From Department of Molecular Medicine and Surgery
Karolinska Institutet, Stockholm, Sweden

GENETIC EPIDEMIOLOGY OF ADIPOSITY AND ABNORMAL GLUCOSE TOLERANCE IN SOUTH ASIAN INDIANS

Senthil K Vasan

Karolinska Institutet

Stockholm 2013

Published by Karolinska Institutet. Printed by Universitets service US-AB

ISBN 978-91-7549-230-8

But I have promises to keep,
And miles to go before I sleep,
And miles to go before I sleep.
Robert Frost.

To
My appa and amma

ABSTRACT

Genetic factors play a substantial role in susceptibility to common diseases such as type 2 diabetes (T2DM) and obesity-related disorders. The current investigation was undertaken to examine the association of genetic variants that indirectly affect diabetes-related traits through their effects on birth weight and adiposity in South Asian Indians. Additionally, the thesis was extended to examine the plausibility of differences in body composition in Asian Indians, compared to the West, as a contributing factor to premature cardio-metabolic risk in Indians.

The ***first study*** investigated the association of two birth-weight lowering genetic variants in the *ADCY5* and near *CCNL1* locus with birth weight and adult glycemic traits. Although the significant associations between both genetic variants and birth weight as observed in Western cohorts were not seen, the *ADCY5* variant displayed an association with increased glucose and decreased fasting insulin response, which supports the fetal-insulin hypothesis proposing a common genetic factor linking birth weight and adult T2DM traits. Recent GWAS have shown that associations of certain variants with birth weight is secondary to their effects on adiposity, and the strong link between obesity and T2DM stimulated further examination of the effect of genetic variants on obesity and diabetes-related traits. In ***Paper II***, it was confirmed that the common *FTO* variant exerts an effect on obesity traits in adult Indians similar to that in Caucasians. A subsequent investigation (***Paper III***) of the effects of *FTO* and *MC4R* in adolescents (mean ± SD age, 17.1 ±1.9 years) demonstrated an association with waist-hip ratio, providing evidence that the preferential accumulation of central fat in Indians is regulated by *FTO* in younger age groups. This could be a constitutional Asian Indian effect, and underpins the importance of ethnic-specific differences in fat distribution. These *FTO* associations with obesity and T2DM traits in adults were further strengthened by a meta-analysis of ~23,000 Asian Indians (***Paper IV***), in which a consistent obesity-related effect was observed, in addition to an effect on T2DM which seemed to be partially mediated through body mass index (BMI). The strong association of *FTO* with waist circumference and subcutaneous adipose tissue (SAT) proxies led to an investigation of the differences in abdominal adiposity depots in Indians compared to Caucasians, using dual energy X-ray absorptiometry (DXA) as described in ***Paper V***. Compared with a BMI-, gender- and age-matched Western cohort, Asian Indians exhibited relatively less visceral fat accumulation and more subcutaneous fat, reinforcing that ethnic differences in fat distribution need to be appreciated when analyzing the connection between obesity and T2DM.

In conclusion, the effect of *ADCY5* on glucose regulation confirms that impaired glucose homeostasis in adulthood is related to birth weight mediated through mechanisms that involve fetal-insulin signaling. Regarding genetic effects on adiposity, the effect estimate of the *FTO* locus on obesity and diabetes-related traits in Asian Indians is similar to that in Western ethnicities. The relative absence of differences in visceral adiposity, and a predominance of subcutaneous fat accumulation along with lower muscle mass and higher overall body fat in Asian Indians, provides novel insights to investigate the role of specific adipose depots and skeletal muscle in relation to heightened cardio-metabolic risk in this population.

LIST OF PUBLICATIONS

I. **Vasan SK**[#], Neville MJ, Antonisamy B, Samuel P, Fall CH, Geethanjali FS, Thomas N, Raghupathy P, Brismar K, Karpe F. Absence of birth-weight lowering effect of *ADCY5* and near *CCNL*, but association of impaired glucose-insulin homeostasis with *ADCY5* in Asian Indians. PLoS One. 2011;6(6):e21331.

II. **Vasan SK**[#], Fall T, Neville MJ, Antonisamy B, Fall CH, Geethanjali FS, Gu HF, Raghupathy P, Samuel P, Thomas N, Brismar K, Ingelsson E, Karpe F. Associations of variants in *FTO* and near *MC4R* with obesity traits in South Asian Indians. Obesity (Silver Spring). 2012 Nov; 20(11):2268-77.

III. **Vasan SK**[#], Fall T, Job V, Gu HF, Ingelsson E, Brismar K, Karpe F, Thomas N. A common variant in the *FTO* locus is associated with waist-hip ratio in Indian adolescents. Pediatr Obes. 2013. Jun; 8(3): e45-9.

IV. **Vasan SK**[#], Karpe F, Gu HF, Brismar K, Fall CH, Ingelsson E, Fall T. *FTO* genetic variants and risk of obesity and type 2 diabetes: a meta-analysis of 28,394 Indians. (*Accepted Obesity 2013*).

V. **Vasan SK**[#], Marinou K, Humphreys S, Karpe F. Comparison of abdominal adiposity in Asian Indians and Caucasians. (*Manuscript*).

#corresponding author

OTHER PUBLICATIONS NOT INCLUDED IN THESIS

I. **Vasan SK**, Thomas N. Developmental origins of adult metabolic disease: The Indian scenario, driving toward a unified hypothesis. Indian J Endocrinol Metab. 2012 Jul;16(4):493-5.

II. Marinou K, Hodson L, **Vasan SK**, Fielding BA, Banerjee R, Brismar K, Koutsilieris M, Clark A, Neville MJ, Karpe F. Functional differences between deep and superficial subcutaneous abdominal adipose tissue. Impact on cardiovascular risk profile (*Submitted*).

III. Shen C, Sharma M, Reid DC, Li P, Celver J, Seman NA, Chen J, **Vasan SK**, Wang H, Gu T, Liu Y, Mohamud WNW, Shen H, Brismar K, Fairbrother WG, Kovoor A, Gu HF. An I/D polymorphism in the RGS9 gene is associated with obesity but not with type 2 diabetes (*Submitted*).

IV. **Vasan SK**, Karpe F. Total body adiposity, fat distribution and their relationship to metabolic complications of obesity in Asian Indians (*Manuscript*).

V. **Vasan SK**[#], Sandhya GI, Thomas N, Selvakumar R, Muliyil J. A 15-year follow-up of fasting blood glucose and diabetes risk prediction: Results from a population-based non-concurrent cohort from rural South India. (*Submitted*).
#corresponding author

CONTENTS

LIST OF ABBREVIATIONS

ADCY5	Adenyl cyclase 5
ANOVA	Analysis of variance
BMI	Body mass index
CCNL1	Cyclin L1
CT	Computed tomography
CVD	Cardiovascular disease
CNV	Copy number variation
DNA	Deoxyribonucleic acid
DXA	Dual energy X-ray absorptiometry
FTO	Fat mass and obesity-associated gene
FIH	Fetal-insulin hypothesis
GWAS	Genome-wide association study
HC	Hip circumference
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
IS	Insulin sensitivity
ISI	Insulin sensitivity index
IMTG	Intra-muscular triglycerides
LA	Linkage analysis
LBW	Low birth weight
LD	Linkage disequilibrium
LDL	Low density lipoprotein
MA	Meta-analysis
MC4R	Melanocortin-receptor 4 gene
MODY	Maturity onset diabetes of the young
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PI	Ponderal Index
RNA	Ribonucleic acid
SAT	Subcutaneous adipose tissue
SFT	Skin fold thickness
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
US	Ultrasonography
VAT	Visceral adipose tissue
WC	Waist circumference
WHR	Waist-hip ratio

1 BACKGROUND

The ongoing world-wide obesity and diabetes epidemic, especially in developing countries such as India, has spurred researchers to investigate potential factors that contribute to the increase disease risk in different populations. Variability in disease susceptibility is attributed to differences in the genetic architecture, environmental interactions and body composition across various ethnic groups. Asian Indians are intrinsically different from the Western population in certain distinct phenotypic characteristics such as lower birth weight (LBW), hyperinsulinemia since birth and increased adiposity at any given body mass index (BMI) (1-3). It is unclear as to which factor actually confers increased risk for cardio-metabolic disease in Asian Indians as all three factors appear to be mutually exclusive and complement one another. The current thesis was therefore undertaken to investigate one possible mechanism that relates specifically to genetic variants that modulate disease risk at three levels: birth weight, adiposity and early alterations in glucose-insulin homeostasis. Such genetic variants that have an effect on one or more of these traits will influence the other(s), thereby acting as a common connecting link between all traits. To complement the genetic findings, the thesis additionally examined differences in body composition, specifically relating to abdominal adiposity, in migrant Asian Indians, compared to Caucasians.

1.1 GENETICS

1.1.1 The Human Genome

The human genome is packaged into 23 pairs of chromosomes within the nucleus of cells, with a minor proportion also found in the mitochondria. The genome comprises of approximately 2.9 billion nucleotides, and includes both the coding genes and the non-coding genome (4). The coding genes contribute to approximately 1.5% of the entire genome, and are made up of nucleotide codons that undergo transcription and translation to form peptides or proteins (5). The non-coding genome contains sequences of diverse function and includes noncoding RNA (transfer RNA and ribosomal RNA), pseudogenes, introns, untranslated regions of mRNA, regulatory DNA sequences, microRNAs, repetitive DNA sequences and sequences related to mobile genetic elements (5-7).

1.1.2 Genetic variation

Both the coding genes and the non-coding genome determine the phenotypic characteristic of an individual (8). However, the majority (above 90%) of the trait-associated variants identified by genetic studies resides within the non-coding genomic sequences (9). Variation within the genome can be identified as single nucleotide polymorphisms (SNPs), insertions, deletions, copy number variations (CNV) and chromosomal rearrangements (10). Indeed, not all genetic variation results in changes in either protein structure or gene regulation, and may not necessarily contribute to the differences in phenotypic characteristics (8, 11). Genomic similarity within humans has been estimated to be about 99.9%, which means that only about 0.1% of the genome sequence is different between individuals (9, 12). However, this 0.1% of the total sequence harbors more than 2 million variants, and these variations confer genetic risk

to common diseases (13). Identification of these genetic variants therefore is important, as they provide mechanistic insights into pathways of disease development.

1.1.3 Monogenic disease

Monogenic diseases occur due to a single mutation in one or both alleles and exhibit a familial clustering with a clear inheritance pattern (14). Maturity onset diabetes of the young (MODY) is a classical monogenic form of diabetes that results from mutations in genes that are expressed in the pancreatic β-cells, or genes that regulate the the insulin secretion pathway (15, 16). Other forms of monogenic diabetes include neonatal and mitochondrial diabetes (17). Rare functional variants in genes involved in the leptin-melanocortin pathway such as leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*), pro-hormone convertase subtilisin/kexin type 1 (*PCSK1*) and the melanocortin receptor-4 receptor (*MC4R)* have been shown to cause severe early-onset monogenic obesity (18).

1.1.4 Genetics of complex disease (Polygenic disease)

Polygenic or complex diseases are diseases that result from the interaction of several genes, each exerting a minor contribution to overall disease risk, with or without the involvement of non-genetic factors (19). Complex disease genetics are studied either by an association approach, in which the frequency of risk alleles or genotypes are compared between cases and controls, or by a genome wide approach, in which most of the genome is surveyed for associations with a phenotypic trait of interest (20, 21). Genetic variants that underlie complex traits usually have subtle effects on disease risk and therefore require large sample sizes to determine the true magnitude of the effect on the trait (21, 22). The usual conclusion derived from such studies is that a detected polymorphism either affects the disease/trait directly, or is a marker of a nearby causal variant that affects disease risk (23).

1.2 GENETIC EPIDEMIOLOGY

Genetic epidemiology facilitates the design and implementation of studies aimed at identifying and characterizing genes that influence phenotypic traits (24). This involves characterization of the effect of genetic variants at a population level by exploring the correlations with trait using two main strategies: i) candidate-gene approach; ii) genome-wide association approach (25).

1.2.1 Candidate gene studies

The candidate gene approach is a simple, straight forward, hypothesis-driven method employed to test the association of a particular genetic variant (allele) with a disease/trait (26). The basis of selection of candidate genes depend on their role in the biological pathways / mechanisms related to phenotype of interest or on their location in previously determined region of linkage. An increased frequency of the risk-determining allele in a population infers that either a causal relationship exists between the genetic variant and the phenotypic trait (disease), or that the variant is in linkage disequilibrium (LD) with the disease gene near the locus in question (27). Candidate gene studies are potentially powerful and represent the most commonly employed method to identify genetic variants that influence disease susceptibility. However, they are plagued by certain limitations such as small sample size, limited number of variants assayed, and the generation of false-positive associations, all contributing to the

inconsistencies in reproducibility between populations (20, 27-29). In the recent past, issues concerning the power of genetic association studies has been tackled by either using larger sample sizes, or by combined meta-analysis of well-designed smaller studies to understand the true magnitude of genetic effect (30, 31).

