance is strengthened by the identification *via* GWAS of common variants near *SH2B1* associated with BMI variation or obesity risk (Berndt et al., 2013; Willer et al., 2009).

BDNF/NTRK2

The brain derived neurotrophic factor (BDNF) model demonstrates the numerous roles of *BDNF* in neural development through activation of TrkB and p75 receptors and involvement in anorexigenic activity (Noble, Billington, Kotz, & Wang, 2011). In the mature central nervous system, *BDNF* is expressed in various hypothalamic nuclei associated with eating behavior and obesity (Kernie, Liebl, & Parada, 2000). To circumvent the problem of early mortality associated with total knock-out of the *BDNF* gene, conditional *BDNF* knockout mice were developed. Conditional knockout of *BDNF* in the brain *via* cre-loxP recombinase system resulted in mice exhibiting hyperphagia, hyperactivity and aggression as well as elevated levels of *POMC* (Rios et al., 2001). Since *BDNF* is only absent in the brain, the resulting obesity can be attributed to the lack of *BDNF* function therein (Rios et al., 2001). In another conditional knockout study, selective knockout of *BDNF* in brains of adult mice resulted in impaired hippocampal function, whereas selective knockout of *BDNF* in earlier stages of development resulted in more drastic phenotypes, such as hyperactivity and severe impairments in hippocampal-dependent learning (Monteggia et al., 2004).

Neurotrophin receptor (TrkB) is a member of the neurotrophin family and is known to be involved in development, maintenance and function of peripheral and central neurons and is

hypothesized to play a role in mediating neuronal plasticity in the hypothalamus (Gray, Yeo, Hung, et al., 2006). TrkB and its ligand BDNF are also known to be involved in the regulation of food intake and body weight (Gray, Yeo, Hung, et al., 2006; Xu et al., 2003). Homozygous mutations in the gene encoding *TrkB* (*Ntrk2*) are lethal in mice, but heterozygous mutations resulting in 25% of *TrkB* expression display hyperphagia, increased linear growth and obesity as well as complex neurobehavioral phenotypes (Xu et al., 2003).

The neurotrophic tyrosine kinase receptor type 2 (*NTRK2)* gene was screened in a boy with early onset obesity, hyperphagia developmental delay, impairments in short-term memory and impaired nociception, revealing a missense mutation in *NTRK2* (G. S. H. Yeo et al., 2004). Further analysis showed an impairment in *BDNF*-stimulated protein kinase phosphorylation (G. S. H. Yeo et al., 2004). The developmental and neurological impairments in this case is consistent with the wide spread of *TrkB* (encoded *via NTRK2*) throughout the central nervous system, where it assumes the responsibility for neuronal survival and differentiation and regulation of synaptic function (Indo et al., 1996). In another case, a girl with loss of one functional copy of *BDNF* presented with hyperphagia, severe obesity, cognitive impairment and hyperactivity (Gray, Yeo, Cox, et al., 2006). Moreover, hyperphagia and obesity observed in a subgroup of patients with WAGR syndrome has been attributed to deletions on chromosome 11 that induce haploinsufficiency of BDNF (Han et al., 2008).

SIM1

Single-minded homolog 1 (SIM1) is a member of the helix-hoop-helix PAS family of nuclear transcription factors (Crews, 1998). Homozygous *Sim1* mice die perinatally (Michaud, Rosenquist, May, & Fan, 1998), but heterozygous mutants exhibit hyperphagic obesity,

increased body fat percentage (J. Holder et al., 2004; Michaud et al., 2001), as well as higher levels of *POMC* expression and resistance to *α-MSH* (Kublaoui, Holder, Gemelli, & Zinn, 2006). They are also more prone to diet-induced obesity (J. Holder et al., 2004) and are associated with defects in the *MC4R* signaling pathway (Kublaoui et al., 2006). To illustrate these signaling defects, *Sim1* heterozygous mouse injected with a melanocortin agonist showed a blunted suppression of food intake, while wild-type mice exhibited a robust reduction in food intake (Kublaoui et al., 2006).

Severe early-onset obesity was observed in a girl with haploinsufficiency of *SIM1*, possibly acting upstream or downstream of MC4R (J. L. Holder, Butte, & Zinn, 2000). Further support for the involvement of *SIM1* in obesity came from studies in which patients displayed Prader-Willie like phenotypes due to heterozygous mutations in *SIM1* (Bonnefond et al., 2013). In another study, heterozygous mutations in *SIM1* were associated with severe obesity accompanied by a neurobehavioral phenotype for a majority of them (Ramachandrappa et al., 2013). Deletions on chromosome 6q16, including *SIM1* region, has been similarly associated with obesity and Prader-Willi like phenotype (Bonaglia et al., 2008). *SIM1* is expressed in kidneys and central nervous system and plays an essential role in formation of PVN of the hypothalamus (Michaud, DeRossi, May, Holdener, & Fan, 2000). This could be a mechanism in which *SIM1* plays a role in energy homeostasis, as PVN neurons express *MC4R* which inhibits food intake (Harris et al., 2001).

3.3.2 Polygenic obesity mouse models and candidate gene studies in human

Given the success in identifying mutations causing severe monogenic obesity from mouse models, in parallel with the development of methods for linkage analysis, other mouse models

have been developed for genetic studies of polygenic obesity. For example, the New Zealand Obese Mouse (NZO) characterizes a combination of hyperphagia, reduced energy expenditure and insufficient physical activity (Herberg & Coleman, 1977). The Kuo Kondo Mouse displays hyperphagia, hyperinsulinemia, insulin resistance (Igel et al., 1998) which precedes onset of obesity (Ikeda, 1994). Later modifications of this model led to development of KKA^{y} from transferring the A^{y} gene, which is now used for obesity and diabetes research and testing of experimental therapies (Okazaki et al., 2002). The Tsumura Suzuki Obese Diabetes Mouse (TSOD) models polygenic obesity with diabetes (hyperglycemia and hyperinsulinemia) (Suzuki et al., 1999). The M16 mouse was developed to characterize the phenotypic consequences of long-term selective breeding for rapid weight gain (Allan, Eisen, & Pomp, 2004). The M16 is an outbred model of early onset polygenic obesity and is characterized by hyperphagia, hyperinsulinemia, and hyperleptinemia (Allan et al., 2004). Lastly, the BSB mouse models are a backcross progeny obtained by crossing C57BL/6J x *Mus spretus* F1 females with C57BL/6J males to model polygenic obesity (Fisler, Warden, Pace, & Lusis, 1993; Warden, Fisler, Pace, Svenson, & Lusis, 1993) . BSB mice range from 1% to 50% body fat with an increase in both intra-abdominal and subcutaneous fat (Fisler et al., 1993). Obesity in BSB model is associated with hyperinsulinemia, hyperglycemia, and hyperlipidemia (Fisler et al., 1993).

Studies of polygenic mouse models have involved the analysis of numerous inbred strains using multiple experimental designs, and dozens of loci have been mapped across all mouse chromosomes (Pomp, 2007; Rankinen et al., 2006). These QTLs affect body weight, body fat, high fat diet-induced weight gain, the severity of obesity, and more specific traits such as food intake, energy expenditure and exercise habits (Fawcett et al., 2008). Only a few studies revealed QTLs in regions that had been previously identified in monogenic studies. For example, a study

using QTL mapping in the BSB mouse model identified a locus that is very proximal to the *LEP* gene, which had been previously identified *via* positional cloning (Warden et al., 1995, 1993; Y. Zhang et al., 1994). To identify the causative variation, each locus identified in a chromosomal region is isolated in a congenic strain, essentially converting it a monogenic study where interactions with other loci are held constant. This facilitates the analysis of the locus under study.

However, positional cloning of genes underlying obesity QTLs has proven to be a difficult task with a limited success rate (Wuschke, Dahm, Schmidt, Joost, & Al-Hasani, 2007). Several factors have contributed to this, including the time and cost required to generate and phenotype sufficient congenic and sub-congenic strains to localize the QTL to a region where a single candidate can be identified. Another challenge has been that many of the QTLs that were originally mapped appear to have resulted from the combined effects of multiple nearby QTLs (Buchner, Geisinger, Glazebrook, Morgan, & Nadeau, 2012; Laplante et al., 2012; Mollah & Ishikawa, 2011; Prevorsek, Gorjanc, Paigen, & Horvat, 2010; Shao et al., 2008; Yazbek et al., 2011). Thus, when isolating the loci in progressively smaller congenic strains, the individual effect sizes (i.e. the phenotypic difference between congenic genotypes) can diminish and could even seemingly disappear if they are within a strain that also harbors a locus acting in the opposite direction. Similar to the case in human polygenic obesity, adiposity in mice seems largely controlled by multiple loci having modest effects. Finally, between any pair of strains, there are haplotype blocks where the strains have numerous genetic differences, both coding and non-coding, that could contribute to a QTL.

Despite the relatively low success rate of positional cloning in identifying polygenic obesity genes, few success stories of employing this approach in mouse models are described below.

Cntnap2 and *Tag1*

Pioneering the use of chromosome substitution strains for positional cloning in mice, the Nadeau laboratory has recently identified two genes associated with diet-induced obesity (Buchner et al., 2012). A mutation in the *Cntnap2* gene which is required for proper potassium channel localization at neuronal nodes of Ranvier was identified through congenic analysis. Depending on the genetic background of the mouse model under investigation, this mutation either protected or predisposed mice to diet induced obesity (Buchner et al., 2012). Using a candidate gene approach based on this finding, the group also assessed its known interacting protein, *Tag1*, in knockout mice and found that this gene also affects obesity by protecting mice against diet induced obesity (Buchner et al., 2012). These studies have provided further evidence linking neuronal function with the regulation of body weight. Copy number variation in *Cntnap2* has recently been identified in a child with syndromic obesity (Vuillaume et al., 2014).

Deptor

The *Fob3a* locus was identified in studies of the Fat and Lean strains generated by long-term selection for these (Stylianou et al., 2004). Recently, through congenic analysis, genetic variation in *Deptor* has been identified as a strong obesity candidate gene at the *Fob3a* locus (Laplante et al., 2012). This gene was previously known for its roles in mammalian target of rapamycin (mTor) signaling, but its role in obesity development was unknown. Through the subsequent generation of transgenic mice, *Deptor* overexpression was associated with increased adipogenesis (Laplante et al., 2012).

Other obesity candidate genes identified through congenic analysis

Studies of the *Nob3* QTL have led to the identification of a microdeletion that eliminates expression of *Ifi202b* (Vogel et al., 2012). The authors showed that this altered the expression of several genes including *11β-Hsd1*. *11β-Hsd1* encodes the cortisone reductase and is a relevant candidate gene for energy balance. Another gene recently identified from mouse positional cloning studies is *Bhlhe40*, which affects muscle fatty acid oxidation (Takeshita, Suzuki, Kitayama, & Moritani, 2012).

Mouse models have also been helpful in elucidating genes that play a role in polygenic obesity risk / protection in humans. For example, candidate gene studies of *MC4R* common genetic variants revealed that the gain-of-function mutation of the variants lower the risk of obesity (Geller et al., 2004; Stutzmann et al., 2007). Additionally, the study of *BDNF* as a candidate gene led to the association of a coding non-synonymous variant (Val66Met) with BMI variation in healthy adults (Gunstad et al., 2006), an association confirmed later on by hypothesis-free GWAS for BMI (G. Thorleifsson et al., 2009; C. J. Willer et al., 2009). Similarly, two non-synonymous variants on *PCSK1* were consistently associated with childhood and adult severe obesity in a study of 13,659 participants of European ancestry, making *PCSK1* a candidate gene for polygenic obesity (Benzinou et al., 2008).

3.4 From human obesity to mouse models: the back and forth

Mouse models have been invaluable in dissecting the genetic origin of human monogenic and polygenic obesity. The reverse approach also holds true as gene discoveries in humans have pioneered new mouse models for obesity. This section will review how genuine genetic

discoveries in humans using high throughput, agnostic approaches such as positional cloning, GWAS or WES have inspired new experiments in mice to investigate the function of the genes.

3.4.1 Positional cloning

BBS

Bardet-Biedl Syndrome (BBS) is a rare recessive developmental disorder, and people with heterozygous mutations in *BBS* gene are more prone to obesity (Croft, Morrell, Chase, & Swift, 1995; Sheffield, 2010). Positional cloning, homozygosity mapping, candidate gene and whole exome sequencing approaches have led to the discovery of 18 genes that are linked to BBS so far (Scheidecker et al., 2014). Follow-up studies in animal models revealed the association of *BBS* genes with cilia function, and in intracellular and intraflagellar trafficking (Sheffield, 2010). More specifically, mice homozygous for a single *BBS* mutation lack spermatozoa flagella (Sheffield, 2010). Further analysis of BBS resulted in development of *BBS* knockout mice (*Bbs2*, *Bbs4* and *Bbs6*) that developed hyperphagia, reduced energy expenditure and increased circulating leptin levels, which suggests that leptin deficiency is not the mechanism of obesity in BBS (Rahmouni et al., 2008). This was further confirmed by administration of leptin, which failed to change body weight or food intake, indicative of leptin resistance in BBS mice (Rahmouni et al., 2008). On the other hand, expression of *Pomc* in *BBS* knock-out mice were significantly lower than that of controls, while *Agrp* and *Npy* levels were comparable to controls, pointing to the idea that *Pomc* is the main player in obesity in *BBS* mice (Rahmouni et al., 2008). This phenomenon is compatible with the role of *BBS* in cilia function, as abrogating cilia in POMC neurons increases food intake and causes obesity in mice (Davenport et al., 2007).

TBC1D1

A major predisposing locus for obesity was identified at 4p.15-14, affecting more females than males (Arya et al., 2004; Meyre et al., 2008; Stone et al., 2002). Positional cloning of this 4p15-14 linkage peak led to the identification of a coding non-synonymous variant (R125W) in the TBC1 domain family member 1 (*TBC1D1*) gene associated with female familial obesity in populations from Utah and France (Meyre et al., 2008; Stone et al., 2006). TBC1D1 is a Rab-GTPase activating protein and is closely related to insulin signaling protein AS160. It is predominantly expressed in skeletal muscle, as it is involved in regulation of lipid utilization in skeletal muscles (An et al., 2010; Chadt et al., 2008; Sano et al., 2003). These discoveries in humans were followed-up by animal work. Studies of the *Nob1* QTL identified a mutation in the *Tbc1d1* gene that protects against obesity (Chadt et al., 2008). *Tbc1d1* knockout mice were shown to exhibit reduction in body weight, impaired glucose utilization, and increased lipid oxidation in skeletal muscles (Dokas et al., 2013). Further analysis of the deleterious effects of the human R125W mutation has been confirmed by *in vivo* overexpression of wild-type and mutant *TBC1D1* proteins in mouse tibialis anterior muscles (An et al., 2010). In this study, the R125W mutation impaired insulin-stimulated glucose transport, but did not impair contraction-stimulated glucose transport. Experiments conducted on phosphorylation sites of other *TBC1D1* mutations had opposing effects, as these mutations impaired contraction-stimulated glucose transport but did not impact insulin-stimulated glucose transport (An et al., 2010). Overall, impairment of muscle glucose transport could lead to increased fat accumulation in adipose tissue and result in subsequent obesity (An et al., 2010).