1.2.2 Genome wide association studies (GWAS)

Genome wide association studies (GWAS) represent a powerful new tool to investigate the genetic architecture of complex diseases. This is a hypothesis-free approach where a dense set of SNP markers (~0.1-5 million) spanning the entire genome is scanned in thousands of individuals, using high-throughput genotyping platforms (32). The design of GWAS is based on the 'common disease, common variant hypothesis', which considers that common diseases are attributable to allelic variants present in more than 1-5% of the population (10). The sequencing of the human genome (33), and the availability of databases of SNPs such as dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), and the International HapMap (http://hapmap.ncbi.nlm.nih.gov/), which provide information on >2.5 million SNPs, have allowed early GWAS to study common variants (minor allele frequency: MAF >1%) that associate with disease. More recently, the 1000 genome project (http://www.1000genomes.org/), which includes more than 37 million variants including both rare and low frequency variants (MAF <0.5% and 0.5-5%, respectively), is expected to increase the success of GWAS in identifying associations of such rare variants in complex disease etiology. The GWAS approach includes a discovery and a replication stage. They resolve genome wide associations at stringent significance levels ($P \leq 5 \times 10^{-8}$), after applying an appropriate correction factor for multiple testing (34). This cut-off is based on the estimated number of significant tests in the genome if all common SNPs in the HapMap were tested either by direct genotyping or by imputation (21). The GWAS approach has no *a priori* assumptions about genes and therefore has been successful in identifying several novel variants that associate with complex disease, but mostly in Caucasians. Large-scale obesity-related GWAS in indigenous Asian Indians are sparse, whilst fewer studies have included migrant Asian Indian populations (35-37). In such migrant populations, the presence of confounding factors such as population admixture and environmental effects have not been dealt with adequately. Therefore, a need to conduct *de novo* studies within Asian Indians is important to confirm the genetic associations that have emerged as strong signals in other ethnicities and also identify novel variants specific to this population. This would provide insights into understanding the genetic contribution to complex diseases that are specific to Asian Indians.

1.3 OBESITY / ADIPOSITY

Obesity results from excessive accumulation of body fat within the subcutaneous and visceral compartments of the human body. With increasing adiposity, enlarged dysfunctional adipocytes within the subcutaneous region lose their ability to appropriately store excessive fat, resulting in spill-over of fat and subsequent accumulation in ectopic compartments such as liver and skeletal muscle (38). The excessive fat at these ectopic sites disrupts normal metabolic signaling, leading to obesity-related complications (39).

1.3.1 Obesity epidemiology

Obesity is a growing world-wide epidemic, and estimates of its prevalence are rapidly increasing both in developing and developed countries. The International Obesity Task Force (IOTF) and the World Health Organization (WHO) report that over 1.1 billion adults worldwide are overweight, of which 312 million are obese (40). The obesity prevalence in adults increasing obesity estimates in childhood, with worldwide prevalence estimated to be 6.7% in 2010 (Global Infobase WHO, May10, 2010). Over the past 20 years, obesity rates have tripled in the developing countries, reflecting substantial changes in behavioral patterns which include adoption of characteristics of a Western lifestyle (decreased physical activity and overconsumption of inexpensive, high calorie foods and drinks) (41). The exact prevalence of obesity among Asian Indians is unknown, but the increase in obesity-related complications in this population is attributed to a higher proportion of body fat, specifically in the abdominal compartment (42, 43).

1.3.2 Obesity and related complications

Obesity is associated with a number of metabolic consequences including hyperinsulinemia, insulin resistance (IR), type 2 diabetes (T2DM), dyslipidemia, hypertension, cardiovascular disease (CVD), and predisposes to higher risk for premature mortality (44-46) including deaths related to cancer (47). Obesity often leads to dysregulation of endocrine, neural and inflammatory pathways that contribute to the development of IR. Low-grade chronic inflammation leading to abnormal inflammatory profile is often observed in the adipose tissue of obese individuals, and are usually associated with adipocyte hypertrophy (increased size) caused by storage of excessive lipids (48) and/or adipocyte hyperplasia (increase in number) (49, 50). This in turn affects pathways such as insulin signaling in target peripheral tissues, leading to a cascade of events that perpetuate metabolic consequences (51, 52).

1.3.3 Subcutaneous and Visceral adiposity

The abdominal fat compartment includes both subcutaneous and visceral adipose tissues (SAT and VAT, respectively). The SAT compartment is further subdivided anatomically into the superficial subcutaneous fat (sSAT) and deep subcutaneous fat (dSAT), separated by a thin facial plane called the scarpia fascia (53). Both SAT and VAT are heterogeneous and differ in their morphology and metabolic characteristics (54). Numerous studies have convincingly reported the association of VAT with metabolic abnormalities such as glucose intolerance, hyperinsulinemia and dyslipidemia in individuals matched for total body fat (55, 56) and SAT (57, 58). However, recently it was shown that SAT correlates with metabolic abnormalities to the same magnitude as VAT (59-61). Although, there is sufficient evidence to support the concept that adiposity *per se* is related to metabolic complications, the relative importance of the different fat depots remains highly debated. Anthropometric measurements such as waist circumference (WC) measure total abdominal adiposity including both SAT and VAT, while skin fold measurements reflect SAT at measured sites. Quantification of abdominal adiposity volumes involving either a single slice (L1-L2 or L3-L4) or sequential abdominal imaging of the abdomen using computed tomography (CT) (62) or magnetic resonance imaging (MRI) (63) have provided a better understanding of relative contributions of adiposity depots to metabolic characteristics. Recently, ultrasound and dual energy X-ray absorptiometry (DXA)

scans (64) have gained popularity for quantification of visceral depots, owing to their ease of use and minimal radiation exposure.

1.3.4 Assessment of obesity phenotypes

Numerous epidemiological studies provide strong evidence for an association between higher BMI and increasing morbidity and mortality, albeit differences in the strength of the associations between various ethnic groups. This has led to ethnic-specific BMI cut-offs for defining obesity (65, 66). Although BMI is the most frequently-used indicator of fatness both in clinical and population studies, its use in defining obesity lacks precision due to its inability to differentiate between fat and muscle mass (67, 68). Also, BMI does not account for heterogeneity of regional body fat deposition. WC, an indicator of abdominal fatness, has also shown a strong association with cardiovascular risk (69, 70). Measurement of WC is particularly important in certain ethnic groups such as Asian Indians, who have a greater predilection to central obesity. The ratio of abdominal waist over hip circumference (WHR) is another index of body fat distribution, and has shown higher predictive ability for cardiovascular and T2DM risk (69). The superiority of one measurement over the other is debated. Nevertheless, BMI measures overall fatness, while other measurements are more specific and reflect regional adiposity.

Table 1: Cardio-metabolic risk cut-offs, based on anthropometric measurements in Indians and Caucasians

Anthropometry	Indians	Caucasians
Body Mass Index (kg/m^2)		
Normal	< 22.9 kg/m^2	≤ 24.9 kg/m^2
Overweight	23-24.9 kg/m^2	25-29.9 kg/m^2
Obese	≥ 25 kg/m^2	≥ 30 kg/m^2
Waist circumference (cm)		
Men	90 cm	102 cm
Women	80 cm	88 cm
Waist-Hip ratio		
Men	0.88	0.90
Women	0.80	0.85

Skin fold thickness (SFT) measured at various sites reflects regional subcutaneous adipose tissue (SAT) (71), and provides information on changes in the distribution of body fat (central and peripheral fat). SFT is also used in the estimation of body fat percentage (BF%) by employing standard prediction equations (72, 73). All anthropometric markers are indirect measures of adiposity and do not quantify fat mass (SAT+VAT). Approaches which quantify adipose tissue volume/masses empirically, such as CT, MRI or DXA scans, are gaining more popularity in obesity research.

1.3.5 Genetics of obesity

Family and twin studies have shown that 40-70% of the inter-individual variation in common obesity is attributable to heritable factors (74). This clearly demonstrates that body weight has a large underlying genetic component which determines an

individual's susceptibility to weight gain. Studies have also reported the heritability estimates of obesity-related traits to be as follows: BMI (50-80%) (74, 75), WC (46-90%) (76, 77), WHR (6-61%) (77, 78), which further strengthens the understanding that numerous genes could impact the expression of obesity-related phenotypes. From a genetic stand point, obesity is classified as monogenic, syndromic and polygenic obesity. Variants associated with monogenic obesity, such as *MC4R* and brain-derived neurotropic factor (*BDNF*), have also shown an association with common obesity (79, 80) The candidate gene approach to identify variants associated with obesity has largely been unsuccessful, whilst GWAS have successfully identified several novel variants that associate with obesity and related traits. Insulin-induced gene 2 (*INSIG2*) was the first locus to be reported in obesity-focused GWAS (81), but replication efforts were inconsistent. Variants in the fat mass and obesity-associated (*FTO)* gene, and variants near the *MC4R* locus, have demonstrated the most robust association with obesity traits, along with consistent replication in several ethnic groups (79, 82). GWAS that followed identified several novel loci that are associated with obesity-related quantitative traits. Employing data from 61 genome wide scans, the Human Obesity Gene Map has reported the identification of 127 candidate genes and 253 quantitative trait loci linked and/or associated with obesity-related phenotypes (83), many of which have only a poorly understood function. The obesity variants identified so far, are usually genes that regulate one or more of the following processes: energy homeostasis and thermogenesis, appetite regulation, adipogenesis, and leptin or insulin signal transduction (84), suggesting that that a majority of such genes are associated with hypothalamic function. However, the combined effect of the variants identified by GWAS accounts only for ~1-2% of genetic variation in BMI due to lower effect sizes (85). The remainder of the unexplained variation, or 'missing heritability', is thought to be explained by genetic variation other than the common DNA variants, such as gene-gene interactions, gene-environment interactions (86), copy number variants (CNV) (87), low frequency genetic variants with larger effect sizes and epigenetic factors (88). The current challenge remains to identify these new variants and understand the underlying biological mechanisms of these loci in conferring obesity risk. Combined approaches using molecular genetics, sophisticated techniques in quantifying adiposity and functional studies would provide a more vivid understanding of common obesity.

1.4 TYPE 2 DIABETES

T2DM is a heterogeneous disorder of carbohydrate and lipid metabolism and progresses through stages of normal glycaemia to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and subsequent T2DM. It is caused by both genetic and environmental factors, of which obesity is overwhelmingly the most important intermediate trait. The life time risk of developing T2DM if one parent has T2DM is about 40%, and this increases to 70% if both parents are affected (89, 90). The concordance rate of T2DM in monozygotic and dizygotic twins is reported to be 70% and 30%, respectively (91, 92).

1.4.1 Epidemiology and pathogenesis

T2DM is now emerging as a pandemic, with an increase in world-wide prevalence from 194 million in 2003 to an estimated 439 million cases by the year 2030 (93). The increase is likely to be most evident in developing countries due to population growth, aging, rapid urbanization, an unhealthy/hyper-caloric diet and a sedentary life style

leading to obesity (94). Estimates based on population growth and rapid urbanization show that India and China will be world leaders in the number of patients with T2DM by 2030, corresponding to 79.4 million and 42.3 million individuals, respectively (95). Obesity leads to insulin resistance (IR) and the inability of the peripheral tissues (muscle, liver and adipose tissue) to respond to physiological concentrations of insulin. The pancreatic β-cell is unable to compensate for the increasing demands for insulin secretion, and a relative deficiency in glucose-mediated insulin release is the main mechanism involved in the pathogenesis of T2DM (96).

1.4.2 Assessment of diabetes related traits

Markers of reduced insulin sensitivity (IS) and insulin secretion are often used as proxy measurements in population-based studies. Studies based on twin and family studies have reported heritability estimates for IS and insulin secretion to range from 50-78% and 47-59%, respectively (96, 97). Direct measure of insulin sensitivity can be carried out either by assessment of glucose disposal during a hyperinsulinemic-euglycemic clamp, or by an insulin suppression test. Insulin secretion may be measured either during the hyperglycemic clamp or by frequent sampling during an intravenous glucose tolerance test (98). Since these tests are laborious, time consuming and require hospital admission of patients, they are not suitable for epidemiological studies. Therefore, indirect measures of IS/IR and insulin secretion derived from fasting insulin and glucose concentrations, or from repeated sampling following an oral glucose tolerance test (OGTT), are often used in field studies.

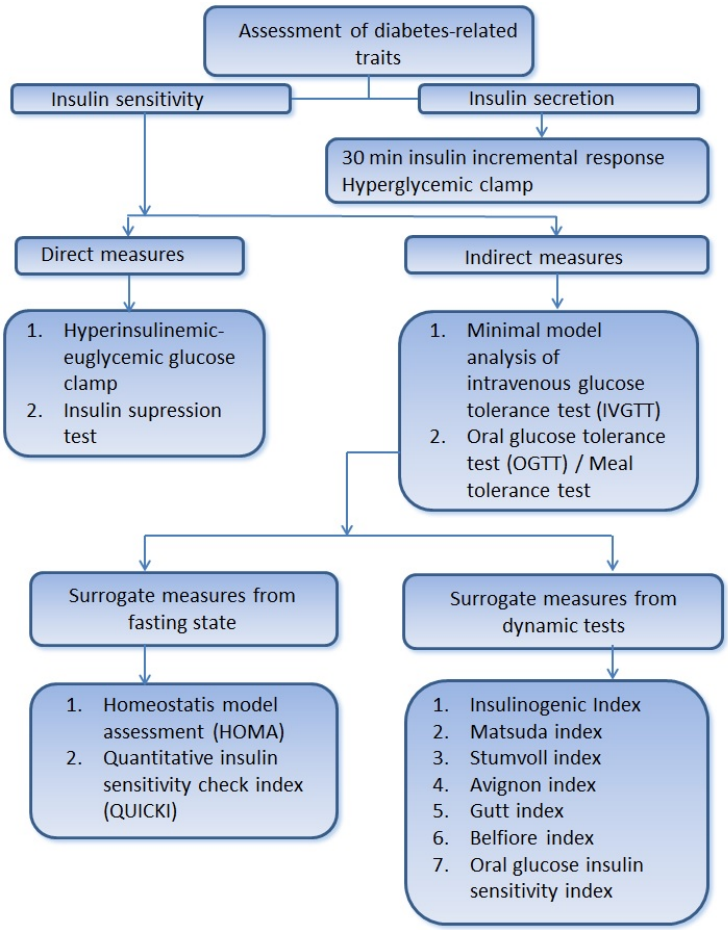


Figure 1: Flow chart showing methods used in the assessment of diabetes-related traits

The commonly used surrogate measures derived from OGTT that were used in the current investigation are discussed subsequently.

1.4.3 Genetics of type 2 diabetes

Genetic variants associated with T2DM usually affect pathways that involve β-cell function, adiposity or insulin resistance *per se* (99). By linkage analysis two genes, namely calpain 10 (*CAPN10*) (100) and transcription factor 7-like 2 (*TCF7L2*) (98) were identified as T2DM associated genes (101). Associations with T2DM of functional or positional candidate genes have also been extensively reported, but so far only 6 genes have shown consistent associations; these include the Pro12Ala variant in the peroxisome proliferator-activated receptor gamma (*PPARG*) gene (102), insulin receptor substrate 1 (*IRS1*) (103), Glu32Lys variant in the potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) (104), Wolfram syndrome 1 (*WFS1*) (105), and HNF1 homeobox A (*HNF1A*) and B (*HNF1B*) (103). The first GWAS on T2DM identified hematopoietically-expressed homeobox (*HHEX*) and solute carrier family (Zinc transporter) member (*SLC30A8*) as being associated with T2DM (106). Subsequent GWAS reported robust associations for cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A/CDKN2B*), insulin-like growth factor 2 binding protein 2 (*IGFBP2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKALI*) and *TCF7L2* with T2DM (103, 104, 107). Several GWAS and large scale meta-analyses thereof confirmed these signals and additionally identified new loci associated with diabetes and related glucose-insulin traits both in European (106, 108, 109) and non-European ethnicity (110, 111). Currently over 60 loci have been associated with T2DM and related traits (112). However, the combined effect of these variants explains only 5-10% of disease risk, since most of the identified variants demonstrate very modest effect sizes ranging from 1.05 to 1.15; the only exception being *TCF7L2*, which has an odds ratio of 1.37 (113). Whether variants included in the 'missing heritability' concept could explain the remaining risk is highly debated, as for any other complex disease. Nevertheless, these variants have provided great insights into the understanding of the pathogenesis that underlies T2DM.

1.4.4 Diabetes and obesity - connecting link

The link between obesity and T2DM involves two main mechanisms: IR and β-cell failure. Obesity that results from an imbalance between energy intake and expenditure, leads to alterations in several pathways involving inflammatory signaling (114), endoplasmic reticulum stress (115), increased production of reactive oxygen species, mitochondrial dysfunction (116) and increased accumulation of triglycerides and free fatty acids (117). These mechanisms may not be mutually exclusive, and are usually inter-linked, with one leading to another. The net result of such alteration is increased free fatty acids in circulation leading to fatty acid overload in the liver and skeletal muscle. This results in decreased responsiveness of the peripheral tissues to insulin leading to IR (52). Genetic factors may additionally predispose an individual to reduced β-cell capacity which could otherwise compensate for the increasing demands on insulin secretion imposed by the IR. Such progressive β-cell dysfunction may further worsen the situation by causing prolonged increases in the concentrations of glucose, free fatty acids and other substances that lead to cellular injury (117). This in turn may trigger an inflammatory cascade, with the recruitment of macrophages and other

immune cells further exacerbating the inflammatory milieu, thus establishing a vicious cycle (118). Genes implicated in obesity susceptibility such as *FTO*, *MC4R*, transmembrane protein 18 (*TMEM18*), glucosamine-6-phosphate deaminase 2 (*GNPDA2*), SH2B adaptor protein 1 (*SH2B1*) and neuronal growth regulator (*NEGRI1*) have also shown associations with T2DM, thus providing a common genetic link between both disease states (82, 119). Typically, the association between these gene variants and T2DM disappears if appropriate adjustment for obesity is made between cases and controls.

1.5 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHaD)

The DOHaD theory proposes that chronic disease susceptibility in adulthood is influenced not only by the inherent genetic and post-natal lifestyle, but also by the *in utero* environment that the fetus is exposed to (120). The *in utero* environment is governed by maternal factors such as maternal nutrition, placental function, substance abuse, maternal stress and illness during pregnancy. These factors regulate fetal growth through mechanisms that cause permanent structural changes, affect epigenetic programming of gene expression and accelerate cellular ageing (121). As a consequence of these effects, the embryo/fetus, being highly sensitive to the perturbations of the maternal environment, undergoes alterations during critical periods of development which have a long-term health impact during adulthood. The developmental origins theory can be linked back to the 'thrifty phenotype' hypothesis proposed by Barker and colleagues, who demonstrated a relationship between birth weight (as a proxy measure of adverse fetal environment) and adult disease. These authors concluded that sub-optimal fetal nutrition during critical time points of intrauterine development may cause permanent alterations in fetal structure, function and metabolism, due to skewed developmental programming (122, 123). These theories are not mutually exclusive, but complement one another. Most epidemiological studies have relied on 'birth weight' as a proxy indicator of *in utero* nutrition. However, the relative contributions of birth weight and subsequent body size to adult disease vary between specific disease markers and between populations, with some ethnic groups demonstrating weak or no significant relationship and other populations showing a stronger relationship (124-127).

1.5.1 Fetal-insulin hypothesis

The fetal insulin hypothesis (FIH) proposed by Hattersley and Tooke (2002) states that genetic variants that associate with glucose-insulin homeostasis may cause impaired insulin-mediated growth prenatally, eventually resulting in low birth weight and adverse metabolic outcomes in adulthood (128). Genetic variants in the glucokinase (*GCK*) gene which are associated with monogenic diabetes were the first to be shown to influence birth weight via maternal genotype (129). The studies that followed provided evidence for more common variants that associate with T2DM to influence birth weight. For example, polymorphisms in the insulin-like growth factor–1 (*IGF-1*) gene promoter (130) and variants in the *CDKAL1* and *HHEX-IDE* loci (131, 132) were shown to influence birth weight via the fetal genotype. This birth weight lowering effect was modulated through altered pancreatic β-cell function and reduced insulin secretion, supporting the fetal-insulin hypothesis. Such evidence has stimulated further

research to investigate common genetic determinant which could explain multiple phenotypes such as low birth weight and adult T2DM. This would be particularly important in vulnerable populations such as Asian Indians, who are born with lower birth weight and also have a higher risk for T2DM and CVD. Such studies could provide a possible explanation for the ongoing obesity and T2DM epidemic in India.

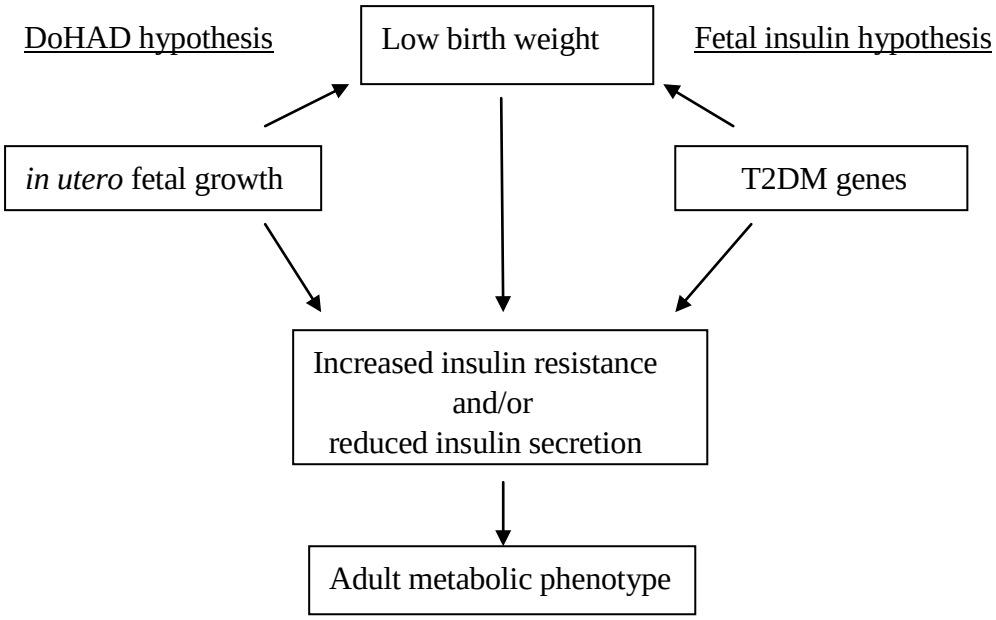


Figure 2: Proposed causal pathway that explain the association between low birth weight and adult metabolic phenotype. (DoHAD: Developmental origins of health and disease)

1.5.2 Genetics of low birth weight

Birth weight heritability estimates vary between 10-40% (133) and are determined by fetal and maternal genes as well as by the intra-uterine environment. To date, two genetic variants (*ADCY5* and *CCNL1*) identified by GWAS have shown robust associations with birth weight via the fetal genotype (134). Other birth weight - modulating variants include, firstly, *CDKAL1* and *HHEX-IDE*, which appear to operate via the fetal genotype, in support of the fetal insulin hypothesis (131), and secondly *GCK* and *TCF7L2*, which appear to act via the maternal genotype (135). Genetic variants that influence birth weight through maternal genotype probably mediate fetal growth by a primary effect on maternal glucose levels, increasing fetal insulin secretion and subsequently increased fetal growth. Besides evidence of association between birth weight and T2DM (*ADCY5* and *CDKAL1*), there is accumulating evidence of such associations with other adult phenotypic traits such blood pressure (*ADRB1*) and height (*HMGA2* and *LCORL)* (136). However, these variants explain only 0.76% of the variance in birth weight amongst Europeans, and 0.32-1.52% of the variation in populations of Middle Eastern, Southeastern Asian and African origin, leaving ample room for non-genetic explanations.

1.5.3 Non-genetic determinants of birth weight

Studies of non-genetic factors that influence birth weight primarily focus on two important events: maternal nutrition during pregnancy and *in utero* epigenetic fetal

programming. Recent reviews on animal models have summarized extensively the role of maternal nutrition, maternal hypoxia and/or placental insufficiency in fetal growth and development (137, 138). Yajnik *et al*. showed that higher folate intake in vitamin B12-deficient mothers increases T2DM risk in off-spring, secondary to increased lipogenesis, increased accumulation of odd-chain fatty acids and greater adiposity (139). The role of epigenetic regulation of differential gene expression (140), particularly in relation to T2DM genes such as *PPARα* (141) and *HNF4α* (142), provides a newer understanding of the role of epigenetic mechanisms involved in modulating birth weight and subsequent disease. Studies in humans have also shown that methylation of the imprinted *IGF2* gene, and of genes implicated in early growth and metabolism in individuals exposed to the Dutch famine in early life, as possible explanations of adult chronic disease susceptibility (143, 144). Therefore, non-genetic determinants of birth weight appear to play a role which is as equally important role as that of genetic factors which link fetal origins and adult disease.

1.6 ETHNICITY AND IMPACT ON DISEASE

Ethnic differences in non-communicable disease susceptibility are attributed to differences in body composition, genetic background and environmental influences, all of which determine metabolic capacity (145, 146). The unique phenotypic characteristics of Asian Indians and the relationship to chronic diseases provide valuable evidence that ethnic-specific differences in body composition have important role in chronic disease risk (147-150).