ENPP1

A positional cloning experiment led to identification of ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) as a possible contributor to obesity and type 2 diabetes in humans. A significant linkage for childhood obesity was detected on chromosome 6q22.23 in French pedigrees (Meyre et al., 2004). By using overlapping published linkage studies on obesity (Atwood et al., 2002), insulin secretion (Abney, Ober, & McPeek, 2002; Duggirala et al., 2001) and type 2 diabetes (Demenais et al., 2003; Ehm et al., 2000; Ghosh et al., 2000; Xiang et al., 2004), the *ENPP1* gene was picked for further analysis. *ENPP1* directly inhibits insulin-induced conformational changes of the insulin receptor, and affects its activation and signaling (B. A. Maddux & Goldfin, 2000; B. Maddux et al., 1995). A risk haplotype of *ENPP1* was associated with childhood obesity, glucose intolerance and type 2 diabetes (Meyre et al., 2005). Subsequent development of *Enpp1* knockout mice highlighted a phenotype of more efficient adipocyte maturation in mesenchymal embryonal cells compared to wild-type (Liang, Fu, Ciociola, Chandalia, & Abate, 2007). Transgenic mice overexpressing human *ENPP1* in muscle and liver tissue exhibited elevation in glucose and insulin levels compared to wild-type, conveying that *ENPP1* plays a role in insulin resistance and hyperglycemia (Dong et al., 2005; B. A. Maddux et al., 2006).

3.4.2 Genome wide association studies

GWAS in obesity field have identified 119 independent loci associated with BMI and obesity status (Choquet & Meyre, 2011; Locke et al., 2015). Table 2 provides a summary of these identified genes and associated SNPs. Interestingly, GWAS showed that almost all genes involved in Mendelian forms of obesity in mice and humans (*LEPR, POMC, MC4R, BDNF, PCSK1, TUB, NTRK2, SH2B1*) display common variants associated with BMI and polygenic

obesity as well (Dickson, Wang, Krantz, Hakonarson, & Goldstein, 2010). Genetic animal models were developed prior to GWAS discoveries for these genes whose functions were well-established. However, most of the genes located in or near GWAS signals were of unknown function. This prompted the scientific community to develop new genetic mouse models for some of these genes that we describe below.

FTO

The fat mass and obesity associated gene (*FTO*) is the first gene that has been convincingly associated with obesity using GWAS. The role of *FTO* as an important contributor to polygenic obesity was confirmed in GWAS for type 2 diabetes, BMI, early onset obesity, and incidentally in a population stratification approach (Dina et al., 2007; Frayling et al., 2007; Hinney et al., 2007; Scuteri et al., 2007). Initial GWAS on *FTO* identified *FTO* as an unknown gene in an unknown pathway (Frayling et al., 2007). The exact molecular mechanism of how *FTO* might contribute to obesity is still under investigation, but high level of expression of *FTO* in the hypothalamus is suggestive of its role in food intake (Fredriksson et al., 2008). This required further studies of *FTO* in knockout and overexpression animal models to understand the mechanism in which *FTO* influences obesity (Choquet & Meyre, 2011).

Fto knockout mice exhibit high perinatal lethality, significant reduction in body length, fat mass and lean mass, indicative of the role of *Fto* in energy homeostasis (Church et al., 2009; Fischer et al., 2009; McMurray et al., 2013). Deletion of *Fto* in the hypothalamus *via* adeno-associated viral vectors encoding Cre recombinase resulted in small reduction in food intake and decreased weight gain with no effect on energy expenditure (McMurray et al., 2013). Overexpression of Fto in mice results in increased food intake, increase in body and fat mass (Church et al., 2010).

Despite the metabolic phenotypes found in FTO overexpression / inactivation rodent models and the location of SNPs associated with human obesity in the intron 1 region of *FTO*, the implication of other neighboring genes in obesity is not excluded. As an illustration, the obesity-associated *FTO* sequence directly interacts with the promoters of *IRX3* as well as *FTO* in the human, mouse and zebrafish genomes (Smemo et al., 2014). Expression QTL studies in human brains revealed that obesity associated SNPs in *FTO* are associated with expression of *IRX3*, but not *FTO* itself (Smemo et al., 2014). *Irx3* knockout mice show reduction in body weight and increase in basal metabolic rate, indicative of a direct link between *Irx3* and body composition (Smemo et al., 2014). The rs8050136 SNP located in the first intron of *FTO* modulates the binding for CUX1 P110 and P200 isoforms which in turn regulate the expression of *FTO* and of the nearby ciliary gene *RPGRIPL1* (Stratigopoulos et al., 2014; Stratigopoulos, LeDuc, Cremona, Chung, & Leibel, 2011). Homozygous mutants of *Rpgripl1* are lethal, but *Rpgrip1l* $^{+/-}$ mice are leptin resistant, hyperphagic and obese (Stratigopoulos et al., 2014). Overall, these animal experiments suggest that the genes *IRX3* and *RPGRIPL1* may mediate at least in part the association of SNPs in *FTO* with obesity.

NPC1

Besides *FTO*, genome wide association studies revealed that two non-synonymous variants in high linkage disequilibrium (H215R / I858V) in the Niemann-Pick C1 (*NPC1)* gene were associated with extreme obesity in European adults (Meyre et al., 2009). Subsequent mouse models of partial inactivation of *Npc1* evidenced significant weight gain in mice fed a high-fat diet, indicating a possible gene-diet interaction (Jelinek et al., 2010). More recently, SNPs at the

Npc1 locus have been associated with differences of body fat (%) in response to high-fat high-sucrose diet in a GWAS performed in mice (Parks et al., 2013).

ETV5

Ets variant 5 (ETV5) is a transcription factor that can act either as an activator or repressor of transcription of genes involved in cell proliferation, differentiation, apoptosis and cell-cell or cell-matrix interaction (Sementchenko & Watson, 2000; Sharrocks, 2001). Two GWAS in populations of European ancestry identified SNPs near *ETV5* as associated with BMI and obesity (Berndt et al., 2013; Speliotes et al., 2010; Thorleifsson et al., 2009). This association triggered further research on *ETV5* in animal models. *Etv5* knockout mice exhibit lean bodies, resistance to diet induced obesity and severe glucose intolerance due to impaired insulin exocytosis and hypoinsulinaemia (Gutierrez-Aguilar et al., 2014).

NEGR1

GWAS identified SNPs near the neuronal growth regulator 1 (*NEGR1*) gene associated with BMI variation and obesity (Berndt et al., 2013; Thorleifsson et al., 2009; Wheeler et al., 2013; Willer et al., 2009). *NEGR1* is expressed in the brain and participates in the neurite outgrowth in the developing brain (Marg et al., 1999). With the use of ENU mutagenesis, mice carrying a loss-of-function of *Negr1* displayed reduced food intake and physical activity, unchanged energy expenditure and reduction in overall body mass (A. W. S. Lee et al., 2012).

3.4.3 Next generation sequencing

SDCCAG8

Nephronophthisis-related ciliopathies (NPHP-RCs) are developmental problems that impact kidneys and are associated with renal degeneration, intellectual disability and obesity. Exome sequencing identified 12 truncating mutations on serologically defined colon cancer antigen 8 (*SDCCAG8)* gene, showing that a loss-of-function of *SDCCAG8* is causal for human retinal-renal ciliopathy (Otto et al., 2010). The candidacy of *SDCCAG8* gene was strengthened by the identification of common variants associated with childhood obesity through a GWAS in German and French populations (Scherag et al., 2010). To better understand the function of *SDCCAG8*, a gene-trap mouse line (*Sdccag8*$^{gt/gt}$) was subsequently developed (Airik et al., 2014). The *Sdccag8*$^{gt/gt}$ mice exhibited the human phenotype of NPHP-RCs and revealed that retinal degeneration associated with the disorder exhibits early and leads to progressive loss of vision, whereas the renal degeneration occur later due to DNA damage from signaling activity (Airik et al., 2014).

Table 2: List of genes and SNPs associated with body mass index (BMI) or binary obesity from genome-wide association studies (GWAS)

Gene	SNPs	Chr.	Phenotype	Reference
ADCY9	rs2531995	16	Obesity	Berndt, SI. 2013. Nat Genet
AGBL4	rs657452	1	BMI	Locke, AE. 2015. Nature
ASB4	rs6465468	7	BMI	Locke, AE. 2015. Nature
BDNF	rs6265, rs4923461, rs10767664, rs2030323, rs988712	11	BMI, Obesity, Overweight	Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Jiao, H. 2011. BMC Med Genomics, Okada, Y. 2012. Nat Genet, Wen, W. 2012. Nat Genet 2012
BRE	rs116612809	2	BMI	Gong, J. 2013. Am J Hum Genet
C9orf93	rs4740619	9	BMI	Locke, AE. 2015. Nature
CADM1	rs12286929	11	BMI	Locke, AE. 2015. Nature
CADM2	rs13078807	3	BMI, Overweight	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
CALCR	rs9641123	7	BMI	Locke, AE. 2015. Nature

CBLN1	rs2080454	16	BMI	Locke, AE. 2015. Nature
CDKAL1	rs2206734, rs9356744	6	BMI	Okada, Y. 2012. Nat Genet, Wen, W. 2012. Nat Genet 2012
CLIP1	rs11057405	12	BMI	Locke, AE. 2015. Nature
CREB1, KLF7	rs17203016	2	BMI	Locke, AE. 2015. Nature
EHBP1	rs11688816	2	BMI	Locke, AE. 2015. Nature
ELAVL4	rs11583200	1	BMI	Locke, AE. 2015. Nature
EPB41L4B, C9orf4	rs6477694	9	BMI	Locke, AE. 2015. Nature
ERBB4	rs7599312	2	BMI	Locke, AE. 2015. Nature
ETS2	rs2836754	21	BMI	Locke, AE. 2015. Nature
ETV5	rs7647305, rs9816226	3	BMI, Obesity, Overweight	Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
FAIM2	rs7138803, rs7132908	12	BMI, Obesity, Overweight	Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Paternoster, L. 2011. PLoS One, Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet
FANCL	rs887912	2	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
FANCL, FLJ30838	rs12617233	2	BMI	Guo, Y. 2012. Hum Mol Genet
FHIT	rs2365389	3	BMI	Locke, AE. 2015. Nature
FIGN	rs1460676	2	BMI	Locke, AE. 2015. Nature
FLJ35779	rs2112347	5	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
FOXO3, HSS00296402	rs9400239	6	BMI	Locke, AE. 2015. Nature
FTO	rs9939609, rs9930506, rs1121980, rs1421085, rs8050136, rs1558902, rs17817449, rs12149832, rs9940128, rs62033400, rs1421085, rs1121980,	16	BMI, Obesity, Childhood Obesity	Dina, C. 2007. Nat Genet, Hinney, A. PLoS One. 2007, Frayling,TM. 2007. Science, Scuteri, A. 2007. PLoS Genet, Loos, RJ. 2008. Nat Genet, Meyre, D. 2009. Nat Genet, Thorleifsson, G. 2009. Nat Genet, Willer, CJ. 2009. Nat Genet, Cho, YS. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Scherag, A. 2010. PLoS Genet, Paternoster, L. 2011. PLoS One, Wang, K. 2011. PLoS One, Bradfield, JP. 2012. Nat Genet, Wen, W. 2012. Nat Genet, Okada, Y. 2012. Nat Genet, Guo,

	rs9936385, rs9941349, rs3751812, rs1558902, rs17817449			Y. 2012. Hum Mol Genet, Graff, M. 2013. Hum Mol Genet, Pei, YF. 2013. Hum Mol Genet, Berndt, SI. 2013. Nat Genet, Wheeler, E. 2013. Nat Genet
GBE1	rs3849570	3	BMI	Locke, AE. 2015. Nature
GDF15, PGPEP1	rs17724992	19	BMI	Locke, AE. 2015. Nature
GIPR	rs2287019, rs11671664	19	BMI	Speliotes, EK. 2010. Nat Genet, Wen, W. 2012. Nat Genet, Okada, Y. 2012. Nat Genet
GNAT2	rs17024258	1	Obesity	Berndt, SI. 2013. Nat Genet
GNPDA2	rs10938397, rs13130484, rs348495	4	BMI, Obesity, Overweight	Willer,CJ. 2009. Nat Genet, Speliotes,EK. 2010. Nat Genet,Graff,M. 2013. Hum Mol Genet, Berndt, SI. 2013. Nat Genet
GP2	rs12597579	16	BMI	Wen, W. 2012. Nat Genet 2012
GPRC5BB	rs12444979	16	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
GRID1	rs7899106	10	BMI	Locke, AE. 2015. Nature
GRP	rs7243357	18	BMI	Locke, AE. 2015. Nature
GRP120	rs116454156	10	Obesity	Ichimura, A. 2012. Nature
HHIP	rs11727676	4	BMI	Locke, AE. 2015. Nature
HIF1AN	rs17094222	10	BMI	Locke, AE. 2015. Nature
HIP1, PMS2L3, PMS2P5, WBSCR16	rs1167827	7	BMI	Locke, AE. 2015. Nature
HMGA1	rs206936	6	BMI	Speliotes, EK. 2010. Nat Genet
HNF4G	rs4735692	8	Obesity	Berndt, SI. 2013. Nat Genet
HOXB5	rs9299	17	Childhood Obesity	Bradfield, JP. 2012. Nat Genet
HS6ST3	rs7989336	13	Obesity	Berndt, SI. 2013. Nat Genet
HSD17B12	rs2176598	11	BMI	Locke, AE. 2015. Nature
IFNGR1, OLIG3	rs13201877	6	BMI	Locke, AE. 2015. Nature
KAT8, ZNF646, VKORC1, ZNF668, STX1B, FBXL19	rs9925964	16	BMI	Locke, AE. 2015. Nature

KCNK3	rs11126666	2	BMI	Locke, AE. 2015. Nature
KCNMA1	rs2116830	101	Obesity	Jiao, H. 2011. BMC Med Genomics
KCTD15	rs11084753, rs29941	19	BMI	Willer, CJ. 2009. Nat Genet, Thorleifsson,G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet
KLF9	rs11142387	9	BMI	Okada, Y. 2012. Nat Genet
LEPR	rs11208659	1	Childhood Obesity	Wheeler, E. 2013. Nat Genet
LMX1B	rs10733682	9	BMI	Locke, AE. 2015. Nature
LOC100287559, BBS4	rs7164727	15	BMI	Locke, AE. 2015. Nature
LOC284260, RIT2	rs7239883	18	BMI	Locke, AE. 2015. Nature
LOC285762	rs9374842	6	BMI	Locke, AE. 2015. Nature
LPIN2	rs643507	18	Obesity (Athmatic patients)	Melen, E. 2013. Clin Exp Allergy
LRP1B	rs2890652	2	BMI	Speliotes, EK. 2010. Nat Genet
LRRN6C	rs10968576	9	BMI, Obesity	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
MAF	rs1424233	16	Obesity	Meyre, D. 2009. Nat Genet
MAP2K5	rs2241423, rs4776970, rs997295	15	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Wen, W. 2012. Nat Genet 2012, Guo, Y. 2012. Hum Mol Genet, Berndt, SI. 2013. Nat Genet
MAPK3, KCTD13, INO80E, TAOK2, YPEL3, DOC2A, FAM57B	rs4787491	16	BMI	Locke, AE. 2015. Nature
MC4R	rs17782313, rs571312, rs12970134, rs2331841, rs6567160, rs8089364, rs7234864, rs723486, rs7227255, rs2229616, rs17782313, rs17700144,	18	BMI, Obesity, Overweight	Loos, RJ. 2008. Nat Genet, Thorleifsson,G. 2009. Nat Genet, Meyre, D. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Scherag, A. 2010. PLoS Gene, Paternoster, L. 2011. PLoS One, Guo, Y. 2012. Hum Mol Genet, Okada, Y. 2012. Nat Genet, Wen, W. 2012. Nat Genet, Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet, Wheeler, E. 2013. Nat Genet, Graff, M. Hum Mol Genet. 2013, Pei, YF. Hum Mol Genet. 2013

	rs663129, rs571312, rs476828			
MIR548A2	rs1441264	13	BMI	Locke, AE. 2015. Nature
MIR548X2, PCDH9	rs9540493	13	BMI	Locke, AE. 2015. Nature
MRPS33P4	rs13041126	20	Obesity	Berndt, SI. 2013. Nat Genet
MTCH2	rs10838738, rs3817334	11	BMI, Obesity, Overweight	Willer,CJ. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
MTIF3	rs4771122	13		Speliotes, EK. 2010. Nat Genet
NAV1	rs2820292	1	BMI	Locke, AE. 2015. Nature
NEGR1	rs2815752, rs2568958, rs1993709	1	BMI, Obesity, Overweight	Willer, CJ. 2009. Nat Genet, Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet, Wheeler, E. 2013. Nat Genet
NLRC3	rs758747	16	BMI	Locke, AE. 2015. Nature
NPC1	rs1805081	18	Obesity	Meyre, D. 2009. Nat Genet
NRXN3	rs10150332	14	BMI, Obesity	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
NT5C2, CYP17A1, SFXN2	rs11191560	10	BMI	Locke, AE. 2015. Nature
NTRK2	rs1211166	9	BMI	Guo, Y. 2012. Hum Mol Genet
NUP54, SCARB2	rs17001654	4	BMI	Locke, AE. 2015. Nature
OLFM4	rs9568856, rs9568867	13	Obesity	Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet
PACS1	rs564343	11	Childhood Obesity	Wheeler, E. 2013. Nat Genet
PARK2	rs13191362	6	BMI	Locke, AE. 2015. Nature
PCSK1	rs261967, rs6232, rs6234, rs6235	5	BMI, Obesity	Benzinou, M. 2008. Nat Genet, Wen, W. 2012. Nat Genet
PLCD4, CYP27A1, USP37, TTLL4, STK36, ZNF142, RQCD1	rs492400	2	BMI	Locke, AE. 2015. Nature