1.6.2 Ethnic variation in genotypes and differential disease risk

The phenotypic expression of a certain trait/disease differs among various ethnic groups, but the reasons for this are unclear. Differences in the DNA sequence variation, gene expression (151), allele frequencies (152, 153), haplotype structure (154) and genetic effect *per se* across populations (155) provide clues that genetic determinants may not impart the same population-attributable risk across different ethnic groups (110, 156). For example, results from the Indian Genome Variation consortium show that a high level of genetic divergence is observed even within Indian subgroups; this divergence clusters on the basis of ancestral descent and language and is quite distinct from HapMap populations (157, 158). Such genetic differences may partially explain the variation in disease susceptibility among Indians and other ethnic groups. These observations also point that gene-disease associations cannot be generalized and need validation across populations of different ethnicity.

1.6.3 Birth weight and adult disease in Asian Indians

A series of studies conducted by Yajnik *et al*. have provided robust epidemiological evidence relating to differences in birth weight, body composition and adult disease risk in Indians compared to Caucasians. Comparison of measurements of new born term Indian and UK-born British babies showed that Indian babies had lower birth weight (2.7 vs. 3.5 kg), and were shorter (47.3 vs. 50.2 cm) and thinner (Ponderal index 24.1 vs. 27.3 kg/cm^2). However, subscapular SFT, which reflects SAT, was comparable (4.2 vs. 4.8 mm). The ‘thin-fat’ phenotype at birth is attributed to lower lean and skeletal muscle mass and to relatively higher adiposity (3, 159, 160). Comparison of pre-pregnancy characteristics of Indian and British mothers provides evidence that Indian mothers are smaller, thinner and consumed much less energy, protein and

micronutrient-rich food (161). Results from the Pune Maternal Nutrition study showed that birth weight in Indians was independent of socio-economic status and nutritional intake during pregnancy, but was predicted by maternal pre-pregnancy weight (160). Put together, this cumulative evidence implies that differences in weight and body composition at birth, and maternal nutritional status during pregnancy, are important determinants of increased disease susceptibility in adulthood, supporting the fetal origins theory. Investigation of the genetic determinants that influence fetal growth through maternal genotype will provide additional clues to the genetic modulation of birth weight and adult disease risk.

1.6.4 Body composition differences in Indians and Caucasians

Body composition includes both the lean (fat-free) and fat masses (162). As explained previously, the distinct body characteristics of Asian Indians is proposed to relate to increased cardio-metabolic risk (67, 163, 164). The metabolic effects attributable to central adiposity in this population is largely reported to be due to visceral fat (165, 166), while recent investigations have demonstrated an excessive SAT contribution to central adiposity (147, 167, 168). Interestingly, glucose disposal has been shown to inversely correlate with fat mass in all compartments of abdominal adipose tissue, suggesting that any given change in abdominal fat correlates with reduced insulin sensitivity in Indians (149). The physiological explanation of ethnicity-related differences in body composition, particularly in adipose tissue, is linked to inherent genetic and epigenetic programming in fat cells to store excess lipids (169).Despite a lower lean mass, it is also shown that South Asians have 30% higher intra-myocellular triglycerides compared to BMI-matched Caucasians, suggesting the presence of dysfunctional skeletal muscle lipid metabolism (170). Both the role of lower skeletal mass in Indians, and its relationship with metabolic risk, needs further investigation.

1.6.3 Genetics of obesity in Asian Indians

The field of obesity genetics in Asian Indians still remains largely unexplored, except for a few independent replications of *FTO* and *MC4R* variants. The largest GWAS on migrant Indians identified a robust association of variants close to the melanocortin-4 receptor (*MC4R*) gene with waist circumference and IR (35). Several independent studies that followed (171, 172), and a recent meta-analysis, have further confirmed this association (173).

Replications of *FTO* in association with obesity traits have been inconsistent in Indians (174-178). A recent GWAS that included migrant Indians in the UK showed an association of *FTO* with BMI, and additionally identified a novel variant in the *IRS1* locus to be associated with obesity-related traits in Indians (37). A meta-analysis by Li *et al.* which included ~97,000 Asians further confirmed that the *FTO*-obesity effect in Asians is the same as that in Caucasians (179). This study, however, combined both indigenous and migrant Indian populations, and a stratified analysis was not performed. Besides these well-validated GWAS signals, few independent studies have reported new variants that associate with obesity traits in Indians, using candidate gene approach; however, these reports were not consistently replicable, and none of the variants detected so far by these studies have shown signals in large scale GWAS of populations from European or Asian countries. Previous studies on obesity genetics in Indians, and results included in this thesis compels some emphasis regarding

investigation of obesity phenotype in Indians: i) it may not be justifiable to combine different ethnicities, even within Asian subgroups, owing to differences in body composition ii) BMI is a composite index of both fat and lean mass and does not reflect adiposity, particularly in Indians, who are centrally obese iii) genetic associations with specific measurements of regional adiposity need further investigation iv) identification of genetic variants that associate with different fat depots, measured using sophisticated techniques, may further provide insights into the role of genetics in the regulation of body weight and fat distribution in Indians.

1.6.4 Genetics of diabetes in Indians

Viswanathan *et al*. showed a strong familial aggregation, amongst most Asian Indians having at least one first-degree relative with T2DM (180). The genetic susceptibility to T2DM has been well studied both by candidate gene and GWAS approach in both indigenous and migrant Indian populations, and common susceptibility variants for T2DM appear to be shared among Indians and other ethnic groups. The protective effect of *PPARG2/ProA12Ala* on IR and T2DM risk was not consistent (181), although in certain sub-populations from North India, a robust association with T2DM was confirmed (176, 182). Several independent attempts to replication replicate some of the well-known GWAS signals in the *KCNJ11*, *CDKALI*, *CDKN2A/B*, HHEX and *BAZ1B* loci among Indians (183-185). A recent GWAS on 12,535 Asian Indians showed that 49/56 previously reported signals showed associations with T2DM which were similar to, and of the same effect sizes as, those observed in other populations; furthermore, a new variant in the 2q21 locus that harbors the *TMEM163, RAB3GAP1* and *ACMSDI* genes was also identified and collectively all these variants explained only about 7.65% of variance in T2DM risk among Indians (111). Such attempts open opportunities to investigate the functional role of newly discovered genes in biological pathways that lead to T2DM and its relevant clinical significance.

2 THE PRESENT STUDY

The effect of genetic variants on phenotypic traits is variable across populations. Although GWAS have helped to identify several genetic variants with disease traits, the results cannot be extrapolated to all ethnic groups due to differences in genetic background, allele frequency, patterns of LD, lifestyle and environmental factors. The validation of positive associations in other independent datasets, and especially those from high-risk ethnic groups, is crucial for identifying true population-specific variants. This would provide an understanding of the magnitude of genetic contribution to specific phenotypic traits in the population of interest. Lower birth weight, hyperinsulinemia, lower muscle mass, greater central adiposity and increased T2DM and CVD risk at any given BMI are intrinsic phenotypic characteristics of Asian Indians compared to the Western populations, and makes this population unique for the study of specific genetic effects in relation to birth weight, adiposity and T2DM.

2.1 AIMS

The overall aim of the current thesis is to investigate the genetic effect of GWAS-identified variants that associate with birth weight and adiposity in South Asian Indians, and also to assess whether these variants influenced glucose-insulin traits. The additionally aimed to study differences in body composition in Indians, compared to Caucasians.

Specific aims:

- To investigate the association of genetic variants (*ADCY5* and *CCNL1*) with birth weight and diabetes-related traits in Asian Indians (***Paper I***).

- To investigate the association of common obesity variants in the *FTO* and *MC4R* loci to obesity-related traits in South Asian Indian adults, and additionally verify their effect on diabetes-related traits (***Paper II***).

- To investigate the association of variants in *FTO* and *MC4R* to anthropometric measurements of adiposity and glycemic traits in Indian adolescents (***Paper III***).

- To estimate the magnitude of the obesity and T2DM effect conferred by *FTO* variants in Asian Indians by meta-analysis of previously published studies (***Paper IV***).

- To assess body composition differences in Asian Indians compared to Caucasians, using DXA. (***Paper V***).

2.2 INVESTIGATION OF GENOME WIDE SIGNALS IN ASIAN INDIANS

The current thesis investigated the association of two birth weight associated genetic variants (*ADCY5* and *CCNL1*), and two obesity associated variants (*FTO* and *MC4R*), with obesity and with diabetes-related traits in a homogenous cohort of individuals recruited from South India.

2.1.1 Adenyl cyclase 5

The *ADCY5* gene is located on chromosome 3 (3q21.1), and encodes a member of the membrane-bound adenylate cyclase enzymes which mediate G protein-coupled receptor signaling. These enzymes are involved in the synthesis of the second messenger cyclic adenosine monophosphate (cAMP). The activity of adenylate cyclases is regulated by post-translational modification, phosphorylation state, the activation of other G-protein coupled receptors and by intracellular calcium signaling, for example through calmodulin. Two GWAS have shown robust associations for rs9883204 in the *ADCY5* locus with birth weight (134, 136), and associations of *ADCY5* variants with T2DM are also clear (104, 186). The association of this variant with birth weight has also been investigated in other populations (131, 187). However, the existence of any similar association has not been explored in Indians. This is of obvious interest, since the average birth weight is very low and the T2DM risk is high in this population. In the current investigation, we chose to study the rs9883204 variant of *ADCY5*, since this variant was shown to be associated with birth weight lowering to the extent of ~30g/allele (95% CI 23-38g) at a genome-wide significance ($P= 7 \times 10^{-15}$) (134). Also, this variant is in strong linkage disequilibrium with another SNP, rs11708067 ($r^2=0.75$), which was previously shown to be associated with T2DM (188).

2.2.2 Cyclin L1

The cyclin L1 gene is located on chromosome 3 (3q25.3), and is a transcriptional regulator which is involved in regulation of pre-mRNA splicing. Cyclin L1 and L2 are members of the cyclin family (serine-rich protein family), and contain arginine and serine-rich domains at their C-termini. They are localized in the compartment of the nucleus which is rich in splicing factors (the so-called 'nuclear speckles'), and are themselves involved in RNA splicing (189, 190). The Early Growth Genetics (EGG) consortium showed that a cluster of SNPs near the *CCNL1* and *LEKR1* genes were associated with low birth weight (~42 g; 95% CI: 35-48g) and reduced PI at birth (134). Ryckman *et al*. showed that rs900400 *(CCNL1)* influenced birth weight in preterm neonates independently of gestational age (187), and this was further confirmed in another independent study by Mook-Kanamori *et al.* (191). Although this robust birth weight-lowering effect was confirmed in another GWAS of Europeans and 11,848 individuals of Asian and African origins (136), the effect of this variant in Asian Indians has not been studied to date. The possible underlying biological mechanisms are yet to be elucidated. The current investigation included analyses of the same SNP of *CCNL1*, rs900400, owing to its robust association with birth weight, as shown in the previous GWAS (134).

2.2.3 Fat mass and obesity associated gene

The *FTO* gene is a nuclear protein of the AlkB-related non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily and is located at 16q12.2 locus (192). *FTO* was first identified as one of the genes within a 1.6 megabase deletion in chromosome 8 and responsible for a fused toe (Ft) phenotype characterized by developmental defects, polydactyly, fused toes and growth retardation (193). The Wellcome Trust Case Control consortium discovered several SNPs within the first intron of *FTO* locus to be associated with BMI in Europeans in a GWAS for T2DM (82). Two GWAS that followed in succession reported three variants in intron 1 of *FTO* to be associated with early-onset childhood and adult obesity (rs1421085 and rs17817449), and with related traits such as body weight and WHR (rs9930506) (194, 195). Subsequently, numerous studies using either GWAS or candidate gene approaches showed consistent associations with *FTO* variants and obesity-related traits in Europeans (119, 196, 197), Africans (198, 199) and Asians (200, 201). However, corresponding studies in an Indian population are inconsistent. The current investigation included an examination of the associations of rs9939609 with obesity-related traits, since this SNP has shown the strongest effect on BMI and a 31% increase in obesity risk. The combined statistical power of association in the aggregate data for *FTO* and BMI is reported to be the most robust for this SNP ($P = 4 \times 10^{-51}$) (82). To date, *FTO* is still the locus with the largest effect size on BMI in the general population. *FTO* is widely expressed across multiple tissues, particularly in the arcuate nucleus of the hypothalamus, and plays a key role in energy homeostasis (202). The mechanisms that underlie the *FTO*-obesity effect have been shown to operate through increased energy intake (203-205), possibly via increased dietary fat, interaction with physical activity (206, 207), and by reductions in satiety (208).