PMS2L11	rs2245368	7	BMI	Locke, AE. 2015. Nature
POMC	rs713586, rs6545814, rs1561288, rs6752378, rs10182181	2	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Wen, W. 2012. Nat Genet 2012, Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet, Graff,M. 2013. Hum Mol Genet
PRKCH	rs1957894	14	Childhood Obesity	Wheeler, E. 2013. Nat Genet
PRKD1	rs11847697, rs12885454	14	BMI	Speliotes, EK. 2010. Nat Genet, Locke, AE. 2015. Nature
PTBP2	rs1555543	1	BMI	Speliotes, EK. 2010. Nat Genet
QPCTL	rs2287019	19	Obesity, Overweight	Berndt, SI. 2013. Nat Genet
RABEP1	rs1000940	17	BMI	Locke, AE. 2015. Nature
RALYL	rs2033732	8	BMI	Locke, AE. 2015. Nature
RARB	rs6804842	3	BMI	Locke, AE. 2015. Nature
RASA2	rs16851483	3	BMI	Locke, AE. 2015. Nature
RMST	rs11109072	12	Childhood Obesity	Wheeler, E. 2013. Nat Genet
RPL27A	rs11042023	11	Obesity	Berndt, SI. 2013. Nat Genet
RPTOR	rs7503807	17	Overweight	Berndt, SI. 2013. Nat Genet
SBK1, APOBR	rs2650492	16	BMI	Locke, AE. 2015. Nature
SCG3, DMXL2	rs3736485	15	BMI	Locke, AE. 2015. Nature
SDCCAG8	rs12145833	1	Childhood Obesity	Scherag, A. 2010. PLoS Gene
SEC16B	rs10913469, rs543874, rs574367, rs516636, rs591120	1	BMI, Obesity, Overweight	Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet, Graff, M. 2013. Hum Mol Genet, Wen, W. 2012. Nat Genet, Okada,Y. 2012. Nat Genet
SH2B1	rs7498665, rs4788102, rs7359397, rs4788099	16	BMI, Obesity, Overweight	Willer, CJ. 2009. Nat Genet, Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Guo, Y. 2012. Hum Mol Genet, Berndt, SI. 2013. Nat Genet
SLC39A8	rs13107325	4	BMI	Speliotes, EK. 2010. Nat Genet
SMG6, N29617	rs9914578	17	BMI	Locke, AE. 2015. Nature
STXBP6	rs10132280	14	BMI	Locke, AE. 2015. Nature
TAL1	rs977747	1	BMI	Locke, AE. 2015. Nature
TCF7L2	rs7903146	10	BMI	Locke, AE. 2015. Nature
TDRG1,	rs2033529	6	BMI	Locke, AE. 2015. Nature

LRFN2				
TFAP2B	rs987237, rs734597, rs2272903	6	BMI, Obesity, Overweight	Speliotes, EK. 2010. Nat Genet, Paternoster, L. 2011. PLoS One, Guo, Y. 2012. Hum Mol Genet, Berndt, SI. 2013. Nat Genet
TLR4	rs1928295	9	BMI	Locke, AE. 2015. Nature
TMEM160	rs3810291, rs3810291	19	BMI, Obesity	Speliotes, EK. 2010. Nat Genet, Berndt, SI. 2013. Nat Genet
TMEM18	rs6548238, rs7561317, rs2867125, rs12463617, rs4854344	2	BMI, Obesity, Overweight	Willer, CJ. 2009. Nat Genet, Thorleifsson, G. 2009. Nat Genet, Speliotes, EK. 2010. Nat Genet, Guo, Y. 2012. Hum Mol Genet, Bradfield, JP. 2012. Nat Genet, Berndt, SI. 2013. Nat Genet, Wheeler, E. 2013. Nat Genet, Graff,M. 2013. Hum Mol Genet
TNKS	rs17150703	8	Childhood Obesity	Scherag, A. 2010. PLoS Gene
TNNI3K	rs1514175, rs12142020, rs1040070, rs1514174	1	BMI, Obesity	Speliotes, EK. 2010. Nat Genet, Bradfield, JP. 2012. Nat Genet, Graff,M. 2013. Hum Mol Genet, Berndt, SI. 2013. Nat Genet
TOMM40, APOE, APOC1	rs2075650	19	BMI	Guo, Y. 2012. Hum Mol Genet
TUB	rs4929949	11	BMI	Speliotes, EK. 2010. Nat Genet
UBE2E3	rs1528435	2	BMI	Locke, AE. 2015. Nature
ZBTB10	rs16907751	8	BMI	Locke, AE. 2015. Nature
ZNF608	rs48361333	5	BMI	Speliotes, EK. 2010. Nat Genet
ZZZ3	rs17381664	1	Obesity	Berndt, SI. 2013. Nat Genet

3.5 The waltz between mouse and human genetic studies

Recent attempts in understanding genetics of obesity utilizing both human and animal genetic approaches are discussed below.

3.5.1 Linkage study

Arrestin domain-containing 3 protein (*ARRDC3*) is a regulator of cell receptor signaling, and also plays a role in metabolism (Luan et al., 2009). Genome wide linkage for human obesity

identified a linkage peak on chromosome 5, and positional cloning identified *ARRDC3* associated with higher BMI in males but not in females (Patwari et al., 2011). Higher *ARRDC3* expression is associated with visceral adipose tissue and obesity in males. Animal models such as the *Arrdc3* deficient mice have validated the role of *ARRDC3* in metabolism by being resistant to obesity in a dosage dependent manner (both genders, but with greater impact on males than females) (Patwari et al., 2011).

3.5.2 Candidate gene approach

G-protein coupled receptor 120 (GPR120) is a receptor for unsaturated long chain fatty acids, and plays a role in adipogenesis, regulation of appetite and food preference (Hirasawa et al., 2005). *Gpr120* deficient mice fed a high fat diet exhibit obesity, glucose intolerance, fatty liver, decreased adipocyte differentiation and lipogenesis (Ichimura et al., 2012), but no difference in body weight between *Gpr120* deficient and wild type mice was observed when both groups were fed a normal diet (Ichimura et al., 2012). When assessed in humans, *GPR120* was expressed in adipose tissue, with obese individuals having a higher expression in both subcutaneous and omental adipose tissue (1.8 fold increase) (Ichimura et al., 2012). In order to study the contribution of *GPR120* to human obesity, the four *GPR120* exons were sequenced in 312 non-consanguineous extremely obese French children and adults. Exon sequencing revealed a deleterious non-synonymous variant (p.R270H) of minor allele frequency (MAF) of 3% that inhibits *GPR120* signaling activity and increases the risk of obesity by 62% in 6,942 obese individuals and 7,654 control subjects from Europe (Ichimura et al., 2012). Thus, *GPR120* plays a role in sensing dietary fat, and is important in energy balance.

Melanocortin 2 receptor accessory protein 2 (MRAP2) is a homologue of MRAP, expressed in the brain and adrenal gland (Chan et al., 2009). *MRAP2* can interact with all melanocortin receptors, which results in *MC2R* surface expression and signaling. *MRAP2* can also reduce the responsiveness of *MC1R, MC3R, MC4R* and *MC5R* to *α-MSH* (Chan et al., 2009). Mouse models deficient in *Mrap2* exhibit obesity (Asai et al., 2013). Selective knockout of *Mrap2* in neurons expressing *Sim1* also exhibit obesity, similar to global knockout of *Mrap2*, consistent with the idea that *Sim1* expressing neurons are key regulators of energy balance (Asai et al., 2013)**.** Four rare heterozygous mutations of *MRAP2* have been identified in obese humans (Asai et al., 2013).

3.5.3 Whole exome sequencing

Kinase suppressor of Ras 2 (KSR2) is a scaffolding protein involved in multiple signaling pathways through kinase cascades (Dougherty et al., 2010; Pearce et al., 2013) that are linked to regulation of food intake, body fat content and glucose homeostasis (Revelli et al., 2011). By using a whole-exome sequencing strategy, *KSR2* loss-of-function mutations were identified in humans and were associated with hyperphagia, early-onset obesity, low heart rate, reduced basal metabolic rate and severe insulin resistance (Pearce et al., 2013). *Ksr* $2^{-/-}$ mice display obesity, high insulin levels, and impaired glucose tolerance (Pearce et al., 2013). Obesity persisted in *Ksr* $2^{-/-}$ mice despite being fed the same amount of diet as *Ksr2*$^{+/+}$ littermates (Pearce et al., 2013).

3.6 Conclusions

We have reviewed the synthesis between mouse and human genetics in the field of obesity. We describe the approaches and techniques that are available for mouse and human geneticists, and provide a striking illustration of the synergy between these approaches that led to successful

obesity causing gene identifications these last decades. We list innovative approaches to not only ensure a higher yield of novel obesity genes, but also a deeper understanding of their function. Figure 4 is an illustration that summarizes the discussions in this review paper. Integrative mouse human strategies have the potential to lead to the identification of more genes responsible for common Mendelian forms of obesity, as well as gene x gene and gene x environment interactions. This may help to unravel the missing heritability of obesity. We believe that an exhaustive understanding of obesity genetics will help to identify novel drug targets and to design more efficient and personalized obesity prevention and management programs that, with the support of populations and stakeholders, will ultimately curb the obesity epidemic (Agurs-Collins et al., 2008).

4.0 The association of obesity candidate genes from mouse models to different obesity measures in EPiDREAM multiethnic cohort

4.1 Introduction

At the moment, single nucleotide polymorphisms (SNPs) explain 2.7% of BMI variations in humans (Adam E Locke et al., 2015), while monogenic obesity mutations in 11 known genes may explain up to 10% of obesity cases (Choquet & Meyre, 2010; Yazdi et al., 2015). Evidence from Bayesian models show that up to 30% of BMI variance may be explained by common SNPs (C. Llewellyn et al., 2013). Most variants identified so far confer relatively small increments in risk, and explain only a small proportion of familial clustering, leading many to question how the remaining, 'missing' heritability can be explained (Manolio et al., 2009). The current view is that the genetic architecture for complex traits is characterized by a very large but finite number of causal variants (Wood et al., 2014) so most of them remain to be discovered at this stage.

The missing heritability phenomenon may be fueled by the many limitations of conventional genome-wide association studies (GWAS). Hypothesis-free GWAS exhaustively test the genotype/phenotype associations across up to 17 million genetic markers and represent to date the most efficient way to identify common variants (MAF> 1%) associated with complex diseases (Genomes Project, 2015; Genomes Project et al., 2010; Visscher, Brown, McCarthy, & Yang, 2012). An important consideration is that the overwhelming majority of GWAS and other genetic studies have been limited to European ancestry populations, whereas genetic variation is greatest in populations of recent African ancestry (Frazer et al., 2007), and studies in non-Europeans have yielded intriguing new variants (Lu & Loos, 2013; Monda et al., 2013; Wen et al., 2012). Limitations in the design of early GWAS, such as imprecise phenotyping, may have

reduced estimates of effect sizes while preserving some ability to identify associated variants (Pearson & Manolio, 2008).

Traditionally, obesity GWAS focus on BMI as the measure of adiposity in their analysis (A Li & Meyre, 2013). GWAS of fat-distribution phenotypes such as waist circumference or waist to hip ratio have revealed almost 50 loci with genome-wide significance and relatively little overlap with those loci influencing BMI (Dmitry Shungin et al., 2015). Disentangling whether the established BMI loci associate with body fat percentage *per se* or with body mass overall will require larger sample sizes (Kilpelainen et al., 2011). In regards to adiposity measure, there has never been a pangenomic analysis on the novel Body-Adiposity Index (BAI) trait, even though it is easy to measure in humans and has been verified as a good measure of adiposity (Bergman et al., 2011). Furthermore, studies on binary obesity indicate that analysing BMI and obesity binary trait in the same sample increases the yield of novel genes, thus, expanding the analysis beyond BMI could lead to identification of novel variants (Berndt et al., 2013; David Meyre et al., 2009). Given the number of SNPs tested in GWAS, an association must achieve a stringent threshold of statistical significance ($P < 5 \times 10^{-8}$) to be considered validated (Dudbridge & Gusnanto, 2008), and contemporary GWAS are underpowered to achieve this genome-wide significance for SNPs with modest effects on disease risk (Stahl et al., 2010). Assuming that disease-associated SNPs follow the distribution of effect sizes suggested by the validated associations, it is probable that many more true positive associations reside within GWAS data (Park et al., 2010) that have only suggestive statistical evidence of association.

In this study, prior biological evidence of the involvement of 121 genes in obesity was gathered from mouse models and the corresponding SNPs were analyzed for their association with BMI,

BAI, waist circumference, hip circumference and waist to hip ratio in the EpiDREAM cohort and replicated in the GIANT consortium.

4.2 Methods

4.2.1 Participants

The EpiDREAM (Epidemiological arm of the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) is a multi-ethnic longitudinal cohort, comprised of individuals with an increased risk for T2D who were screened for trial eligibility (Gerstein, Yusuf, Holman, Bosch, & Pogue, 2004). In the EpiDREAM study, genetic and baseline clinical information of 17,423 subjects from six ethnic groups (South Asian, East Asian, European, African, Latin American and Native North American) have been included. Self-reported ethnicity has been validated in 17,423 individuals using the eigensoft software (http://genepath.med.harvard.edu/~reich/Software.htm). Further information about the cohort design can be found in other publications (Gerstein et al., 2004).

The Genetic Investigation of ANthropometric Traits (GIANT) consortium is one of the largest international collaborations to study complex traits and diseases. It currently consists of meta-analysis of GWAS data from 125 studies, incorporating up to a total sample size of 339,224 individuals from European, East Asian, South Asian, and African American ancestry (Dmitry Shungin et al., 2015; Thomas W Winkler et al., 2014). For each study, local institutional committees approved study protocols and confirmed that informed consent was obtained. Further information about GIANT can be found elsewhere (Dmitry Shungin et al., 2015).

4.2.2 Genotyping

DNA was extracted from buffy coats in participants of the EpiDREAM study using the Qiagen Gentra System. The EpiDREAM cohort was genotyped using the cardiovascular gene-centric 50K SNP ITMAT-Broad-CARe (IBC) array (Keating et al., 2008). In GIANT, 236,231 individuals from European ancestry were genotyped using genome-wide SNP array, while the remaining 103,047 Europeans as well as other ethnic groups were genotyped using the Metabochip (Dmitry Shungin et al., 2015; Voight et al., 2012).