2.2.4 Melanocortin-receptor 4 gene

The *MC4R* gene belongs to the melanocortin receptor family, and is located at the 18q22 locus. The encoded protein interacts with the adrenocorticotropic and melanocyte-stimulating hormone (MSH) receptors, and mediates anorexigenic responses to leptin in the brain (209). Endogenous antagonists include agouti and agouti-related protein (AgRP). Mutations in the coding region of *MC4R* have been implicated in monogenic forms of obesity (18). Recent GWAS have identified a common variant at ~188 kb downstream of the gene to be associated with BMI and obesity traits in adults and children (79). *MC4R* is expressed throughout the brain and in particularly in the hypothalamus, hippocampus and hindbrain regions (210), and possibly mediates an obesity phenotype through an altered balance between food intake and energy expenditure (209). Besides its functional role in obesity, it is shown to be involved in several functional pathways that regulate pigmentation, energy homeostasis, inflammation, immunomodulation, steroidogenesis and temperature control (211). Loos *et al*. demonstrated that a variant near the *MC4R* locus was associated with early-onset obesity, and this was convincingly replicated in 10 studies comprising of 60,352 adults and 5,988 children (79). Another GWAS on migrant Asian Indians showed an association with waist circumference (35). One cross-sectional study among Indian children provided evidence of an obesity association with *MC4R* both at younger ages and in adulthood (172). A recent meta-analysis of 49 studies further confirmed the association of *MC4R* variants with obesity traits (212). The mechanisms by which *MC4R* variants alter obesity risk are unclear, but are likely to alter *MC4R*

function or expression. In the current investigation, we genotyped the rs17782313 variant of *MC4R*, which is the most convincingly replicated *MC4R* variant associated with obesity traits across different populations.

2.3 SUBJECTS

2.3.1 The 'Vellore Birth cohort'

The 'Vellore Birth Cohort (VBC)' was established in 1969-74, with a primary interest to study maternal health and pregnancy outcomes in geographically defined communities in and near Vellore, Tamil Nadu, South India (213). The study area included farming villages from the KV Kuppam Panchayat Union (120 miles^2), approximately 30 kilometers from Vellore town and three districts of varying socio-economic strata within Vellore town. The rural recruitment included 26 out of 42 contiguous villages belonging to single region of the KV Kuppam rural development block. The three districts represented about one third of Vellore town population (the total population of Vellore town during 1969-73 is not known). According to the 2011 population census of India, Vellore town had a total population of 3,938,106 individuals. During the initial screening (1969-73) all married non-pregnant women of reproductive age were identified by house-to-house survey, and a total of 20,626 eligible women (rural 11,628; urban 8,998) were recruited in the study (214). All singleton live births (n=10,670) born during the study period were included for longitudinal follow-up. Recruitment and follow-up are illustrated in Figure 3 below:

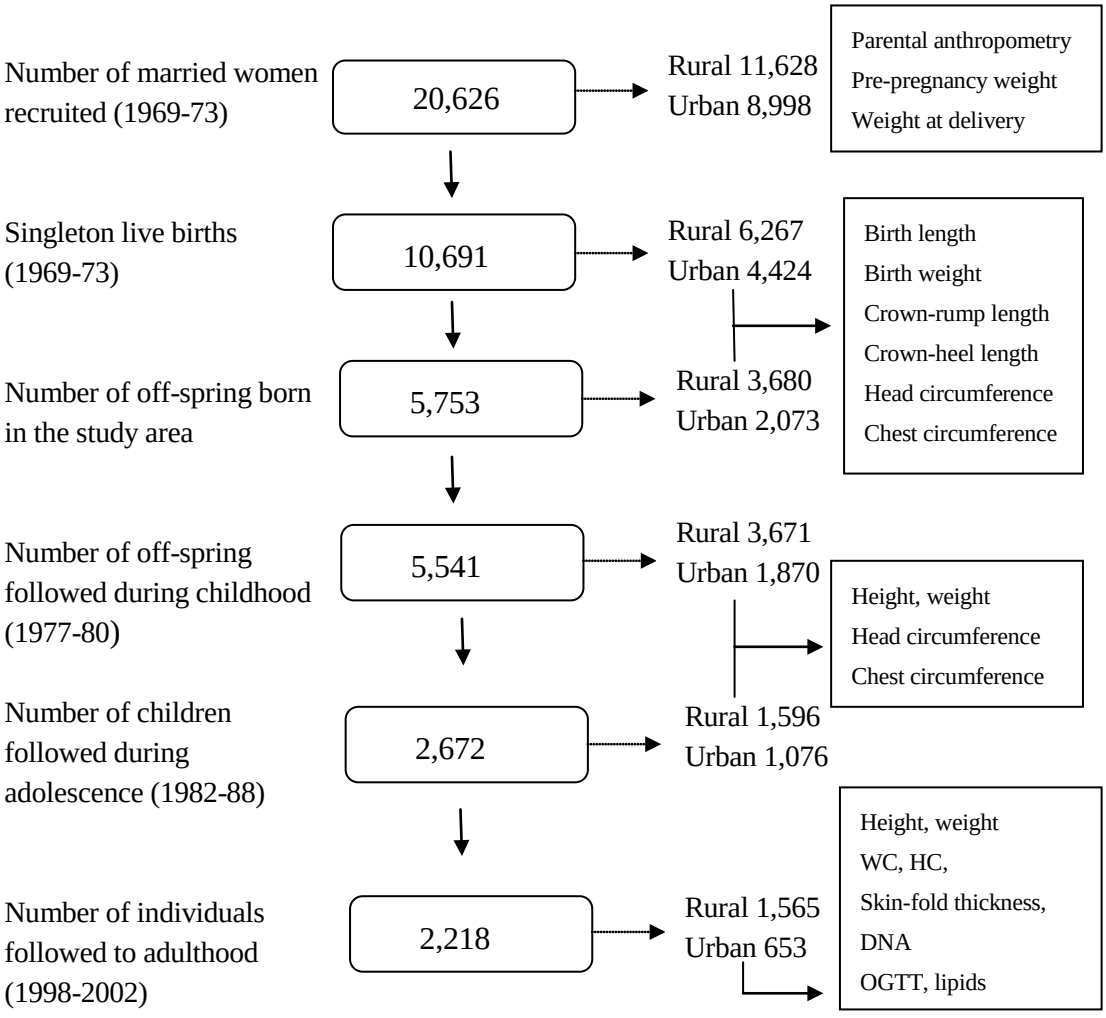


Figure 3: Flow chart showing subject recruitment in the VBC at different time points

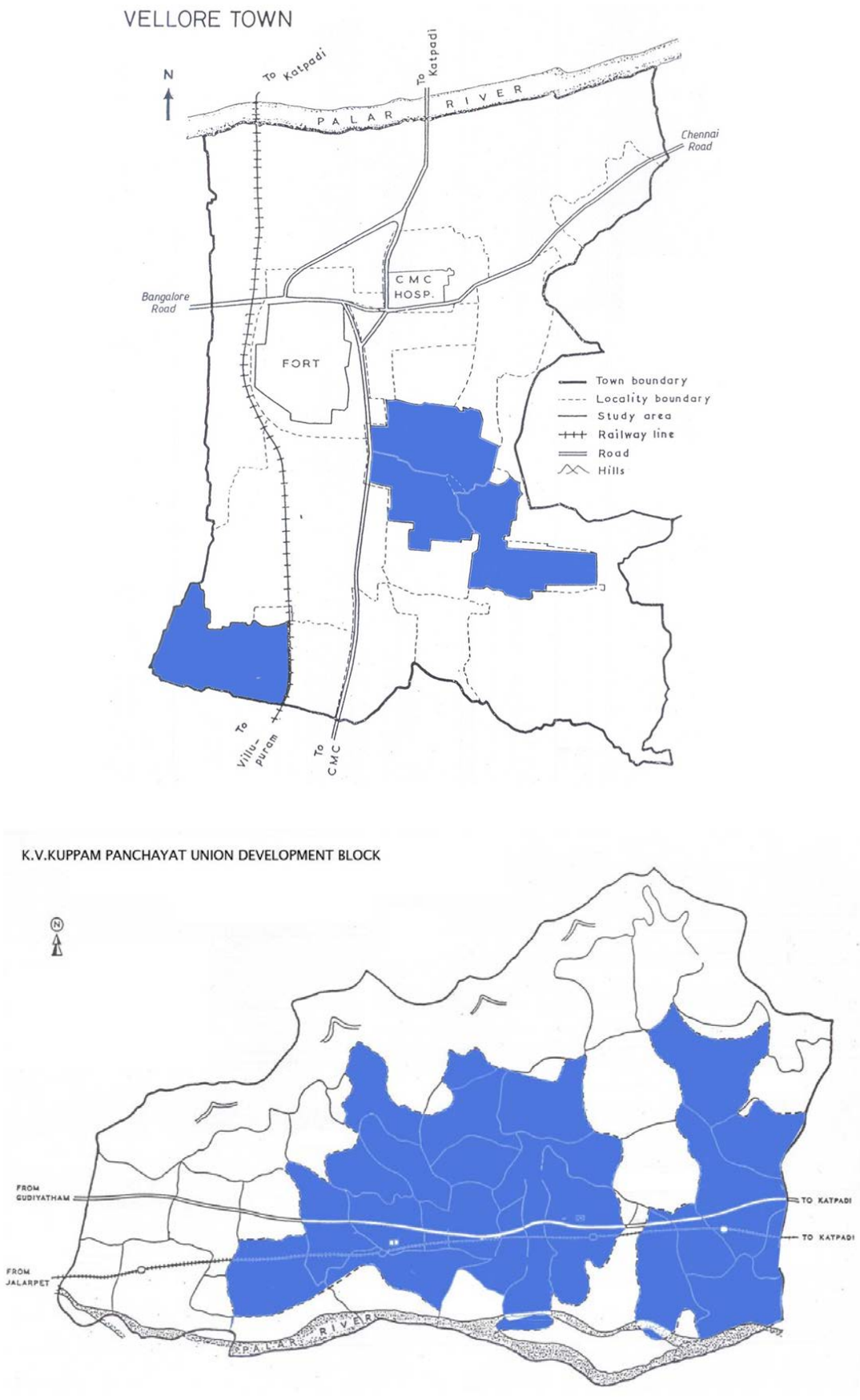


Figure 4: Map showing areas of recruitment for the Vellore Birth Cohort

2.3.2 The 'SPADES' cohort

The SPADES cohort was established as a part of the ongoing "School Program for Awareness in Diabetes, Education and Screening". The primary objective of this study is to implement routine screening for diabetes and promote health awareness among school children in Vellore town. The data for the current thesis was obtained during SPADES-Phase I screening, which included 11 schools and vocational training centers in Vellore, South India (215). The administrations of all schools, in and around Vellore town were contacted either by letter or telephone, to explain the objectives of SPADES program. All children aged over 14 years were asked for consent to participate in a comprehensive health check-up and biochemical testing. Out of the total 4,832 children, 1,822 provided consent, for which complete data was available for 1,359 subjects. Anthropometric measurements included height, weight, waist and hip circumference and skin fold thickness at 4 regions. Tanner's staging was recorded as assessment of pubertal staging. Biochemistry included fasting and 2-hour post-prandial blood glucose levels and lipid profile (total cholesterol, triglyceride, high and low density lipoprotein cholesterol (HDL and LDL, respectively). Whole blood was used for DNA extraction.

2.3.3The Oxford Biobank

The Oxford biobank (OBB) includes a random sample (n=5,000) of men and women aged between 30-50 years, residing in Oxford, Oxfordshire, UK. The OBB was the first, and one of the largest, 'recruit by genotype' biobanks in the UK, and was established to study genetic, epigenetic and functional mechanisms of obesity and its related complications such as CVD and T2DM. Data includes detailed anthropometry and biochemical testing. Relevant tissue samples are stored for future studies (see http://www.oxfordbiobank.org.uk/) (216).

2.4 Methodological considerations

2.4.1 Anthropometry

Anthropometric measurements are simple measurements of body composition. BMI reflects overall obesity, while measurements such as WC, HC and skin-fold thicknesses measure regional adiposity. These measurements are extensively used in field studies because of their ease of measurement and general applicability. In the current epidemiological investigation, anthropometry measures as listed below were used as proxy indicators of obesity traits. Each measurement was recorded thrice, and an average of three readings was used for analyses.