We selected 125 mouse obesity candidate genes that were available for analysis on the 50k cardiometabolic chip. SNPs with a higher than 95% call rate, MAF <0.05 and a significant deviation from Hardy-Weinberg equilibrium (HWE) within each ethnic group ($P < 2.28 \times 10^{-5}$) were removed. Individual samples with a missing rate ≥90%, heterozygosity rate ≥30%, inconsistent sex information, or duplicates (estimated kinship >90%) were also excluded from our analysis. All quality control steps were completed using PLINK (Purcell et al., 2007). Supplementary Figure 4 is a flow chart of the EpiDREAM sample collection.

4.2.3 Phenotyping

Participants were screened using a 75 gram oral glucose tolerance test (OGTT), as well as a questionnaire that included demographic data, medical history and physical activity behaviors at baseline and follow-up. Anthropometric measurements including height, weight, waist and hip circumference were performed using a standardized protocol (Gerstein et al., 2004). Height (m), weight (kg) and hip circumference (HC) (cm) were measured by trained medical staff. Standing height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg in light clothing. Hip circumference was measured in duplicate at the level of the greater trochanters

using a non-flexible tape measure with an attached spring balance with a mass of 750g. Averages of the two measures were used in all analyses. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters (m) squared. We used Body Adiposity Index (BAI) in EpiDREAM, which estimates body adiposity percentage directly based on height and hip circumference (Bergman et al., 2011). Specifically, BAI = [(hip circumference) / ((height)$^{(1.5)}$) - 18], with hip circumference and height being expressed in centimeters and meters, respectively (Bergman et al., 2011). Waist circumference (WC), hip circumference (HC) and the ratio of waist and hip circumferences (WHR) were also analyzed in EpiDREAM. We selected BMI, WC, HC and WHR measures in GIANT, as BAI was not available in this consortium for analysis.

The 2003 American Diabetes Association (ADA) criteria were used to classify participants as having normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or T2D at baseline, as confirmed by an oral glucose tolerance test (ADA, 2004).

4.2.4 Statistical Analysis

Descriptive statistics were used to characterize participants included in this study. Mean values and percentages were used to summarize continuous and categorical variables respectively (Table 3). The association of SNPs with BAI, BMI, WC, HC and WHR were assessed using linear mixed regression models adjusted for confounding variables: age, sex, relatedness and ethnicity / population substructure. Sensitivity analysis for glucose status was also completed. Population stratification was accounted for using the first 5 axes of variation from Principal Components Analysis, determined using GCTA (Price et al., 2006). The first 5 principal components explained 92% of the total genetic variability from among the first 20 components, and are sufficient to account for population substructure in our study (Supplementary Figure 5).

Family clusters were assigned based on individual pairs having kinship estimates >0.1875 (half way between 2nd and 3rd degree relatives). Linear mixed models were fitted assuming an exchangeable correlation structure using the c++ based *lme4* package in R (http://CRAN.R-project.org/package=lme4>) (Bates et al., 2015). Corrections for multiple hypothesis testing were applied using False-Discovery-Rate (FDR) based on Benjamin and Hochberg method (Benjamini & Hochberg, 1995). In the replication analysis, the GIANT Consortium GWAS 2015 Metadata files, using all ancestries, were downloaded from https://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files. All the SNPs that passed the FDR threshold with promise of association with varying obesity phenotypes were assessed in this consortium in order to verify the stability of our findings. All statistical analyses were done using R-3.1.0 software (http://www.r-project.org).

Table 3. Descriptive statistical analysis of the EpiDREAM cohort participants

Trait	Descriptive Statistic
Age (Years)	52.6 (11.4)
Sex	
Female	10560 (61.0)
Male	6744 (39.0)
BMI (Kg/m^2)	30.2 (6.2)
BAI (% adiposity)	33.0 (7.5)
Glucose Status	
NGT	9200 (53.2)
IFG/IGT	5551 (32.1)
T2D	2553 (14.7)
Ethnicity	
South Asian	2748 (15.9)
East Asian	224 (1.3)
European	9319 (53.9)
African	1245 (7.2)
Latin American	3270 (18.9)
Native North American	498 (2.9)

4.3 Results

4.3.1 Candidate Gene Selection

A thorough literature review on PubMed was conducted in search for genetic manipulation experiments in mouse models that have resulted in obesity or obesity related phenotype such as increase in fat mass. Knock-out, knock-in or transgenic experiments were screened for collection of candidate genes that met our selection criteria (Supplementary Table 1). The focus of our search was on weight gain and increased adiposity as opposed to weight loss, as weight reduction in mouse models could be related to toxicity from genetic manipulation techniques (Reed, Lawler, & Tordoff, 2008).

4.3.2 Association of Obesity Predisposing SNPs with BMI

A list of 221 genes related to obesity were selected from mouse models, as previously reported (Yazdi et al., 2015), which 125 were available on the 50K array for analysis in the EpiDREAM cohort. After quality control steps, 2194 SNPs in 121 genes were analyzed in the cohort. After adjustments for gender, age, relatedness and ethnicity *via* the 5 axis of the PCA, we found 22 SNPs that are associated with BMI, after adjustments to multiple testing using false discovery rate (p-value: 0.0003), in the EpiDREAM cohort that correspond to five genes (*FTO*, *ALMS1*, *RSRC1*, *GHR* and *POMC*). The strongest association was with the previously identified *FTO* gene (p-value: 9.61E-15, β: 0.5211, 95% CI: 0.3894, 0.6528). Table 4 is an outline of these SNPs with their respective p-values.

4.3.3 Association of Obesity Predisposing SNPs with BAI, WC, HC and WHR

Similarly, after quality control and FDR adjustments (p value: 0.0001), we found 16 SNPs in *FTO* and *RSRC1* associated with BAI. The strongest association was with *FTO* (p value: 1.95E-10, β: 0.4476, 95% CI: 0.3098, 0.5854). Eighteen SNPS were associated with WC (p value: 0.0003) in *FTO* and *POMC*, with the strongest association in *FTO* (p value: 6.40E-12, β: 1.0759, 95% CI: 0.769, 1.3828), and 17 SNPS associated with HC (p-value: 0.0002) in *FTO* and *RSRC1* with the strongest association in *FTO* (p value: 1.09E-10, β: 0.932, 95% CI: 0.6489, 1.2151). There were no significant SNPs associated with WHR in our analysis. Supplementary Tables 2-4 provide a list of these associated SNPs.

Table 4: SNPs associated with BMI in EpiDREAM cohort

BMI SNP	Gene	Chrom. Location	Effect Size	Risk Allele	p-value
rs3751812	*FTO*	16:52375961	0.5211 (0.3894, 0.6528)	T/G	9.61E-15
rs11075989	*FTO*	16:52377378	0.4989 (0.3709, 0.6269)	A/G	2.36E-14
rs9939609	*FTO*	16:52378028	0.497 (0.369, 0.625)	A/T	2.93E-14
rs7185735	*FTO*	16:52380152	0.4957 (0.3675, 0.6239)	G/A	3.45E-14
rs1421085	*FTO*	16:52358455	0.5011 (0.3704, 0.6318)	G/A	6.15E-14
rs9941349	*FTO*	16:52382989	0.4916 (0.3628, 0.6204)	T/C	7.49E-14
rs12149832	*FTO*	16:52400409	0.4877 (0.3566, 0.6188)	A/G	3.04E-13
rs7193144	*FTO*	16:52368187	0.4713 (0.3431, 0.5995)	C/T	5.64E-13
rs17817449	*FTO*	16:52370868	0.4622 (0.3338, 0.5906)	G/T	1.69E-12
rs9922708	*FTO*	16:52388647	0.4555 (0.3279, 0.5831)	T/C	2.55E-12
rs1121980	*FTO*	16:52366748	0.4144 (0.2888, 0.54)	A/G	9.91E-11
rs9923147	*FTO*	16:52359050	0.4092 (0.2835, 0.5349)	T/C	1.75E-10
rs7206790	*FTO*	16:52355409	0.3894 (0.2636, 0.5152)	G/C	1.28E-09
rs11642841	*FTO*	16:52402988	0.3862 (0.2518, 0.5206)	A/C	1.76E-08
rs17817288	*FTO*	16:52365265	0.3579 (0.2332, 0.4826)	C/T	1.86E-08
rs11075987	*FTO*	16:52372662	0.3035 (0.1776, 0.4294)	G/T	2.32E-06
rs8047395	*FTO*	16:52356024	0.278 (0.1531, 0.4029)	A/G	1.28E-05
rs1477196	*FTO*	16:52365759	0.2643 (0.1315, 0.3971)	G/A	9.60E-05
rs4594864	*GHR*	5:42659895	0.3477 (0.1637, 0.5317)	A/G	0.0002122

rs1729998	*RSRC1*	3:159410718	0.2387 (0.1092, 0.3682)	G/A	0.000301
rs3820700	*ALMS1*	2:73570318	0.3409 (0.1503, 0.5315)	T/C	0.0004548
rs3769671	*POMC*	2:25243657	0.5101 (0.2229, 0.7973)	G/T	0.0004992

4.3.4 Replication in GIANT

Given that GIANT is a meta-analysis of a number of GWAS studies, we were able to replicate the SNPs that were associated with BMI, WC, and HC based on the availability of data. In our analysis of BMI, 20 SNPs replicated in GIANT, corresponding to *FTO, RSRC1* and *GHR*. The strongest association was observed in *FTO* (p value: 2.17E-158, β: 0.0803, 95% CI: 0.0744, 0.0862). For hip circumference and waist circumference, all the SNPs from our discovery cohort replicated. Tables 5-7 provide a breakdown of the statistical values in our analysis.

Table 5. Replication results of SNPs associated with BMI in GIANT Consortium.

BMI SNPs	Gene	Effect Size	P value	N
rs1421085	*FTO*	0.0803 (0.0744,0.0862)	2.17E-158	333087
rs17817449	*FTO*	0.0753 (0.0696,0.081)	1.41E-146	339127
rs9923147	*FTO*	0.0758 (0.0699,0.0817)	3.28E-143	338212
rs1121980	*FTO*	0.0756 (0.0697,0.0815)	1.49E-142	338325
rs3751812	*FTO*	0.077 (0.0711,0.0829)	3.67E-142	337016
rs7193144	*FTO*	0.0754 (0.0695,0.0813)	6.18E-142	338935
rs9939609	*FTO*	0.0754 (0.0695,0.0813)	1.86E-141	328213
rs7185735	*FTO*	0.0748 (0.0689,0.0807)	1.07E-139	338642
rs11075989	*FTO*	0.0746 (0.0687,0.0805)	1.01E-138	338953
rs12149832	*FTO*	0.0737 (0.0678,0.0796)	1.43E-133	338700
rs9941349	*FTO*	0.0736 (0.0677,0.0795)	2.01E-133	338017
rs9922708	*FTO*	0.0701 (0.0642,0.076)	8.06E-123	338071
rs17817288	*FTO*	0.0667 (0.0608,0.0726)	7.05E-113	338133
rs11642841	*FTO*	0.0709 (0.0648,0.077)	8.92E-113	338300
rs8047395	*FTO*	0.0656 (0.0595,0.0717)	2.12E-102	337327
rs11075987	*FTO*	0.0616 (0.0559,0.0673)	1.26E-98	338737

rs7206790	*FTO*	0.066 (0.0597,0.0723)	3.02E-95	335108
rs1477196	*FTO*	0.0575 (0.0514,0.0636)	7.53E-75	337975
rs1729998	*RSRC1*	0.0119 (0.0046,0.0192)	0.001299	236180
rs4594864	*GHR*	0.0105 (0.0009,0.0201)	0.03212	235534
rs3769671	*POMC*	0.0178 (0.0026,0.0382)	0.08698	223492
rs3820700	*ALMS1*	0.0028 (0.0074,0.013)	0.5903	236150

Table 6. Replication results of SNPs associated with WC in GIANT Consortium.

WC SNPs	Gene	Effect Size	p-value	N
rs1421085	*FTO*	0.072 (0.0653,0.0787)	1.30E-102	242142
rs3751812	*FTO*	0.07 (0.0633,0.0767)	1.30E-93	242402
rs7193144	*FTO*	0.067 (0.0605,0.0735)	7.40E-93	244232
rs9923147	*FTO*	0.069 (0.0623,0.0757)	8.50E-93	244129
rs1121980	*FTO*	0.068 (0.0615,0.0745)	1.00E-92	244198
rs17817449	*FTO*	0.067 (0.0605,0.0735)	1.30E-92	244385
rs9939609	*FTO*	0.067 (0.0605,0.0735)	1.10E-90	236716
rs7185735	*FTO*	0.067 (0.0605,0.0735)	2.40E-90	244034
rs11075989	*FTO*	0.067 (0.0605,0.0735)	9.60E-90	244208
rs9941349	*FTO*	0.067 (0.0603,0.0737)	3.10E-89	243914
rs12149832	*FTO*	0.066 (0.0593,0.0727)	3.60E-86	244275
rs9922708	*FTO*	0.064 (0.0575,0.0705)	1.10E-82	244026
rs11642841	*FTO*	0.062 (0.0553,0.0687)	4.80E-73	244160
rs17817288	*FTO*	0.059 (0.0525,0.0655)	9.20E-72	244173
rs8047395	*FTO*	0.059 (0.0525,0.0655)	5.40E-70	243787
rs7206790	*FTO*	0.06 (0.0531,0.0669)	2.20E-64	242899
rs11075987	*FTO*	0.055 (0.0485,0.0615)	3.10E-63	244250
rs3769671	*POMC*	0.024 (0.0005,0.0475)	0.055	142166

Table 7. Replication results of SNPs associated with HC in GIANT Consortium.

HC SNPs	Gene	Effect Size	P value	N
rs1421085	*FTO*	0.069 (0.0619,0.0761)	2.60E-81	225139
rs1121980	*FTO*	0.065 (0.0581,0.0719)	3.10E-77	227198
rs3751812	*FTO*	0.067 (0.0599,0.0741)	4.50E-77	225667
rs9923147	*FTO*	0.066 (0.0589,0.0731)	1.60E-76	227120
rs9939609	*FTO*	0.065 (0.0579,0.0721)	5.90E-74	219677
rs17817449	*FTO*	0.064 (0.0571,0.0709)	1.10E-73	227371

rs7193144	*FTO*	0.064 (0.0571,0.0709)	1.20E-73	227217
rs9941349	*FTO*	0.065 (0.0579,0.0721)	7.70E-73	226898
rs7185735	*FTO*	0.064 (0.0569,0.0711)	2.50E-72	227038
rs11075989	*FTO*	0.064 (0.0569,0.0711)	3.70E-72	227196
rs12149832	*FTO*	0.064 (0.0569,0.0711)	1.00E-71	227281
rs9922708	*FTO*	0.062 (0.0549,0.0691)	1.30E-66	227041
rs17817288	*FTO*	0.058 (0.0511,0.0649)	5.30E-62	227163
rs11642841	*FTO*	0.06 (0.0527,0.0673)	6.30E-61	227160
rs11075987	*FTO*	0.055 (0.0481,0.0619)	5.00E-56	227245
rs7206790	*FTO*	0.059 (0.0516,0.0664)	6.20E-54	225920
rs1729998	*RSRC1*	0.013 (0.0042,0.0218)	0.0031	145454

4.4 Discussion

Conventional GWAS require a stringent p-value to identify a variant as associated with a certain phenotype. Many variants that do not reach this threshold are discarded in GWAS, such that the 97 variants associated with BMI identified so far only explain 2-3% of variance (Adam E Locke et al., 2015) as opposed to their predictive value which is between 30-40% (J. Yang, Bakshi, Zhu, Hemani, Vinkhuyzen, Lee, Robinson, Perry, Nolte, van Vliet-Ostaptchouk, Snieder, LifeLines Cohort, et al., 2015). As conventional GWAS may not have the power to detect some of the causal variants associated with complex diseases, it is important to ameliorate this issue by conducting studies in large sample sizes, or employ a candidate gene approach, or use new methods such as gene-based associations rather than SNP-based associations to reduce the burden of multiple testing (Hägg et al., 2015; Manolio et al., 2009).

In this study, we used biologically relevant genes based on gene manipulation techniques in mouse models and performed statistical analysis to find variants associated with a variety of obesity measures in the multi-ethnic EpiDREAM cohort. We expanded our analysis beyond the typical BMI study, as other studies have found little overlap between SNPs associated with BMI and other obesity measures (Dmitry Shungin et al., 2015). In order to strengthen our discovery

and also to overcome some of the shortcomings of conventional GWAS studies, we replicated our results from our discovery cohort in the GIANT international consortium. In this analysis, we found a novel variant (rs1729998) on the *RSRC1* gene associated with BAI, BMI and hip circumference that were previously not reported in other GWAS. Replication in GIANT further strengthened this association to BMI and hip circumference. It is interesting to note that previous GWAS associated variants on *RSRC1* to height, and previous studies have showed an association between taller stature and higher BMI (Freedman et al., 2002; Freeman, Power, & Rodgers, 1995). Furthermore, BAI is a measure of hip circumference in relation to height, and *RSRC1* was associated with all three phenotypes in our analysis. Other studies on *RSRC1* have pointed out the role of this gene in pre and postnatal brain development (Fallon et al., 2000; Rakic & Zecevic, 2003). Imaging techniques that provide information on the structural and function of specific genes in activating different regions of the brain have associated SNPs on *RSRC1* with schizophrenia and neural development (Potkin et al., 2010; Potkin et al., 2009). The rs1729998 on *RSRC1* is an intron variant that also influences mRNA splicing and gene expression, supported by the involvement of *RSRC1* in pre-mRNA splicing (Chen et al., 2015).

The next step of this project would be to replicate this variant in multiethnic cohorts. Although GIANT is a multi-ethnic cohort, a majority of the subjects (95%) are from European ancestry. We have selected a sub-set of cohorts in GIANT, The Women's Health Initiative (WHI), The Atherosclerosis Risk in Communities (ARIC) and The Multi-Ethnic Study of Atherosclerosis (MESA) that will not only provide us with a relatively large sample size (approximately 88,000 subjects), but it will also be a better representation of other ethnicities. Replicating our findings in a cohort compromised of these studies can further confirm the generalizability of our variant in the general public.

In conclusion, our results emphasize the importance of animal models in leading the way in gene discovery associated with human complex disorders. Our candidate gene approach resulted in the discovery of a novel variant associated with BMI, BAI and hip circumference in a large sample size. This discovery, paired with animal model studies can improve our understanding of the physiological pathways involved in obesity development. This approach is informative for designing other genetic studies of obesity or other complex diseases, and will guide future research aimed at unravelling the complex biology of obesity.

5.0 Conclusions and Perspectives

Studying genetics of obesity is a complex and multi-dimensional pursuit, but research attempts have been able to shed light on some of the main players in obesity development. Chapter 2 of this thesis overviewed the current knowledge in genetics of obesity, from a monogenic, oligogenic, polygenic and epigenetics perspective. It also emphasized on the importance of biological studies in understanding the functionality of obesity genes, in order to implement this knowledge in clinical practice. Chapter 3 emphasized on the essential role of animal models, particularly mouse models, in our attempt to discover new obesity genes. It also elaborated on the integration between mouse and human studies, such that gene discoveries in mouse are further investigated in human studies and *vice versa*. Chapter 4 detailed a candidate gene approach based on mouse model studies that led to the discovery of a novel variant associated with a number of anthropometric measures.

Although completing our understanding of the genetic architecture of obesity requires more investigation, this thesis will be concluded by providing some insights into accelerating the identification of obesity predisposing genes.

The use of approaches integrating multiple types of data (system biology / functional genomics) could boost the identification of genes predisposing to obesity (D. Yang, Jiang, & He, 2009). Such studies could benefit from the use of mice to access tissues that are not readily available in humans or to perform deep phenotyping that is too expensive in humans. The co-mapping of gene expression levels (eQTLs), protein levels (pQTLs) and even metabolites (mQTLs) to a location in the genome associated with a disease may generate novel hypotheses for direct testing in humans and mice (Davis et al., 2012; Mackay, Stone, & Ayroles, 2009; X. Yang et al., 2009). As an illustration, a study combining positional cloning and high-throughput transcriptome

approaches identified two novel candidate genes driving adiposity in mice (*Akr1b8* and *Rgs2*) that deserve further investigation in humans (Derry et al., 2010).

Stringent P-value thresholds are classically used in GWAS ($P < 5 \times 10^{-8}$) to adjust the experiment for the many hypotheses tested (Dudbridge & Gusnanto, 2008). As a result, many true associations that do not reach stringent P-value thresholds are missed by conventional GWAS (Stahl et al., 2012). Similar issues are now experienced in next generation sequencing (NGS) studies (Do, Kathiresan, & Abecasis, 2012). Identifying these true positive associations is challenging and so far has been addressed by the ever expanding meta-analyses (Stahl et al., 2012). Another way to 'separate the wheat from the chaff' could include the utilization of hypothesis-driven GWAS and next generation sequencing (NGS) approaches as opposed to hypothesis-free strategies (Li & Meyre, 2013). This method is beneficial in its decreased number of statistical tests and less stringent significance thresholds for the hypotheses being tested (Li & Meyre, 2013), similar to the approach in the EpiDREAM study. Narrowing down the hypothesis to a specific linkage region or molecular pathway for example, could lead to identification of association signals previously missed by conventional GWAS (Li & Meyre, 2013). These high-throughput hypothesis-driven experiments would greatly benefit from the inclusion of data collected in mouse models of obesity (Li & Meyre, 2013). This approach goes beyond the reduction in the number of tests and supports the statistical significant SNPs with biologically relevant data, which ultimately increase the possibility that the results stem from a true association and are not false positives (Ioannidis, 2005).

It is also important to emphasize on the value of expression studies in future experiments. Expression studies in mice can add valuable knowledge of the expression and regulation of genes under diverse environmental exposures (Yoganathan, Karunakaran, Ho, & Clee, 2012) especially

when studying the expression of a gene in a certain tissue is difficult to obtain in human studies. In a recent study, the expression of a subset of GWAS obesity candidate genes was observed to be different in the hypothalamus and/or adipose tissue of fed vs. fasted animals (Yoganathan et al., 2012). These experiments are helpful in moving from GWAS association signals to relevant candidate genes for obesity (Yoganathan et al., 2012).

Improving on methodology and techniques used in animal studies could also provide better insight in upcoming genetics studies. Employing more recent tools in genome editing such as CRISPR/Cas9 could allow for more precise targeted mutagenesis (F. Zhang, Wen, & Guo, 2014). This method depends on small RNA for sequence-specific cleavage (Jinek et al., 2012) for DNA targeting which is relatively cheap and easy to produce (F. Zhang et al., 2014). It involves a non-specific Cas9 nuclease and a set of programmable sequence-specific CRIPS RNA (crRNA) which can guide Cas9 to cleave the DNA and generate double-strand breaks at target sites (F. Zhang et al., 2014). CRISP/Cas9 is able to simultaneously allow for genomic modifications at multiple independent sites (Cong et al., 2013), but it can also induce non desired insertion deletions (Lin et al., 2014).

In light of improvement in animal models, utilizing tissue or time specific knockouts, knock-in or transgenic mice could help in better understanding the function of genes that were previously associated with obesity. For example, mice with global *PPARγ* inactivation showed reduced adipose tissue and mild glucose intolerance (Koutnikova et al., 2003). In comparison, fat-specific *PPARγ* knockout animals showed complete lipoatrophy, impaired adipokine secretion, profound insulin resistance and hyperglycemia, abnormal bone, mammary gland and skin metabolism (Wang, Mullican, Dispirito, Peed, & Lazar, 2013). Although not frequently used in the obesity genetics field, time-specific knockouts are helpful in understanding gene expression at different

developmental stages (Loebel, Hor, Bildsoe, & Tam, 2014). For instance, complete post-natal inactivation of *BDNF* in mice was associated with hyperphagic obesity, whereas pre-natal inactivation of the same gene was lethal (Rios et al., 2001).

Understanding the importance of gene-gene interactions in development of obesity is another key area of investigation. Studying gene-gene interactions in humans are experimentally demanding because they require large sample sizes to detect significant interactions, and are statistically challenging due to multiple testing issues (Hu, Wang, & Wang, 2014). Therefore model organisms are an important tool for studying gene-gene interactions (MacKay, 2014). Genetic studies in mice may also facilitate the discovery of gene-gene interactions or loci whose effects are only evident in the context of specific alleles at other loci. This approach is based on the hypothesis that a QTL for a trait in mouse that maps to a homologous location for the same trait in humans, is most likely caused by the same gene (Leduc et al., 2011). The BSB mouse model is an example of mouse model used to study epistatic effects on obesity QTLs (Yi et al., 2004). Mapping and identification of gene x gene interactions in mice could be examined in humans, again, since the homologous regions of mouse and human chromosome regions are well-defined (Chiu et al., 2006). Double-knockout mouse models, where two genes are inactivated, can reveal valuable information about gene-gene interactions (Chiu et al., 2006). Double-knockout mice have not been commonly studied in obesity field, so we present an example from the diabetes field. $Irs^{1-/-}$ have mild glucose intolerance and $Irs^{3-/-}$ have no detectable phenotype, but $Irs^{1-/-}/Irs^{3-/-}$ are hyperglycemic and display severe lipoatrophy (Terauchi et al., 2003), indicative of their interaction in developing the type 2 diabetes phenotype. Complex mouse models showing evidence of epistasis can be tested in human genetic epidemiology studies. Although the number of known gene-gene interactions in human obesity is relatively small, we hope that increased

sample size in epidemiological studies could help elucidate more of these interactions. Double knock out or transgenic mouse models are an attractive model to confirm the interactions identified in humans.

Aside from the importance of gene-gene interactions, recognizing the importance of gene-environment interactions in developing obesity is crucial (Andreasen & Andersen, 2009). For instance, physical activity could offset the aggregated genetic risk of multiple obesity loci (Shafqat Ahmad et al., 2013; S. Li et al., 2010). Gene-environment studies can help in targeting populations that may respond well or poorly to a specific lifestyle or therapeutic interventions (Shafqat Ahmad et al., 2013). Establishing large case-control studies in population-based cohorts, precise phenotyping of quantitative trait studies with precise measurements of lifestyle exposure, and target testing of interaction in existing lifestyle trials will provide the best understanding of gene-environment interactions (Franks, Mesa, Harding, & Wareham, 2007).

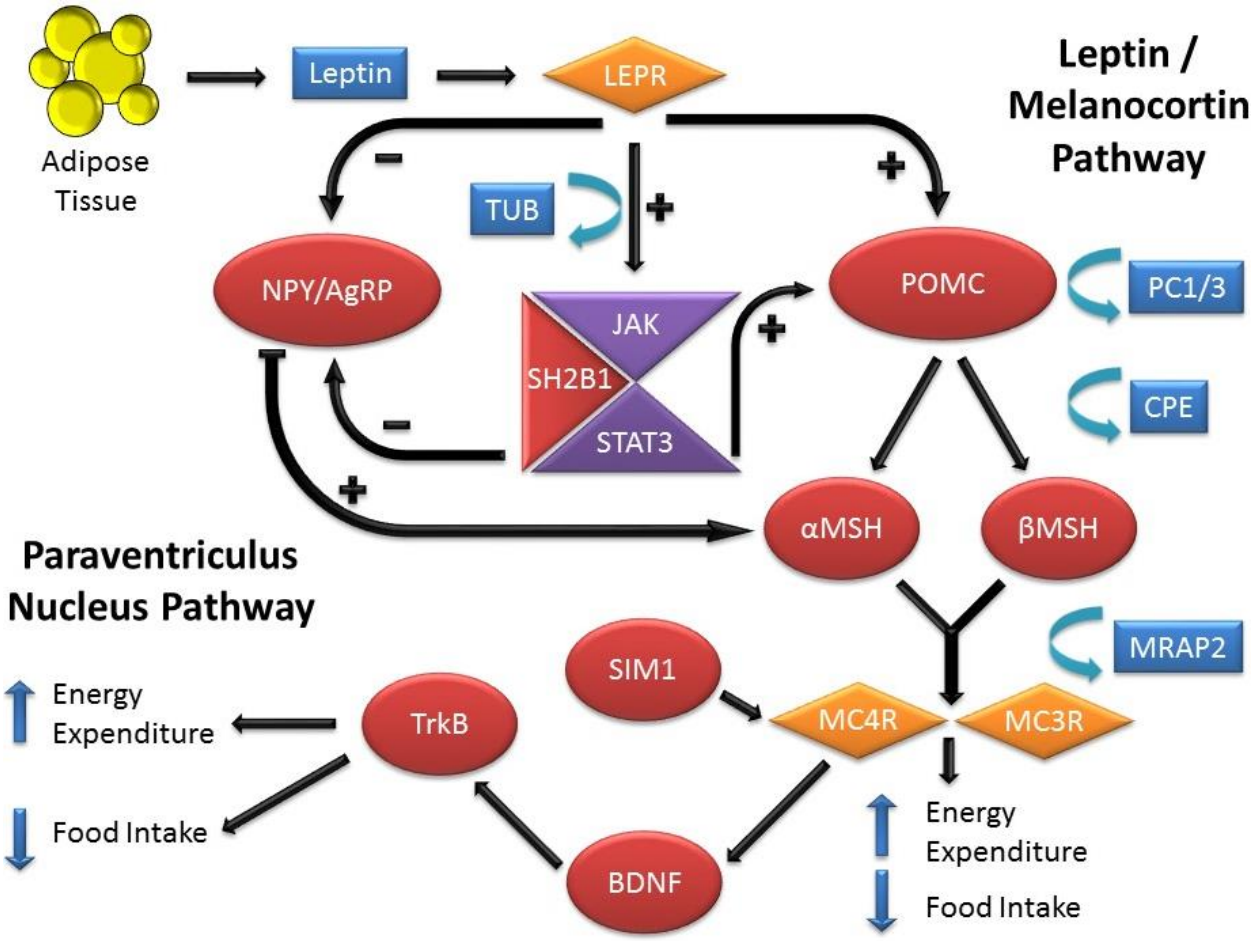
To effectively demonstrate how genetic variations at a specific locus modify the effect of an environmental stimuli on a metabolic trait requires a combination of environmental modifications on animal models and human etiological trials (Franks & Roth, 2008). Different environmental modifications can be tested in homozygous or heterozygous knock out or knock in models and they can eventually be studied in inbred strains with naturally occurring allelic variations. Testing the gene-environment interactions in genes with prior evidence of their role in interacting with an environmental stimuli in mouse models can improve the probability that an observed effect is true (Franks & Roth, 2008), which may help to better control for the high likelihood of false positives in gene-environment interactions in human studies. Studying gene-environment interactions in knock-out animal models may be used as a first step to prioritize genes for gene environment interaction studies in human populations. For instance, *Gipr*

knockout mice are protected against obesity and disturbance to their glucose homeostasis under a high-fat diet (Sonestedt et al., 2012). Human studies of *GIPR* common gene variants found a significant interaction between the rs10423928 SNP and a high-fat/low carbohydrate diet on risk of type 2 diabetes (Sonestedt et al., 2012).

On a different note, genes involved in diet and immune response have been preferential targets in positive selection during mammalian evolution, highlighting the importance of nutrient availability and pathogens as powerful driving forces of evolution (Kosiol et al., 2008). Evolutionary assessment of mutations associated with highly penetrant from of obesity through mammal evolution (including rodents) may be complementary to *in vitro* and *in vivo* characterization studies in evaluating their functional relevance (Stäubert et al., 2007). This approach may be less relevant to common variants of obesity with modest effect. For instance, more than 90% of 70 missense mutations in *MC4R* identified in obese patients were located at amino acid positions that are highly conserved during 450 million years of MC4R evolution in vertebrates (Stäubert et al., 2007).