Table 2: Anthropometric measurements used in the current investigation

Anthropometry	Measurement Method	Unit
Height	Measured using a standard measuring tape and head positioned at Frankfurt plane	cm
Weight	Measured using a standard weighing scale to the nearest 0.1 kg	kg
Body Mass Index (BMI)	Calculated as ratio of weight (kg) and height (m) squared	kg/m^2
Waist Circumference (WC)	Measured at the smallest girth between lower costal margin and iliac crest using a non-stretchable tape	cm
Hip Circumference (HC)	Measured at the widest circumference between the superior iliac crest and ischial tuberosity using a non-stretchable tape	cm
Waist-Hip ratio (WHR)	Ratio of waist circumference to hip circumference	
*SFT - Subscapular	Measured 1-2 cm diagonally from the inferior angle of scapula	mm
*SFT – Triceps	Measured at the mid-point of the upper arm between the acromian process and the tip of the olecranon process	mm
*SFT – Abdomen	Measured laterally at about 2 cm from the umbilicus	mm

*SFT: Skin fold thickness measured using Harpenden Calliper (CMS Instrument, London)

2.4.2 Biochemistry

Biochemical measurements of interest in the current investigation (VBC cohort) included glucose and insulin, measured at serial time points (fasting, 30, 60 and 120 minutes) following a standard 75 g oral glucose tolerance test (OGTT). Glucose was measured by the glucose oxidase/peroxidase method following an 8-hour fast. In *paper III*, fasting and 2-hour capillary glucose levels were measured using an Accu-Check© glucometer [Co-efficient of variation (CV): 1.9-2.3%]. For studies that included VBC samples, plasma insulin was measured by an immune-radiometric assay using Coat-a-Count kits (Diagnostic Products Corporation, USA). Lipids were measured in fasting venous blood samples. Total serum cholesterol and triglycerides were measured by

standard enzymatic procedures, and HDL and LDL cholesterol by a direct assay method. The biochemical tests were performed on a Hitachi 912 auto-analyzer, using reagents from Roche Diagnostics (Mannheim, Germany), with the use of appropriate controls. The quality of these measurements was assessed using Roche Precinorm and Precipath controls for glucose and lipids, and BioRad Lyphocheck Immunoassay controls for insulin. Intra- and inter-assay coefficients of variation for insulin estimations were 8.0–14.5 and 8.2–13.0%, respectively. All biochemical measurements were done centrally at the Department of Clinical Biochemistry, Christian Medical College and Hospital, Vellore.

2.4.2.1 Oral glucose tolerance test (OGTT)

The OGTT is a method used to diagnose glucose intolerance and T2DM following administration of a standard dose (75 g) of anhydrous oral glucose, followed by serial measurements of plasma glucose and insulin at fasting and 30, 60 and 120 minutes. The OGTT mimics the normal physiological response, and reflects both β-cell insulin secretory capacity and the sensitivity of tissues for insulin-mediated disposal of the glucose load (217). IR is a common phenomenon observed in about 25% of non-diabetic individuals, and the degree of IR is directly related to the degree of hyperinsulinemia (218). Several prospective studies have shown that hyperinsulinemia/borderline elevations in glucose levels in normal individuals are significant predictors of T2DM. Resistance to insulin-mediated glucose uptake is one of the earlier changes that can be documented before any discernible decrease in the insulin secretory response (219, 220). Although plasma glucose levels during an OGTT is determined by insulin sensitivity and insulin secretion, other factors such as incretins, the neural response to nutrient ingestion, gastrointestinal motility and emptying also influence the OGTT response. The following indices derived from OGTT were used in the current thesis as markers of diabetes-related traits:

I. Homeostasis Model assessment (HOMA): is a model developed in 1985 which utilizes fasting glucose and insulin levels to predict IR and β-cell function. HOMA assumes a feedback loop between the liver and β-cells (221). In the normal state, the β-cells respond to plasma glucose concentrations and produce insulin. Insulin in turn mediates suppression of hepatic glucose production. This loop is altered in glucose intolerance. HOMA%IS reflects IS and HOMA%B reflects β-cell function, but both of these models assume a dynamic β-cell function, which is not usually the case in states of impaired glucose tolerance. Therefore, a more simplified equation, HOMA-IR, is often used, which is a surrogate marker of IR. HOMA-IR demonstrates a linear correlation with glucose clamp data and minimal model estimates of IS/IR (222).

II. Matsuda Index: was originally proposed by Matsuda and DeFronzo. It is an insulin sensitivity index that reflects estimates of hepatic and muscle insulin sensitivity determined from OGTT data (223). The fasting measurements reflect hepatic insulin sensitivity, while the mean of dynamic data reflects skeletal muscle insulin sensitivity. IS calculated using Matsuda Index have been shown to highly correlate with whole body glucose disposal rate assessed during a hyperinsulinemic euglycemic clamp (223).

III. Stumvoll Index: is a measure used to predict IS and β-cell function based on glucose and insulin measurements obtained during an OGTT (224, 225). The original

equation was derived using a multiple linear regression analysis, and is based on the time of sample collection during OGTT and demographic parameters (BMI, age). In the current investigation, the modified SI based on data obtained at 0 and 120 minutes was used without demographic data, since the latter is not validated in the Indian population.

IV. Insulinogenic Index: reflects the early phase insulin response, and is an index of β-cell function (insulin secretion). The insulinogenic index is shown to correlate well with first phase insulin release following an intravenous glucose tolerance test ($r=0.61$, $p<0.001$) (226).

V.AUC-glucose and Insulin: The area under the curve represents total glucose or insulin levels over 120 minutes recorded during an OGTT. Impaired glucose tolerance is reflected as a larger or increased AUC-glucose disappearance curve. Hepatic insulin sensitivity contributes to the shape of the glucose curve (227).

Table 3: Measures of IS/IR derived from oral glucose tolerance test

Glycemic Index	Measures of Insulin Sensitivity / Insulin Resistance
HOMA	$\text{HOMA IR} = \{[I_{\text{fasting (uU/ml)}}] \times [G_{\text{fasting (mmol/l)}}]\} / 22.5$
Matsuda Index	$\text{ISI (Matsuda)} = 10{,}000 / \sqrt{[(G_{\text{fasting(mg/dl)}} \times I_{\text{fasting(mU/l)}} \times (G_{\text{mean}} \times I_{\text{mean}})]}$
Stumvoll Index	$\text{ISI (Stumvoll)} = 0.156 - 0.0000459 \times I_{120\ (\text{pmol})} - 0.000321 \times I_{0\ (\text{pmol})} - 0.00541 \times G_{120\ (\text{mmol})}$
Insulinogenic Index	$\text{II} = [(I_{30\ (\text{pmol})} - I_{0\ (\text{pmol})})/(G_{30\ (\text{mg})} - G_{0\ (\text{mg})}]$
Area under the curve – Glucose	$\text{AUC}_{\text{glucose}} = \{(30-0) \times [G_{0\ (\text{mmol})} + G_{30\ (\text{mmol})}] + (60-30) \times [(G_{30\ (\text{mmol})} + G_{60\ (\text{mmol})}] + (120-60) \times [G_{60\ (\text{mmol})} + G_{120\ (\text{mmol})}]\}/2$
Area under the curve - Insulin	$\text{AUC}_{\text{Insulin}} = \{(30-0) \times [I_{0\ (\text{mmol})} + I_{30\ (\text{mmol})}] + (60-30) \times [(I_{30\ (\text{mmol})} + I_{60\ (\text{mmol})}] + (120-60) \times [I_{60\ (\text{mmol})} + I_{120\ (\text{mmol})}]\}/2$

HOMA-IR: Homeostasis model assessment - Insulin Resistance; G: Glucose; I: Insulin; ISI: Insulin sensitivity index
G_0, G_{30}, G_{60}, G_{120}: Glucose measured at 0, 30, 60 and 120 minutes following an oral glucose tolerance test
I_0, I_{30}, I_{60}, I_{120}: Insulin measured at 0, 30, 60 and 120 minutes following an oral glucose tolerance test

2.4.3 Genotyping methods

DNA was extracted from peripheral blood using Qiagen kits. The samples were genotyped using 10-20ng genomic DNA in a 384-well format on an ABI 7900HT machine, using a final volume of 4 µl. The genotyping was performed using the following TaqMan® SNP Genotyping Assays: C1860681_10 and C3035719_20 (rs900400 near *CCNL1*) and rs9883204 (*ADCY5*), C30090620_10 (rs9939609 *FTO*), C32667060_10 (rs17782313 *MC4R*). The TaqMan® Genotyping Master Mix was used, following the manufacturer's conditions. The genotype failure rate was 3.0% for rs900400, 2.8% for rs9883204, 1.5% for rs9939609 and 2.8% for rs17782313, probably due to low quality DNA for the platform used. In samples from the SPADES study, the same SNP genotyping assays were used, and the genotyping failure rate was 1.1% for rs9939609 and 4.6% for rs17782313.

2.4.4 Body composition estimation using DXA

In ***Paper V***, the body composition of migrant Asian Indians and matched controls from the OBB was estimated using DXA (GE Health Care Lunar iDexa, with software version 14.1). The algorithm used by the iDXA software performs quantification of VAT and trunk/android fat mass with high accuracy (64).

2.4.5 Statistical methods

In all studies, baseline characteristics were summarized using descriptive statistics. Data for all normally distributed variables are presented as mean and standard deviation (SD). For skewed variables, the data were either presented as median and inter-quartile range (IQR), or log transformed and presented as mean and SD.

In ***paper I***, the association between the genotypes and phenotypic traits were tested using analysis of variance (ANOVA) for normally distributed traits, and the Kruskall-Wallis test for skewed variables. Adjustments for covariates were performed only in ANOVA models. In ***Papers II and III***, linear regression analysis was performed to compute the effect size for the additive models, using quantitative traits as the dependent variable and genotypes as the independent variables. Possible confounding factors (as appropriate for the study) were adjusted. An additive genetic model was considered in all the genetic studies based on the assumption that the overall genetic variation contributing to the phenotype variability is due to the sum of individual effects of multiple loci (genotypes) (228) and that there is no interaction between the alleles within a locus or between loci (228). The genotypes of all SNPs were analyzed for deviation from Hardy-Weinberg equilibrium using $\chi 2$- analyses (229). We did not adjust for multiple comparisons, as the variants were chosen based on strong prior hypotheses, and all genetic variants tested were well-established lead signals in previous GWAS.

2.4.5.1 Meta-analysis

Meta-analysis (MA) is a method of combining results from different studies. MA is usually performed either on extracted data from published studies or individual data sets. Results may be combined with an inverse-variance weighted fixed effect model or a random effect model. In the fixed effect model, it is assumed that only one true effect size underlies all studies, and the differences in observed effects are due to sampling errors, while in the random effect model the true effect could actually vary between

studies (230). The results of the meta-analysis are usually represented graphically with forest-plots, in which the study-specific results are shown together with the estimated combined effect. Unfortunately, smaller studies or studies with negative results are often unpublished, and this might affect the study estimates of a meta-analysis. Funnel plots are a way of assessment of publication bias in a meta-analysis, and plots the measure of study size on the vertical axis and function of effect size on the horizontal axis (231). Two tests are commonly employed to quantify the amount of publication bias captured by the funnel plot, namely the Beggs and Mazumdar's rank correlation test, and Egger's regression test. The trim-and-fill method is a nonparametric method used to estimate the effect of the missing studies on the final outcome (232). Meta-analysis of genetic association studies has gained considerable importance, especially when independent candidate genetic studies have shown inconsistent results, obviously as a result of their lower statistical power (233). Heterogeneity tests are generally performed to determine if there are genuine differences in the cumulative results or whether variation in the findings is a chance occurrence (234, 235). This is usually tested using a Cochran's Q-test. This is quantified using I^2 statistics, which describes the total variation (degree of inconsistency) across the studies that is due to heterogeneity rather than by chance. Heterogeneity is considered low, moderate or high, based on I^2 values of 25%, 50% and >75%, respectively (234). Meta-regression was performed to identify sources of heterogeneity and examine moderators of overall association. In ***Paper IV,*** extracted data from original publications were used for meta-analysis. We performed random effects model and the results are presented as forest plots with corresponding estimates as either β or odds ratio (OR) along with 95% CI as applicable. Appropriate tests for heterogeneity and publication bias were included to validate the conclusions.

2.5 RESULTS

2.5.1 Paper I - *ADCY5* and *CCNL1* study

The association between the common variants rs9883204 (*ADCY5*) and rs900400 (near *CCNL1*) with birth weight and adult diabetes-related traits was investigated in 2,151 South Asian Indians. Despite the relatively low mean birth weight of the cohort (mean ± SD: 2.7 ± 0.5 kg), genetic variants in *ADCY5* and near *CCNL1* did not demonstrate a birth weight -lowering effect in this population. The MAFs of the risk alleles were comparable to that of the original population included in the GWAS by Freathy *et al.* (MAF rs900400: Indians 21%, Europeans in GWAS 32-47%; MAF rs9883204: Indians 81%, Europeans in GWAS 71-83%), and no gene-dose effect with respect to birth weight was observed. In line with the previous GWAS publication by Freathy *et al*. in which variants near the *CCNL1* locus demonstrated an association with reduced ponderal index (PI) at birth ($p=5 \times 10^{-21}$), we re-analyzed the data and found an association with reduced PI at birth for both rs900400 and rs9883204 genotypes (p=0.0009 and p=0.005 respectively, Table 4), but no significant association with birth length or head circumference. Examination of the relationship with diabetes-related traits showed rs9883204 to be associated with increased fasting and 120 minute glucose values, and with reductions in the insulinogenic index and 120 minute insulin levels, all of which argue for a common genetic link between birth weight lowering variants and risk of T2DM (236). The near *CCNL1* variant did not show any association with T2DM-related traits, consistent with the Freathy *et al.* report (134).

Table 4: Association of rs900400 (CCNL1) and rs9883204 (near CCNL1) with Ponderal index

	0	1	2	P value
rs900400				
Birth weight (kg)	2.79 (0.47)	2.80 (0.45)	2.80 (0.53)	0.87
Birth length (cm)	48.1 (2.7)	48.0 (2.7)	47.9 (2.5)	0.53
Ponderal Index	25.2 (4.3)	25.8 (4.6)	24.2 (3.9)	0.0009
Adult weight	53.2 (11.58)	53.3 (11.60)	55.5 (12.18)	0.13
Adult height	160.4 (9.0)	160.0 (8.8)	162.2 (5.3)	0.059
Adult BMI	20.6 (3.79)	20.8 (3.81)	21.0 (3.94)	0.42
rs9883204				
Birth weight (kg)	2.78 (0.46)	2.80 (0.46)	2.77 (0.55)	0.54
Birth length (cm)	49.1 (3.0)	48.5 (2.8)	47.8 (2.6)	<0.00001
Ponderal Index	25.4 (4.2)	25.4 (4.6)	23.8 (4.7)	0.005
Adult weight	53.5 (11.65)	53.2 (11.44)	52.4 (11.97)	0.80
Adult height	160.2 (8.8)	160.1 (9.0)	160.5 (9.3)	0.90
Adult BMI	20.7 (3.76)	20.7 (3.80)	20.3 (4.16)	0.64

rs900400 represents near CCNL1 variant and rs9883204 represent ADCY5 variant. 0, 1, 2 represent number of risk alleles. Ponderal index calculated as ratio of weight (kg) divided by height (m) cubed.

2.5.2 Paper II – *FTO* and *MC4R* in adults study

An examination of the obesity- and diabetes-related effects of the rs9939609 (*FTO*) and rs17782313 (*MC4R*) variants in a cohort of 2,151 (males: 1,118 and females: 1,034) individuals (mean ± SD age: 28.3 ± 1.1 years) from South India showed that rs9939609 (*FTO*) is associated with obesity-related anthropometric traits in adulthood, and that this effect appeared to be greater in the urban setting. Similar effects were not observed in younger age groups. The relatively stronger association between rs9939609 and skin fold thickness measurements which reflect SAT is reported for the first time in Asian Indians. Obesity associations with the *MC4R* variant was significant for total weight and regional adiposity measurements (WC and HC), but these were abolished when adjusted for height demonstrating that these associations are driven by body frame. The study was adequate powered at 86% to detect the 0.4% variance explained by *MC4R* previously (237). Analysis of longitudinal data, showed a significant association for rs9939609 with adult Z-scores of weight (p=0.024) and BMI (p=0.006), and rs17782313 with adult and adolescent height (p=0.047 and p=0.002 respectively). The association with height observed with rs17782313, is consistent with the previous observations in other populations, and suggests that polymorphisms in the *MC4R* locus relate to accelerated linear growth

2.5.3 Paper III – *FTO* and *MC4R* in adolescents study

Detailed investigations of the association between regional adiposity measurements and obesity-related genetic variants in younger age groups were not possible in *Paper II*, due to the limited availability of specific anthropometric measurements that reflect body fat distribution. The focus of the former study therefore was limited to only associations with overall obesity (Z-scores of BMI) in younger age groups. In *Paper*

III, the independent effects of these variants with respect to specific adiposity measurements were investigated in a cohort of 1,226 adolescents (mean ± SD Age: 17.1 ± 1.9 years) from South India (the SPADES cohort) (238). The results showed a significant association between rs9939609 (*FTO*) and WHR in adolescents, and this was significant even after adjusting for BMI and height. *FTO* demonstrated an increased abdominal obesity risk, based on the 95th percentile cut-off of WHR (OR 1.46; 95%CI 1.03-2.04, p =0.031). Although this association may point towards an effect on fat redistribution, it is unlikely considering, firstly, that *FTO* has not demonstrated any similar association in large datasets or GWAS, and secondly, that genetic loci that regulate body fat distribution are distinct from those that associate with obesity and BMI. Nevertheless, this finding could be important because the independent association of WHR with increased cardio-metabolic risk is well documented among Indians (59, 145). The study was not sufficiently powered to detect associations between with *MC4R* variant and obesity-related traits in this population.

2.5.4 Paper IV – Meta-analysis of *FTO* variants study

There is a considerable discrepancy in the literature about the association of *FTO* variants with obesity-related traits in Asian Indians. Therefore, in *Paper IV*, a meta-analysis of all published literature on *FTO* and diabetes and/or obesity traits in Asian Indians (both migrant and indigenous) was performed to confirm/refute the association of *FTO* variants with obesity traits and T2DM in Indians. Pooled data from eight studies (n=28,394) showed an increased risk with obesity (OR 1.15, 95% CI 1.08 -1.21, $p=2.14 \times 10^{-5}$) and related traits such as BMI ($\beta=0.30$ kg/m^2, 95% CI 0.21- 0.38, $p = 4.78 \times 10^{-11}$), WC ($\beta=0.74$ cm, 95% CI 0.49-0.99), HC ($\beta=0.52$, 95% CI 0.26-0.78) and WHR ($\beta=0.002$, 95% CI 0.001-0.004). The results of our meta-analysis also showed an increased risk for T2DM (OR 1.11; 95%CI 1.04-1.19, p=0.002), which attenuated when adjusted for age, gender and BMI (OR 1.09; 95% CI 1.02-1.16, p=0.01). This points towards a *FTO*-diabetes-effect that is partially mediated through BMI, contrary to the early literature that showed the effect of *FTO* on T2DM to be independent of BMI in Asians (176, 178). The estimates of *FTO* effects on obesity related traits and T2DM are summarized in Table 5. Heterogeneity was observed for T2DM and WC, and meta-regression did not identify any potential confounders. There was no evidence of publication bias observed for obesity-related traits. Publication bias as assessed by funnel asymmetry was observed for T2DM. The adjusted estimate as analyzed using trim and fill technique re-estimated overall diabetes risk after imputation of the missing studies and was not significant (OR 1.07: 95%CI 0.97-1.18, p=0.162).

Table 5: Summary of overall estimates of FTO on obesity-related traits in Asian Indians

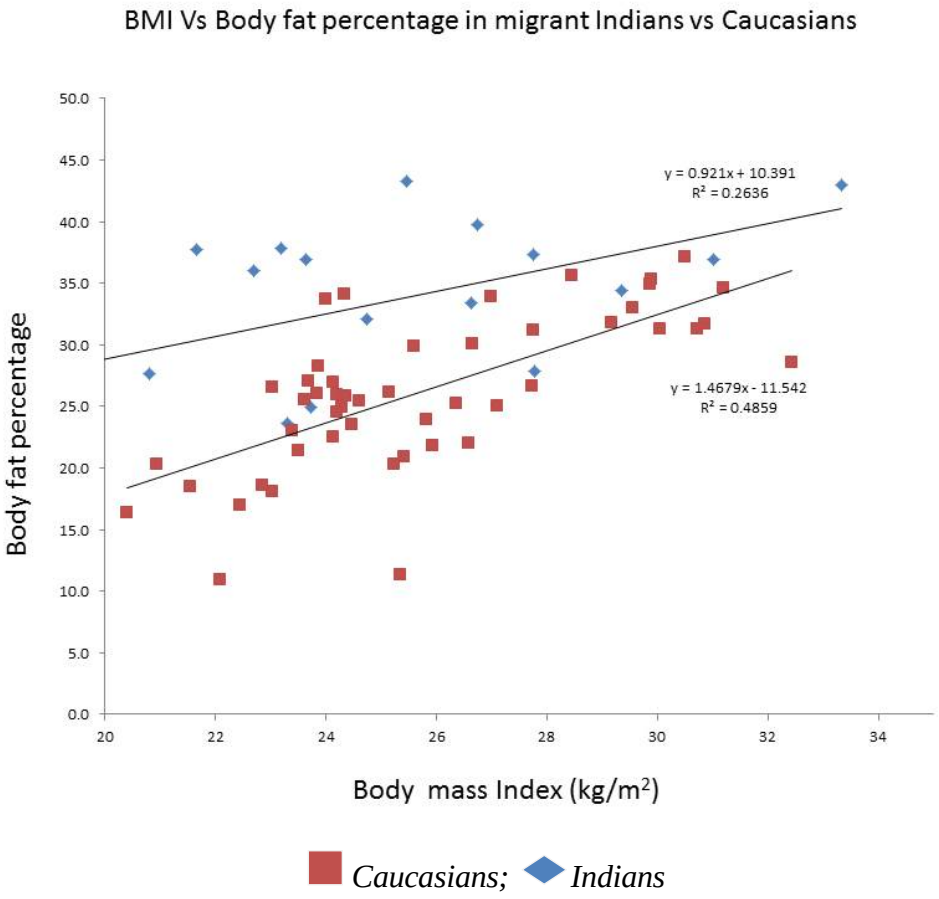
Phenotypic traits	n	β / OR (95% CI)	P value
Body mass index (kg/m^2)	28,394	0.30 (0.21, 0.38)	4.78×10^{-11}
Waist circumference (cm)	28,394	0.74 (0.49, 1.00)	0.001
Hip circumference (cm)	28,394	0.52 (0.26, 0.78)	0.0009
Waist-hip ratio	28,394	0.002 (0.0001,0.004)	0.001
*Obesity risk	24,987	1.15 (1.08, 1.21)	2.14×10^{-05}
*Type 2 diabetes risk†	24,987	1.11 (1.04, 1.19)	0.002
*Type 2 diabetes risk‡	24,987	1.09 (1.02, 1.16)	0.013

*† Type 2 diabetes risk adjusted for age and gender ‡Type 2 diabetes risk adjusted for age, gender and BMI. *Estimates represented as odds ratios (OR).*

2.5.5 Paper V – Body composition in Asian Indians study

Ethnic differences in body composition are an important determinant of cardio-metabolic risk especially in Indians. The approach described in *Paper V* was designed to test whether any differences exist in VAT in migrant Asian Indians, compared to Caucasians. Using iDXA, detailed body composition characteristics were compared between 26 migrant Asian Indians in Oxford, UK and 78 Caucasians from the Oxford Biobank (OBB), matched for age, gender, BMI and height. As expected, at any given BMI, the total body fat percentage as estimated by iDXA was higher in Asian Indians, compared to Caucasians. For example, as shown in the Figure5, at a BMI of 22, the body fat percentage of Indians is ~30%, while in Caucasians this is much lower (corresponding to ~20%). However, at higher BMI, the difference between the ethnicities appears to be steeper i.e. at a BMI of 30 kg/m^2, the body fat percentage in Indians and Caucasian corresponds to approximately 36% and 30%, respectively. Despite a lower lean mass (-11%, p=0.0001) and higher total fat mass (+25%, p=0.0008), no difference in VAT was observed was observed. Similarly the visceral/android ratio, which describes the proportion of fat that is inside the body cavity within the DXA defined 'Android' area, did not show any significant difference between both ethnicities and genders (42% in Asian Indians 125 and 55% in Caucasians). With increasing adiposity, Asian Indians demonstrated a more homogenous distribution of body fat which was largely subcutaneous. The association between total body fat and leg fat was linear, pointing towards a more homogenous distribution of excessive body fat (mainly in the form of SAT) similar to that observed in Caucasians.

Figure 5: Correlation between body mass index (BMI) and DXA measured body fat percentage in Indians and Caucasians.



3 DISCUSSION

The current thesis investigated the genetic contribution to birth weight, obesity and diabetes-related traits in Asian Indians. This was addressed by examining the genetic effects of well validated gene-disease associations in large homogenous cohorts of Asian Indians. The current investigation is an attempt to verify the extent to which common variants of genome-wide significance in Caucasians have similar effects in Asian Indians.