Lastly, beyond classical human and mouse genetics, other recent disciplines have emerged such as epigenetics that may improve the understanding of the complex aetiology of obesity, and aid us in better predicting the disease by being integrated in big data analysis. Finally, even though genomic studies have triggered a great enthusiasm, translational applications remain subpar. To achieve clinical relevance, we not only require more scientific knowledge, but also a shift in the way of education, health expenses, agro-industrial lobbying, medicine and our individual lifestyle choices.

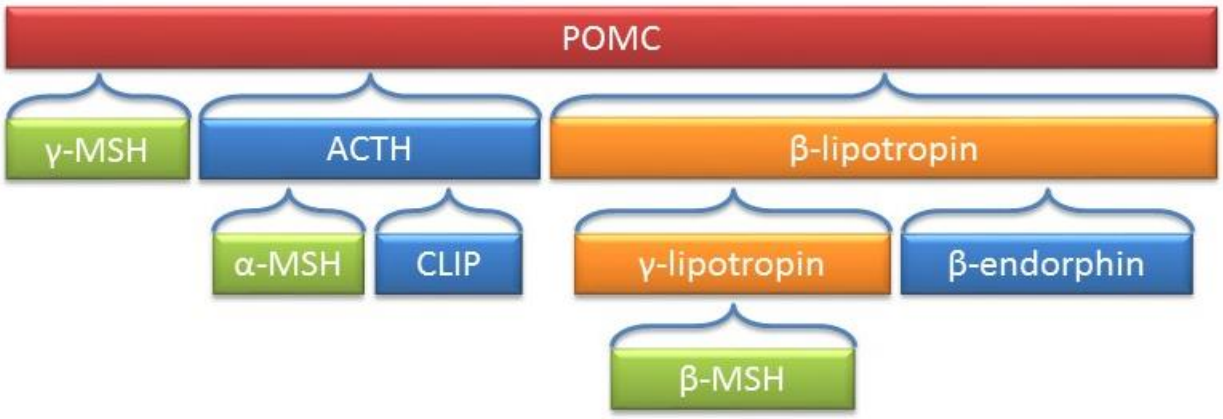
6.0 Supplementary Material



Supplementary Figure 1: Genes involved in the leptin-melanocortin pathway that have been associated with monogenic obesity through their influence on food intake and energy expenditure.

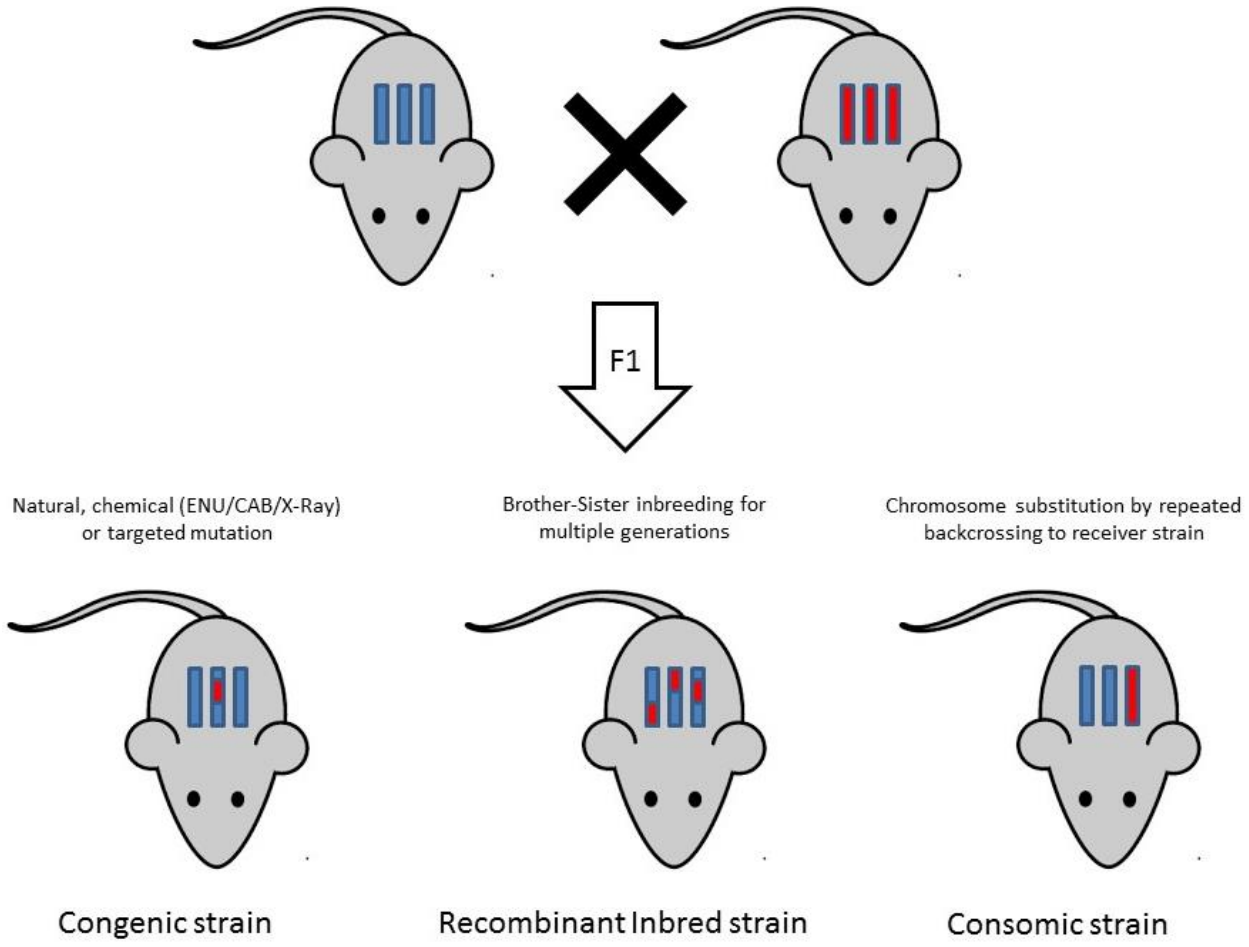
Leptin secreted from adipose tissue binds to the leptin receptor in the hypothalamus. Leptin binding inhibits the neuropeptide Y / agouti-related protein (NPY/AgPR) production and stimulates pro-opiomelanocortin (POMC) production, which undergoes post-translational modifications to produce peptides such alpha and beta-melanocyte-stimulating hormone (α and βMSH) *via* the processing of prohormone convertase 1(PC1/3) and carboxypeptidase E (CPE)

enzymes. Alpha and βMSH bind to melanocortin 3 and melanocortin 4 receptors (MC3R and MC4R) and induce their activity. Melanocortin 2 receptor accessory protein 2 (MRAP2) can reduce the responsiveness of both MC3R and MC4R to α and βMSH and result in obesity. On the other hand, Single-minded 1 (SIM1) acts as a facilitator of MC4R activity. Increase in the MC3R and MC4R activities result in a decrease in food intake and increase in energy expenditure. MC4R activity also stimulates release of Brain-derived neurotrophic factor (BDNF) which will bind to the neurotrophin receptor (TrkB) and influence food intake and energy expenditure. Aside from activation of the POMC, leptin binding to its receptor also activates the Janus kinase / signal transducer and activator of transcription (JAK/STAT) signaling. This pathway, through the help of Src homology 2 B adapter protein 1 (SH2B1), results in activation of Signal transducer and activator of transcription 3 (STAT3). STAT3 will then migrate to the nucleus with the help of Tubby bipartite transcription factor (TUB) and activate its target genes related to energy homeostasis and mediate in the anorexigenic effects of leptin.



Supplementary Figure 2: Processing of the POMC precursor protein.

Adrenocorticotropic hormone (ACTH) and β-lipotropin are products generated in the corticotrophic cells of the anterior pituitary under the control of corticotropin releasing hormone (CRH). Alpha-melanocyte stimulating hormone (α-MSH), corticotropin-like intermediate lobe peptide (CLIP), γ-lipotropin and β-endorphin are products generated in the intermediate lobe of the pituitary under the control of dopamine. α-, β- and γ-MSH are collectively referred to as melanotropin or intermedin.



Supplementary Figure 3: General overview of mutagenesis and inbreeding in mice.

Congenic, recombinant inbred and consomic mice are obtained when part of the genome of one mouse strain is transferred to another strain by backcrossing the donor mouse to the receiver strain. In congenic mouse, the offspring resembles the parent strain except for the mutated chromosomal segment, whereas in consomic strain, the offspring carries an entire chromosome from the donor strain. Recombinant inbred strains are obtained by cross breeding of inbred mice to increase their genotypic diversity and carrying a series of brother-sister mating for multiple generations.

Supplementary Table 1: List of genes experimented in mouse models for obesity or obesity-related phenotype

Num	Cand. Genes	Phenotypic Details	Technique	Ref
1	*ACADVL*	Adult-onset fat mass gain	Knock-out	Exil, VJ. 2003. Circ Res
2	*ADAR2*	Obesity under HFD*	Transgenic	Singh, M. 2007. J Biol Chem
3	*ADRA1B*	Accelerated weight gain on HFD	Knock-out	Burcelin, R. 2004. J Biol Chem
4	*ADRA2A*	Obesity in homozygous mutation	Transgenic	Valet, P. 2000. J Biol Chem
5	*ADRB1*	Obesity	Knock-out	Bachman, ES. 2002. Science
6	*ADRB2*	Obesity	Knock-out	Soloveva, V. 1997. Mol Endocrinol
7	*ADRB3*	Obesity on HFD	Knock-out	Susulic, VS. 1995. J Biol Chem
8	*AEBP1*	Obesity in females	Transgenic	Zhang, L. 2005. Mol Med
9	*AGRP*	Elevated weight gain & obesity	Transgenic	Ollman, MM. 1997. Science
10	*ALMS1*	Obesity	Knock-out	Collin, GB. 2005. Hum Mol Genet
11	*ALP1*	Accelerated weight gain on HFD	Knock-out	Narisawa, S. 2003. Mol Cell Biol
12	*ANGPTL6*	Obesity and insulin resistance	Knock-out	Oike, Y. 2005. Nat Med
13	*ANKRD26*	Obesity in homozygotes	Transgenic	Bera, TK. 2008. Acad Sci USA
14	*APOB*	Increased BW*	Knock-out	Siri, P. 2009. J Biol Chem
15	*APOC3*	Obesity on HFD	Transgenic	Jong, MC. 2001. J Lipid Res
16	*APOE*	Obesity	Knock-out	Zhang, T. 2013. Reproduction
17	*AQP7*	Adult-onset obesity	Knock-out	Hibuse, T. 2005. Proc Natl Acad Sci USA
18	*AR*	Obesity, decreased energy expenditure	Cre/LoxP	Fan, W. 2005. Diabetes
19	*ASIP*	Increased BW & Fat mass -- Obesity	Transgenic	Mynatt, RL. 1997. Natl Acad Sci USA
20	*AT2R*	Increase in BW in females only	Knock-out	Samuel, P. 2013. PLoS One
21	*ATX*	Increase in adiposity in fat-	Cre/LoxP	Dusaulcy, R. 2011. J Lipid Res

		specific knockout under HFD		
22	*ATXN2*	Obesity under HFD	Knock-out	Kiehl, T. 2006. Biochem Biophys Res Commun
23	*BBS1*	Adult-onset obesity in 10% of mutants	Knock-out	Kulaga, HM. 2004. Nat Genet
24	*BBS2*	Adult-onset fat mass gain	Knock-out	Nishimura, DY. 2004. Proc Natl Acad Sci USA
25	*BBS4*	Adult-onset obesity	Knock-out	Mykytyn, K. 2004. Proc Natl Acad Sci USA
26	*BBS7*	Obesity	Knock-out	Zhang, Q. 2013. J Cell Sci
27	*BDNF*	Adult-onset obesity in heterozygotes	Knock-out	Coppola, V. 2004. Neuroreport
28	*BRD2*	Obesity	Knock-out	Wang, F. 2009. Biochem J
29	*BRS3*	Obesity	Knock-out	Ohki-Hamazaki, H. 1997. Nature
30	*CAPN10*	Increase in body weight	Knock-out	Cheverud, JM. 2010. J Lipid Res
31	*CART*	Adult-onset obesity	Knock-out	Wierup, N. 2005. Regul Pept
32	*CAV3*	Increased adiposity	Knock-out	Capozza, F. 2005. Am J Physiol Cell Physiol
33	*CB2R*	Increase in body weight and hyperphagia	Knock-out	Agudo, J. 2010. Diabetologia
34	*CCKBR*	Obesity	Knock-out	Lavine, J. 2010. Endocrinol
35	*CDH2*	Increased adiposity	Transgenic	Castro, CH. 2004. J Cell Sci
36	*CDKN1A (B)*	Increased adiposity	Knock-out	Naaz, A. 2004. FASEB J
37	*CEP19*	Obesity	Knock-out	Shalata, A. 2013. Am J Hum Genet
38	*CHEMR23*	Adult-onset obesity	Knock-out	Rouger, L. 2013. J Endocrinol
39	*CHGA*	Increased adiposity	Knock-out	Bandyopadhyay, G. 2012. J Biol Chem
40	*CHOP*	Obesity under HFD	Knock-out	Grant, RW. 2014. J Biol Chem
41	*CLOCK*	Obesity	Knock-out	Turek, F. 2005. Science
42	*CORIN*	Increased bodyweight	Knock-out	Chan, JC. 2005. Proc Natl Acad Sci USA
43	*CPE*	Obesity	Knock-out	Cawley, NX. 2004. Endocrinology
44	*CPT1*	Obesity under HFD	Knock-out	Gao, FX. 2009. Diabetologia

45	*CRH*	Excess fat accumulation & muscle atrophy	Transgenic	Stenzel-Poore, MP. 1992. Endocrinology
46	*CRY (1/2)*	Obesity under HFD	Knock-out	Barclay, JL. 2013. Am J Physiol Endorinol Metab
47	*CSF2*	Adult-onset obesity	Knock-out	Reed, JA. 2005. J Clin Invest
48	*CTRP9*	Increased bodyweight & adiposity	Knock-out	Wei, Z. 2014. Am J Physiol Endocrinol Metab
49	*CYP19A1*	Elevated gonadal fat pad weight	Knock-out	Misso, ML. 2005. Horm Metab Res
50	*D2*	Increased BW & adiposity	Knock-out	Marsili, A. 2011. PLoS One
51	*DGAT1*	Increased gonadal but not subcutaneous fat	over-expression	Yamazaki, T. 2005. J Biol Chem
52	*DPT*	Increased subcutaneous fat	Knock-out	Takeda, U. 2002. J Invest Dermatol
53	*DRD3*	Increased adiposity and obesity	Knock-out	McQuade, JA. 2004. Behav Brain Res
54	*dup(17)*	Obesity	Transgenic	Walz, K. 2003. Mol Cell Biol
55	*ECSCR*	Obesity under HFD	Transgenic	Akakabe, Y. 2013. Nat Commun
56	*ESR1*	Obesity	Knock-out	Heine, PA. 2000. Proc Natl Acad Sci USA
57	*FABP4*	Obesity in homozygotes under HFD	Knock-out	Hotamisligil, GS. 1996. Science
58	*FATP4*	Obesity in homozygotes under HFD	Knock-out	Lenz, LS. 2011. J Biol Chem
59	*FKBP51*	Increase in body weight under HFD	Transgenic	Yang, L. 2012. Am J Physiol Endocrinol Metab
60	*FOXA2*	Heterozygotes develop obesity under HFD	Knock-out	Wolfrum, C. 2003. J Clin Invest
61	*FOXO1*	Obesity	Transgenic	Kamei, Y. 2004. J Biol Chem
62	*FOXO3A*	Obesity	Knock-out	Fang, C. 2008. Am J Physiol
63	*FSHR*	Obesity	Knock-out	Danilovich, N. 2000. Endocrinology
64	*FTO*	Obesity	Over-expression	Church, C. 2010. Nat Genet
65	*GAL-3*	Late-onset obesity	Knock-out	Pang, J. 2013. PLoS One
66	*GAST*	Obesity	Knock-out	Cowey, SL. 2005. Cancer
67	*GCK*	Increased BW under HFD	Transgenic	Ferre, T. 2003. Diabetologia
68	*GDF3*	Increased BW under HFD	Over-expression	Wang, W. 2004. Biochem Biophys Res Commun

69	*GFPT1*	Increased adiposity	Transgenic	McClain, DA. 2005. Am J Physiol Endocrinol Metab
70	*GH*	Obesity	Transgenic	Pomp, D. 1996. Transgenic Res
71	*GHR*	Increased adiposity in males	Knock-in	Rowland, JE. 2005. Mol Cell Bio
72	*GHRH*	Increased adiposity	Transgenic	Cai, A. 1999. Endocrinology
73	*GIRK4*	Increased BW & adiposity	Knock-out	Perry, CA. 2008. Proc Natl Acad Sci USA
74	*GNAS*	Maternal inheritance of mutant allele leads to obesity	Knock-out	Germain-Lee, EL. 2005. Endocrinology
75	*GNB3*	Increased BW and adiposity	Transgenic	Goldlust, S. 2013. Proc Natl Acad Sci USA
76	*GPD2*	Increased BW & adiposity in females	Knock-out	Alfadda, A. 2004. Am J Physiol Regul Integr Comp Physiol
77	*GPR10*	Adult-onset obesity	Knock-out	Ishii, M. 2003. Proc Natl Acad Sci USA
78	*GPR120*	Obesity under HFD	Knock-out	Hirasawa, A. 2005. Nat Med
79	*GPR26*	Obesity	Knock-out	Chen, D. 2012. PLoS One
80	*GPR39*	Obesity	Knock-out	Moechars, D. 2006. Gastroenterology
81	*GPR7*	Adult-onset obesity	Knock-out	Gu, W. 2004. J Mol Neurosci
82	*GPX1*	Increased BW & adiposity	Transgenic	McClung, JP. 2004. Proc Natl Acad Sci USA
83	*GRM8*	Increased adiposity	Knock-out	Duvoisin, RM. 2005. Eur J Neurosci
84	*GRP*	Resistant to diet induced obesity	Knock-out	Ye, R. 2010. Diabetes
85	*GRPR*	Reduced food intake	Knock-out	Hampton, LL. 1998. Proc Natl Acad Sci USA
86	*GSK3B*	Increased BW & adiposity in males	Transgenic	Pearce, NJ. 2004. Metabolism
87	*HDC*	Increased BW & adiposity	Knock-out	Hara, J. 2001. Neuron
88	*HIF1α*	Obesity	Transgenic	Zhang, X. 2010. J Biol Chem
89	*HRH1*	Late onset obesity	Knock-out	Masaki, T. 2004. Diabetes
90	*HRH3*	Increased BW & adiposity	Knock-out	Takahashi, K. 2002. J Clin Invest
91	*HSD1-11β*	Obesity	Transgenic	Zhang, L. 2012. Transgenic

				Res
92	*HSD11β2*	Increased adiposity	Transgenic	Masuzaki, H. 2001. Science
93	*HTR2C*	Late onset obesity	Knock-out	Nonogaki, K. 2003. Diabetes
94	*ICAM1*	Late onset obesity/accelerated under HFD	Knock-out	Gregiore, FM. 2002. AM J Physiol Endocrinol Metab
95	*IDH1*	Obesity	Transgenic	Koh, HJ. 2004. J Biol Chem
96	*IFRD1*	Increased adiposity	Transgenic	Wang, Y. 2005. J Biol Chem
97	*IL18*	Increased BW	Knock-out	Netea, M. 2006. Nature Medicine
98	*IL18R*	Increased BW	Transgenic	Netea, M. 2006. Nature Medicine
99	*IL-1RI*	Adult-onset obesity	Knock-out	McGillicuddy, FC. 2013. Am J Physiol Endocrinol Metab
100	*IL6*	Increased BW & adiposity	Knock-out	Wallenius, V. 2002. Nat Med
101	*INSR*	Increased adiposity & obesity	Cre/LoxP	Cariou, B. 2004. Endocrinol
102	*IRS1*	Increase weight gain	Knock-out	Shirakami, A. 2002. J Endocrinol
103	*IRS2*	Increased adiposity	Cre/LoxP	Lin, X. 2004. J Clin Invest
104	*JAK2 (Adipose)*	Increased adiposity	Cre/LoxP	Sy, S. 2014. Diabetalogia
105	*KCNJ11*	Increased BW & adiposity	Knock-out	Kanezaki, Y. 2004. Endocr J
106	*KDM3A*	Obesity	Knock-out	Okada, Y. 2010. J Androl
107	*KRAS*	Obesity under HFD	Transgenic	Dawson, DW. 2013. Cancer Prev Res
108	*KSR2*	Obesity	Knock-out	Revelli, JP. 2011. Obesity
109	*LEP*	Obesity	Knock-out	D'Souza, AM. 2014. Endocrinol
110	*LEPR*	Obesity	Knock-in	Bates, SH. 2003. Nature
111	*LH (B)*	Obesity in females	Transgenic	Kero, JT. 2003. Am J Physiol Endocrinol Metab
112	*LIPC*	Increased adiposity	Knock-out	Farahani, P. 2004. Obes Res
113	*LPIN1*	Obesity due to increased fat storage	Transgenic	Phan, J. 2005. Cell Metab
114	*LRH-1*	Mild obesity	Knock-out	Hattori, T. 2014. Endocr J
115	*LSR*	Obesity in heterozygotes	Transgenic	Yen, FT. 2008. J Biol Chem
116	*MAGEL2*	Increased BW & adiposity	Knock-out	Bischof, JM. 2007. Hum Mol Genet

117	*MAGP-1*	Increased BW & adiposity	Knock-out	Weinbaum, JS. 2008. J Biol Chem
118	*MAS*	Increased adiposity	Knock-out	Santos, SH. 2008. Diabetes
119	*MC3R*	Obesity	Knock-out	Butler, AA. 2000. Endocrinolopgy
120	*MC4R*	Obesity	Knock-out	Huszar, D. 1997. Cell
121	*MED13*	Obesity	Cre/LoxP	Grueter, C. 2012. Cell
122	*MEST*	Increased adiposity	Transgenic	Takahashi, M. 2005. Am J Physiol Endocrinol Metab
123	*MKKS*	Obesity	Knock-out	Fath, MA. 2005. Hum Mol Genet
124	*MMP11*	Obesity	Knock-out	Andarawewa, KL. 2005. Cancer Res
125	*MMP19*	Accelerated weight gain on HFD	Knock-out	Pendas, AM. 2004. Mol Cell Biol
126	*MPO*	Increased BW	Transgenic	Castellani, LW. 2006. J Lipid Res
127	*MRAP2*	Obesity	Knock-out	Asai, M. 2013. Science
128	*MT1A (B)*	Adult-onset obesity	Knock-out	Beattie, JH. 1998. Proc Natl Acad Sci USA
129	*MT-HGH*	Obesity	Transgenic	Wolf, E. 1991. Growth Dev Aging
130	*NBEA*	Increased BW & adiposity in heterozygotes	Knock-out	Olszewski, P. 2012. PLoS Genet
131	*NEIL1*	Obesity	Knock-out	Sampath, H. 2011. Am J Physiol Endocrinol Metab
132	*NEP*	Adult-onset obesity	Knock-out	Becker, M. 2010. PLoS One
133	*NGN3*	Obesity	Knock-out	Anthwal, N. 2013. Dis Model Mech
134	*NHLH2*	Adult-onset obesity	Knock-out	Jing, E. 2004. Endocrinology
135	*NMU*	Increased BW & adiposity	Knock-out	Handa, R. 2004. Nat Med
136	*NPB*	Mild obesity	Knock-out	Kelly, MA. 2005. Proc Natl Acad Sci USA
137	*NPC1*	Dose-dependent weight gain under HFD	Knock-out	Jelinek, D. 2010. Obesity
138	*NPY*	Obesity under high-sucrose diet	Transgenic	Kaga, T. 2001. Diabetes
139	*NPY1R*	Obesity	Knock-out	Kushi, A. 1998. Proc Natl Acad Sci USA
140	*NPY2R*	Obesity	Knock-out	Lin, D. 2006. Endocrinol

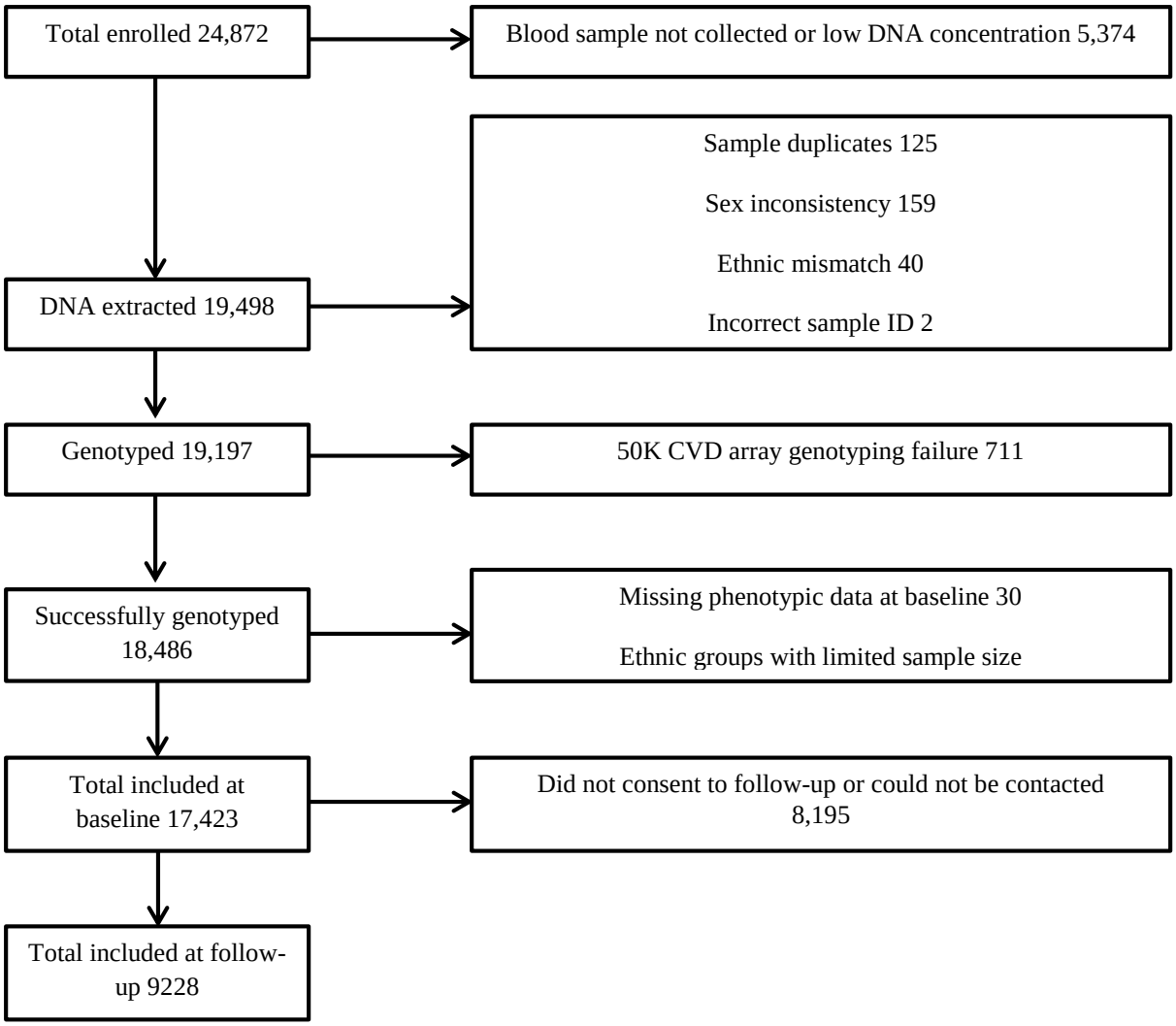
141	*NPY5R*	Increased adiposity	Knock-out	Marsh, DJ. 1998. Nat Med
142	*NR5A1*	Adult-onset obesity	Knock-out	Majdic, G. 2002. Endocrinol
143	*NTSR1*	Adult-onset obesity	Knock-out	Remaury, A. 2002. Brain Res
144	*OGG1*	Increased adiposity in HFD	Knock-out	Sampath, H. 2012. PLoS One
145	*OMA1*	Obesity	Knock-out	Quiros, PM. 2012. EMBO
146	*OSMRβ*	Increase in BW and hyperphagia	Knock-out	Gotardo, EM. 2013. J Nutr Sci Vitaminol
147	*OXT*	Obesity	Knock-out	Nishimori, K. 2008. Prog Brain Res
148	*P62*	Adult-onset obesity and hyperphagia	Knock-out	Harada, H. 2013. J Neurosci
149	*PARP1*	Adult-onset obesity	Knock-out	Devalaraja-Narashimha, K. 2010. J Endocrinol
150	*PC1/3*	Increased adiposity in heterozygotes	Knock-out	Zhu, X. 2002. Proc Natl Acad Sci USA
151	*PCK1*	Obesity	Transgenic	Franckhauser, S. 2002. Diabetes
152	*PCSKIN*	Adult-onset obesity	Transgenic	Wei, S. 2004. J Endocrinol
153	*PCYT2*	Obesity	Knock-out	Fullerton, MD. 2009. J Biol Chem
154	*PEG3*	Obesity	Knock-out	Curley, JP. 2005. FASEB J
155	*PGC-1α*	Obesity	Knock-out	Leone, TC. 2005. PLoS Biol
156	*PGDS*	Obesity	Knock-out	Tanaka, R. 2009. Biochem Biophys Res Commun
157	*PGP*	Increased BW & adiposity	Knock-out	Foucaud-Vignault, M. 2011. PLoS One
158	*PHB*	Obesity	Transgenic	Ande, SR. 2014. Diabetes
159	*PI3K (p110α)*	Increased adiposity, hyperphagia	Knock-in	Foukas, L. 2006. Nature
160	*PLAC 8*	Increase in adiposity	Knock-out	Jimenez-Preitner, M. 2011. Cell Metab
161	*PLSCR1*	Increased adiposity	Knock-out	Zhou, Q. 2002. Blood
162	*PLSCR3*	Increased BW & adiposity	gene-trap	Wiedmer, T. 2004. Proc Natl Acad Sci USA
163	*POMC*	Obesity under HFD	Knock-out	Challis, BG. 2004. Proc Natl Acad Sci USA
164	*PPARA*	Increase in adiposity	Knock-out	Miyazaki, M. 2004. J Biol Chem
165	*PPARG2*	Obesity under HFD	Knock-in	Heikkinen, S. 2009. Cell

				Metab
166	*PPARGC1A*	Increased adiposity in young females & old males	Knock-out	Leone, TC. 2005. PLoS Biol
167	*PPARδ*	Obesity under HFD	Knock-out	Kocalis, H. 2012. PLoS One
168	*PPIF*	Late-onset obesity	Knock-out	Luvisetto, S. 2008. Neurosci
169	*PPIR3A*	Increased BW & adiposity	Knock-out	Delibegovic, M. 2003. Diabetes
170	*PPKAA2*	Increased adiposity	Knock-out	Villena, JA. 2004. Diabetes
171	*PREF1*	Obesity	Knock-out	Moon, YS. 2002. Mol Cell Biol
172	*PRKCQ*	Obesity	Transgenic	Serra, C. 2003. J Cell Physiol
173	*PRL*	Increased BW	Knock-out	Perez-Villamil, B. 1992. J Endocrinol
174	*PROX1*	Obesity in heterozygotes	Knock-out	Harvey, NL. 2005. Nat Genet
175	*PRRP*	Increased BW	Knock-out	Lawrence, C. 2002. Endocrinol
176	*PTPN11*	Obesity	Knock-out	Zhang, EE. 2004. Proc Natl Acad Sci USA
177	*PYY*	Obesity	Knock-out	Batterham, R. 2002. Nature
178	*RAGE*	Increased BW	Knock-out	Leuner, B. 2012. Z Gerentol Geriatr
179	*RAI1*	Obesity in heterozygotes	Knock-out	Bi, W. 2005. Hum Mol Genet
180	*REN*	Adult-onset obesity	Transgenic	Uehara, S. 2003. Int J Mol Med
181	*RETN*	Increased adiposity	Transgenic	Kim, KH. 2004. Proc Natl Acad Sci USA
182	*RPGRIP1L*	Obesity	Knock-out	Vadnais, C. 2013. BMC Genomics
183	*RSC1A1*	Obesity	Knock-out	Osswald, C. 2005. Mol Cell Biol
184	*RSL1*	Females prone to diet induced obesity	Transgenic	Krebs, CJ. 2014. Mol Cell Biol
185	*SAR1B*	Obesity under HFD	Transgenic	Levy, E. 2014. J Nutr Biochem
186	*SDC1*	Adult-onset obesity	Transgenic	Reizes, O. 2001. Cell
187	*SELM*	Increased BW & adiposity	Transgenic	Pitts, MW. 2013. J Biol Chem
188	*SFRP1*	Increase in BW and adiposity under HFD	Knock-out	Gauger, KJ. 2013. PLoS One
189	*SH2B*	Obesity	Knock-out	Ren, D. 2005. Cell Metab
190	*SHP*	Obesity and increased	Transgenic	Tabbi-Anneni, I. 2010. Am J

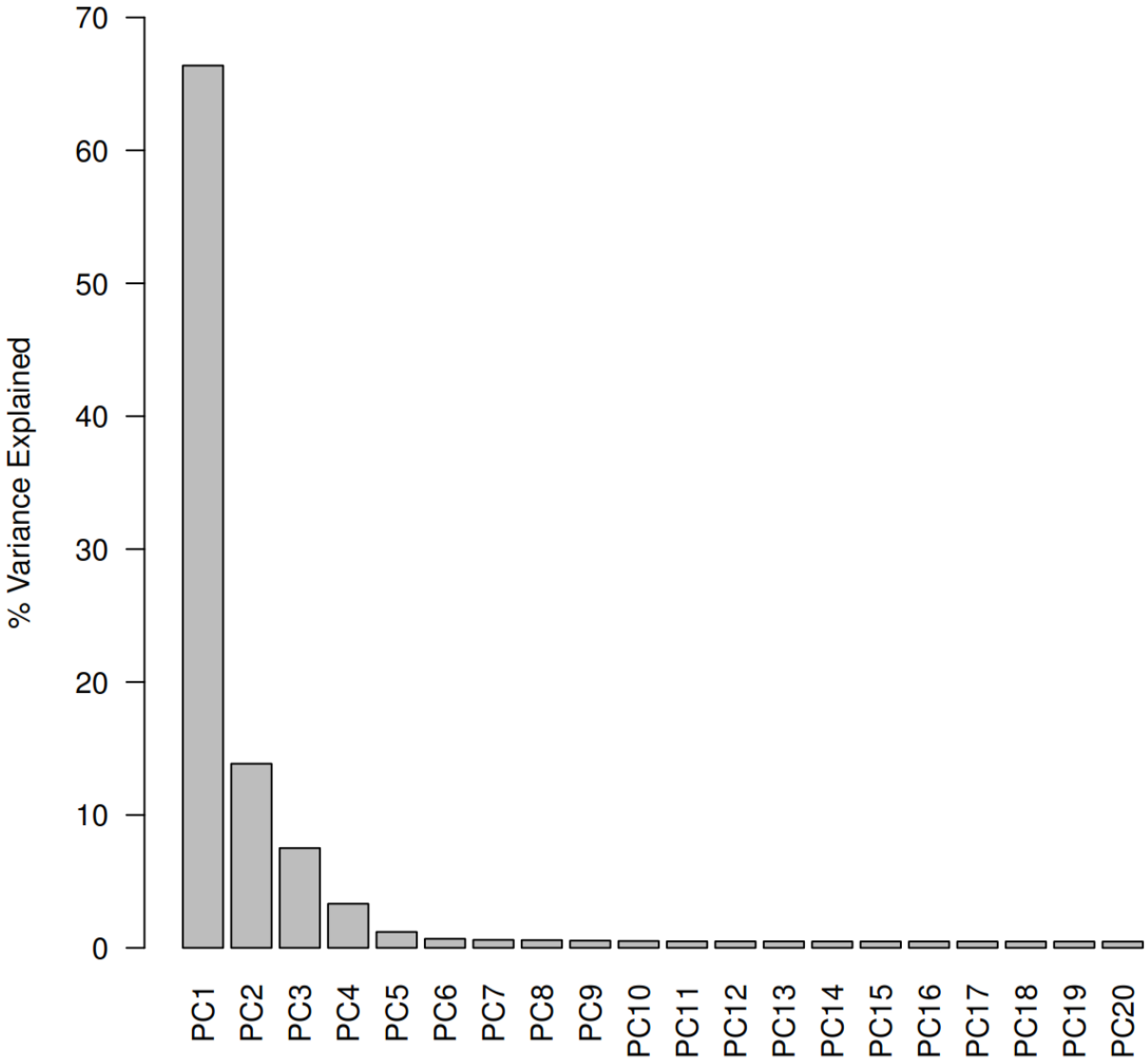
		adiposity		Physiol Endocrinol Metab
191	*SIM1*	Obesity in heterozygotes	Knock-out	Michaud, JL. 2001. Hum Mol Genet
192	*SIRT6*	Adult-onset obesity	Transgenic	Schwer, B. 2010. PNAS
193	*SLC2A4*	Increased adiposity	Transgenic	Carvalho, E. 2005. Am J Physiol Endocrinol Metab
194	*SLC6A1*	Obesity	Transgenic	Ma, YH. 2000. Cell Res
195	*SOCS1*	Liver degeneration, obesity	Knock-out	Starr, R. 1998. Proc Natl Acad Sci USA
196	*SOCS3*	Obesity under HFD	Knock-out	Sachithanandan, N. 2010. Hepatology
197	*SPARC*	Increased adiposity	Knock-out	Bradshaw, AD. 2003. Proc Natl Acad Sci USA
198	*SPONDIN 2*	Obesity	Knock-out	Zhu, LH. 2014. J Hepatol
199	*SRC-1*	Obesity	Knock-out	Picard, F. 2002. Cell
200	*STAT3*	Obesity	Cre/LoxP	Cui, Y. 2004. Mol Cell Biol
201	*STAT5B*	Increased adiposity	Knock-out	Gao, Q. 2004. Proc Natl Acad Sci USA
202	*TAp63*	Obesity	Knock-out	Su, X. 2012. Cell Metab
203	*T-BET*	Obesity	Knock-out	Kim, K. 2013. J Nutr Biochem
204	*THRA*	Increased BW & adiposity	Knock-out	Udy, GB. 1997. Proc Natl Acad Sci USA
205	*TIMP-2*	Obesity and hyperphagia	Knock-out	Stradecki, HM. 2011. J Neuroendocrinol
206	*TIS7*	Increased BW & adiposity	Transgenic	Wang, Y. 2005. J Biol Chem
207	*TNF*	Increased BW & adiposity	Transgenic	Liu, YY. 2003. J Biol Chem
208	*TNF-α*	Increased BW & adiposity	Knock-out	Salles, J. 2012. J Nutr Biochem
209	*TRKβ*	Increased BW & adiposity	Knock-in	Byerly, MS. 2013. PLoS One
210	*TRPV4*	Increased BW & adiposity	Knock-out	O'Conor, J. 2013. Ann Rheum Dis
211	*TUB*	Adult-onset obesity	Knock-out	Voros, G. 2004. J Thromb Haemost
212	*TW*	Obesity in heterozygotes	Knock-out	Kurima, K. 2011. PLoS Genet
213	*TXNIP*	Increased fat to muscle ratio	Knock-out	Stubdal, H. 2000. Mol Cell Biol
214	*UCP1*	Late-onset obesity with HFD	Knock-out	Kontani, Y. 2005. Aging Cell

215	*VDR*	Obesity	Transgenic	Wong, KE. 2011. J Biol Chem
216	*WDTC1*	Obesity in heterozygotes	Knock-out	Hader, T. 2003. EMBO
217	*XOR*	Increased BW & adiposity	Knock-out	Murakami, N. 2014. Arterioscler Thromb Vasc Biol
218	*ZEB1*	Obesity	Knock-out	Saykally, JN. 2009. PLoS One
219	*ZFP90*	Obesity	Transgenic	Schadt, EE. 2005. Nat Gen
220	*ZNT7*	Obesity in males only	Knock-out	Huang, L. 2012. J Biol Chem
221	*ZNT8*	Obesity under HFD	Knock-out	Lemaire. K, 2009. Proc Natl Acad Sci USA

*BW = Body weight, HFD = High fat diet



Supplementary Figure 4: Flow chart of EpiDREAM cohort.



Supplementary Figure 5. **Percentage of variability explained by the Principle Component Analysis (PCA).**

The x-axis represents the PCA adjustment and the y-axis represents the population variance explained by each component.

Supplementary Table 2: SNPs associated with BAI in EpiDREAM cohort

BAI SNP	Gene	Chrom.Location	Effect Size	Risk Allele	p-value
rs11075989	*FTO*	16:52377378	0.4476 (0.3098, 0.5854)	A/G	1.95E-10
rs9939609	*FTO*	16:52378028	0.446 (0.3082, 0.5838)	A/T	2.25E-10
rs7185735	*FTO*	16:52380152	0.4458 (0.308, 0.5836)	G/A	2.30E-10
rs3751812	*FTO*	16:52375961	0.4548 (0.3129, 0.5967)	T/G	3.30E-10
rs9941349	*FTO*	16:52382989	0.4343 (0.2958, 0.5728)	T/C	7.94E-10
rs12149832	*FTO*	16:52400409	0.4401 (0.2991, 0.5811)	A/G	9.58E-10
rs9922708	*FTO*	16:52388647	0.4153 (0.2781, 0.5525)	T/C	2.96E-09
rs7193144	*FTO*	16:52368187	0.4153 (0.2775, 0.5531)	C/T	3.46E-09
rs1421085	*FTO*	16:52358455	0.4239 (0.2831, 0.5647)	G/A	3.59E-09
rs17817449	*FTO*	16:52370868	0.4047 (0.2667, 0.5427)	G/T	9.13E-09
rs1121980	*FTO*	16:52366748	0.3655 (0.2304, 0.5006)	A/G	1.13E-07
rs9923147	*FTO*	16:52359050	0.3493 (0.2141, 0.4845)	T/C	4.10E-07
rs7206790	*FTO*	16:52355409	0.3362 (0.201, 0.4714)	G/C	1.10E-06
rs11642841	*FTO*	16:52402988	0.341 (0.1964, 0.4856)	A/C	3.78E-06
rs17817288	*FTO*	16:52365265	0.2759 (0.1418, 0.41)	C/T	5.53E-05
rs1729998	*RSRC1*	3:159410718	0.2665 (0.1274, 0.4056)	G/A	0.00017348

Supplementary Table 3: SNPs associated with WC in EpiDREAM cohort

SNP	Gene	Chrom. Location	Effect Size	Risk Allele	p-value
rs3751812	*FTO*	16:52375961	1.0759(0.769 ,1.3828)	T/G	6.40E-12
rs1421085	*FTO*	16:52358455	1.0666(0.7621 ,1.3711)	G/A	6.67E-12
rs12149832	*FTO*	16:52400409	1.0616(0.7565 ,1.3667)	A/G	9.07E-12
rs11075989	*FTO*	16:52377378	1.0325(0.7342 ,1.3308)	A/G	1.16E-11
rs9939609	*FTO*	16:52378028	1.0314(0.7331 ,1.3297)	A/T	1.22E-11
rs7185735	*FTO*	16:52380152	1.0297(0.7314 ,1.328)	G/A	1.32E-11
rs9941349	*FTO*	16:52382989	0.9801(0.6804 ,1.2798)	T/C	1.46E-10
rs9922708	*FTO*	16:52388647	0.9579(0.6611 ,1.2547)	T/C	2.53E-10
rs7193144	*FTO*	16:52368187	0.9484(0.6501 ,1.2467)	C/T	4.62E-10
rs17817449	*FTO*	16:52370868	0.9326(0.6339 ,1.2313)	G/T	9.40E-10
rs1121980	*FTO*	16:52366748	0.8403(0.548 ,1.1326)	A/G	1.74E-08
rs7206790	*FTO*	16:52355409	0.8398(0.5472 ,1.1324)	G/C	1.85E-08
rs9923147	*FTO*	16:52359050	0.8284(0.5359 ,1.1209)	T/C	2.85E-08
rs11642841	*FTO*	16:52402988	0.8112(0.4982 ,1.1242)	A/C	3.78E-07
rs17817288	*FTO*	16:52365265	0.7074(0.4172 ,0.9976)	C/T	1.77E-06
rs3769671	*POMC*	2:25243657	1.3105(0.6416 ,1.9794)	G/T	0.000123
rs8047395	*FTO*	16:52356024	0.5261(0.2356 ,0.8166)	A/G	0.0003865
rs11075987	*FTO*	16:52372662	0.5297(0.2368 ,0.8226)	G/T	0.0003935

Supplementary Table 4: SNPs associated with HC in EpiDREAM cohort

HC SNP	Gene	Chrom. Location	Effect Size	Risk Allele	p-value
rs12149832	*FTO*	16:52400409	0.932 (0.6489,1.2151)	A/G	1.09E-10
rs11075989	*FTO*	16:52377378	0.9055 (0.6288,1.1822)	A/G	1.42E-10
rs7185735	*FTO*	16:52380152	0.9047 (0.628,1.1814)	G/A	1.47E-10
rs9939609	*FTO*	16:52378028	0.9033 (0.6266,1.18)	A/T	1.57E-10
rs3751812	*FTO*	16:52375961	0.9282 (0.6434,1.213)	T/G	1.68E-10
rs9941349	*FTO*	16:52382989	0.9027 (0.6246,1.1808)	T/C	1.99E-10
rs9922708	*FTO*	16:52388647	0.8688 (0.5934,1.1442)	T/C	6.31E-10
rs1421085	*FTO*	16:52358455	0.8684 (0.5858,1.151)	G/A	1.71E-09
rs7193144	*FTO*	16:52368187	0.8381 (0.5615,1.1147)	C/T	2.89E-09
rs17817449	*FTO*	16:52370868	0.8257 (0.5486,1.1028)	G/T	5.21E-09
rs1121980	*FTO*	16:52366748	0.7482 (0.4771,1.0193)	A/G	6.30E-08
rs9923147	*FTO*	16:52359050	0.7206 (0.4492,0.992)	T/C	1.95E-07
rs7206790	*FTO*	16:52355409	0.7041 (0.4326,0.9756)	G/C	3.71E-07
rs11642841	*FTO*	16:52402988	0.7376 (0.4473,1.0279)	A/C	6.33E-07
rs17817288	*FTO*	16:52365265	0.5925 (0.3233,0.8617)	C/T	1.60E-05
rs11075987	*FTO*	16:52372662	0.5434 (0.2717,0.8151)	G/T	8.85E-05
rs1729998	*RSRC1*	3:159410718	0.5259 (0.2466,0.8052)	G/A	0.0002236

7.0 References

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