Birth weight is a robust indicator of neonatal well-being, and there are well-documented epidemiological observations that show an inverse correlation between birth weight and adult cardio-metabolic risk. Evidence of genetic regulation of birth weight independent of intra-uterine environment, is illustrated by correlations between paternal height or weight and offspring birth weight (239, 240). The common genetic link between birth weight and increased risk for adult T2DM additionally accounts for some of the observed correlation between these phenotypes (128, 132). In the current investigation, the absence of any birth weight-lowering effect observed for both *ADCY5* and near *CCNL1* variants is most likely due to the lower statistical power of the study (power: 74% for variant near *CCNL1*, and 32% for the *ADCY5* variant). Other possible reasons could be related to non-genetic factors (intra-uterine environment, maternal nutrition) that predominate over any genetic effects in Asian Indians. Although an association with birth weight was not observed, we extended our investigation to see if these variants had any effect on skeletal growth or fat mass at birth. We observed an association with reduced PI for both *ADCY5* and *CCNL1* variants consistent with the original GWAS observations by Freathy *et al*. where *CCNL1* was related to PI (134). In the absence of a birth weight lowering effect, we believe that this association must be spurious. PI is a measure of leanness at birth, and studies have shown that it reflects nutritional status of a new born independently of gestational age (241). However, by definition, PI appears to be the same as BMI, and both measurements provide information on weight controlled for height. Length at birth being is a specific measurement representing skeletal growth, and weight is a composite measure of the newborns fat, lean, bone and visceral mass. A significant association with a derived measure such as PI fails to provide any meaningful explanation regarding the genetic regulation of fetal growth in Indians and appears to neither reflect 'leanness' nor 'adiposity' at birth. The genetic association of PI with near *CCNL1* variants has been inconsistent in other studies (191, 242). A recent GWAS demonstrated an association between variants in the genomic region of 3q25 (*LEKRI* and near *CCNL1* genes) and skin fold thicknesses measured at birth, suggesting that the birth weight -lowering effect of these variants is secondary to an effect on subcutaneous fat (243). Data on SFT at birth is not available in the VBC, and it would be interesting to test this relationship among Asian Indians, who preserve greater subcutaneous fat even at birth (126, 244).

The association with higher fasting and 120 minute glucose levels suggests a possible role for *ADCY5* in the regulation of hepatic glucose production and/or insulin secretion, and the association with reduced insulinogenic index additionally points towards a role in insulin secretion. In a well-powered study among Indians, if an association is

demonstrable for both birth weight and adult glycemic traits, then the mechanism explaining such an association might be due to a direct effect of the fetal risk allele on fetal growth via reduced insulin secretion, consistent with fetal insulin hypothesis. The current study has few limitations and needs emphasis beside limited statistical power. The SNPs genotyped in the current study do not represent the causal variant, implying a need for the identification of other variants that could explain low birth weight in Indians. We acknowledge that our study was limited to examining the strongest GWAS signals only, and other possible candidates such as *CDKAL1* and *HHEX-IDE*, which showed large effect sizes in meta-analysis (132), were not chosen since the independent effect of these variants on birth weight is relatively small.

To date, the most robust and consistently replicated genetic variants of obesity are SNPs within the *FTO* and *MC4R* locus. *FTO* variants increased obesity risk by 1.20-1.30 fold in Europeans, and the risk allele was associated with a 0.39 kg/m^2 (~1000 g/allele) increase in BMI (75, 78). *MC4R* variants were associated with BMI increases of 0.23 kg/m^2 (~600 g/allele) in Caucasians. However, replication of these associations in Indians has been variable. In ***Paper II***, per allele (rs9939609) increases of ~1% for BMI, WC, HC and WHR and skin-fold thickness (SFT) during adulthood are similar to effect estimates reported in the Caucasian population. The association of rs9939609 with more specific measures of SAT suggests that SFT measurements probably reflect adiposity better than other conventional anthropometric measurements in the thin-fat Indian phenotype. This is important, because previous studies have shown that SAT, as measured by subscapular skin folds, is well preserved even at birth (126, 245) and persists through adulthood in Indians (245, 246). The relatively stronger associations with regional adiposity traits compared to overall obesity, allows us to reconsider the definition of obesity in Indians since traditional BMI or WC cut-offs may only be applicable within populations, but not when inter-ethnic comparisons are made. We observed a relatively more stronger *FTO*-obesity effect in the urban than rural setting which suggests that the impact of these genetic effects are more pronounced in individuals who are genetically susceptible when they are exposed to an obesogenic environment such as urban living. This also partially explains the increasing prevalence of obesity and overweight in urban Indian cities, compared to rural India. Our study could not demonstrate *FTO* effects at younger age groups, probably due to two reasons ii) limited sample size in younger populations ii) that the effect of *FTO* genotypes on obesity is stronger during adulthood among Indians, while these effects may be weak and undetectable in younger age groups, unlike other ethnic groups where *FTO* effect was observed both during childhood and in adults.

The association of genetic variants in the *FTO* and *MC4R* loci in younger age groups is evident both from GWAS (79, 82, 247) and longitudinal studies (248-250). Hardy *et al*. demonstrated an age-dependent effect of these variants, with stronger effects observed during childhood and adolescence and weaker effects during adulthood (248). A recent meta-analysis of 13,071 children and adolescents identified 15 genetic variants, including *FTO* and *MC4R*, which were associated with obesity-related traits (251). The results from our study showed an effect of *FTO* on WHR. The *FTO* effect on overall obesity does not emerge in our study, possibly because the individuals in this cohort were relatively leaner (mean BMI 20.4 ± 4.0). The increased risk for central adiposity (independent of height and BMI) conferred by rs9939609 signifies that body fat

distribution is independent of overall obesity, and in populations such as Asian Indians, who are vulnerable to central adiposity, WHR may be an important measurement of adiposity particularly in younger age groups with normal BMI. However, this association needs to be viewed with caution, since *FTO* did not significantly emerge at the genome-wide level for body fat distribution in European ancestry (251), and because overall obesity regulating-variants are distinct from variants that modulate fat distribution. We also acknowledge that the *FTO*-WHR relationship was modest, and did not hold for Bonferroni correction, and therefore larger datasets are required to reconfirm a strong statistical association of *FTO* with regional measurements of adiposity in younger Indians. It is important to note that a significant association with one of the traits, and a non-significant association with another, does not necessarily preclude the overall obesity effects of *FTO*, and points towards a complex interplay between genes and environment in obesity regulation at different ages. This study was also limited by statistical power to test for a gender-specific relationship for the *FTO*-WHR association.

The lack of demonstrable associations with the *MC4R* variant and obesity-related traits in the current investigation may be due to insufficient statistical power. The earliest GWAS which demonstrated an association of a locus near *MC4R* required a much larger sample size, firstly because the risk allele frequency is lower, and secondly because the effect size is smaller than that observed for the *FTO* locus (79). The allele frequency of *MC4R* variants in Asians is relatively higher, compared to that in other ethnic groups (~40% in Asians vs. ~27% in Europeans) (35, 171, 237), yet much larger samples appear to be required to estimate the true effect of *MC4R* on obesity-related traits. It is also possible that the ethnic-specific association of rs17782313 with obesity-related traits could be weak in Asian Indians, and the existence of a causal SNP with a much stronger effect needs further investigation. Investigations concerning the association of *MC4R* variants with obesity traits have been reported among Indians, but both reports have been confounded by the inclusion of cases with T2DM with higher BMIs (171, 172). Contrary to our findings in younger age groups, a recent report among 1362 children (mean age ± SD: 20.6 ± 5.1 years) from North India has shown a strong association of two SNPs in the *MC4R* locus with adiposity related traits (172).

The combined meta-analysis of 28,394 Asian Indians further confirmed that *FTO*-obesity risk in similar in both Indians and Caucasians, with an approximate 1% increment in all adiposity traits. Previous independent investigations of the effects of *FTO* in Asian Indians were largely confounded by factors such as the i) inclusion of T2DM cases, a large majority of whom exhibited increased BMI ii) population heterogeneity due to the admixture of Indians from different geographical regions and cultural backgrounds, iii) by lower statistical power (174, 176-178, 252). The meta-analysis has addressed some of these issues by improving statistical power and testing for heterogeneity across studies using appropriate statistical methods and by the use of meta-regression to identify potential confounders. Therefore, the results of this meta-analysis are robust and confirm the *FTO*-obesity risk in Indians. Examination of the *FTO*-T2DM association additionally suggests that the association between *FTO* and T2DM is partially mediated through the genetic effects on BMI contrary to the earlier belief among Asian Indians that *FTO* is a T2DM risk loci. One important caveat in our study was that we were unable to evaluate the effect of regional adiposity on T2DM,

since the meta-analysis was performed on data extracted from literature, and a combined analysis of independent data sets would have provided the opportunity to investigate an association of T2DM with regional adiposity in Asian Indians. This would be important in a centrally obese Indian population. We acknowledge our study overlapped with samples included in the recent meta-analysis of ~ 97,000 Asians by Li *et al*. However, we did not find any major inconsistencies between our results based solely on Indians for *FTO* effect, which strengthens the validity of the overall estimates obtained in the current study. Also we consider that the results of the meta-analysis by Li *et al*. cannot be generalized to all Asian populations because phenotypic differences, genetic heterogeneity and population specific environmental influences are reported extensively even within the Asian subgroups, and these differences are likely to account for the heterogeneity observed in the Asian study (179).

Further to the validation of genetic associations and the relatively stronger association of *FTO* variants with SFT that reflects subcutaneous adiposity, the current investigation was extended to clinically evaluate differences in regional abdominal adiposity depots in migrant Asian Indians, compared to Caucasians. The results of this study establish two important findings in relation to body composition in Indians: i) against expectation, and despite the presence of considerably higher overall body fat content in Indians, no meaningful differences in VAT content in Asian Indians is observed, compared with Caucasians. ii) the lower muscle mass, as reported by us, is consistent with several previous reports in Asian Indians, and further confirms that sarcopenic obesity is a characteristic feature of Asian Indians.

In the absence of excessive VAT, fat tissue may be distributed more homogenously in Indians, probably in the form of larger SAT depots, allowing the speculation that excessive SAT in the presence of lower lean mass (specifically, skeletal muscle) could possibly be a detrimental determinant of increased cardio-metabolic risk. Only a few reports among indigenous (167, 253) and migrant Indian populations (1, 62, 147) have highlighted the relationship of SAT with cardio-metabolic risk. The characteristics of the seemingly expanded SAT depot seen with obesity in Indians need more detailed investigation in larger datasets. It allows speculating that adiposity *per se* cannot fully account for the increased chronic disease risk, and the existence of a factor beyond adiposity needs identification.

The accumulation of active lipid intermediates such as long chain fatty acyl-CoA, diacylglycerol and ceramides in the skeletal muscle is shown to play a causal role in IR (254). Forouhi *et al*. showed excessive intra-muscular triglycerides (IMTG) (~30%) in Asian Indians compared to Caucasians, pointing towards increased cytosolic lipid accumulation and defective lipid metabolism in skeletal muscle (170). Muscle mass is known to inversely relate to IS and correlates significantly with reduced glucose disposal in Indians (255). Hall *et al*. in a series of experiments on skeletal muscle function and its role in IR among south Asians compared to Caucasians, showed that Asians oxidized less fat (~40%) during submaximal exercise, had reduced skeletal muscle expression of key insulin signaling proteins (IRS-1), and that $V0_{2max}$ and fat oxidation correlated significantly with whole-body IS (256). They additionally report that the IR phenotype cannot be explained by reduced skeletal muscle expression of genes involved in oxidative and lipid metabolism, since individuals with the highest

expression of these genes oxidized the least fat during exercise. Nair *et al*. reported that mitochondrial dysfunction does not account for the IR phenotype in Asian Indians, despite higher skeletal muscle capacity for oxidative phosphorylation, and higher mtDNA content was seen in muscle biopsies. Collectively these studies suggest that the reduced capacity to oxidize fat during submaximal exercise is a more important determinant of IR phenotype in Asian Indians, rather than mitochondrial capacity to oxidize lipids (257). We speculate that lower muscle mass is probably unable to compensate for the higher lipid content in the muscle and therefore exhibits a reduced capacity to oxidize fat. This warrants future investigation concerning the role of skeletal muscle mass in the presence of higher fat mass in the pathogenesis of T2DM in Asian Indians.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

The results of this thesis provide important conclusions on T2DM risk determinants in Asian Indians: i) the association between *ADCY5* variant and adult glycemic traits reinforces that that fetal-insulin hypothesis could be an important mechanism that underlies T2DM etiology in Indians ii) obesity determining genetic variants demonstrate a relatively stronger effect on regional adiposity and proxies of SAT than overall obesity which underscores that SAT may have an important role in obesity-related complications in Indians iii) the absence of differences in VAT, and the relatively higher subcutaneous adiposity along with lower lean mass (particularly skeletal muscle), are intrinsic features of Indians and the exact role in the pathogenesis of IR/T2DM needs detailed investigation.

Genetic association studies amongst Indians have largely been replication attempts of genome-wide significant signals that emerged from GWAS in populations of European ancestry. Recent efforts to integrate independent cohorts into larger consortia by the Indian Genomic Variation initiative (157, 158), hopes to facilitate validation of genetic markers in a large scale and also allow discovery of new genetic variants with use of high throughput genomic methods including the 'omics' platforms. This approach would provide important biological insights into the pathogenesis of complex disease in this Asian Indians.

In a broader perspective, conventional genetics in general appears to explain very little of the phenotypic variation in complex disease phenotypes, and variants, individually or in combination, confer relatively small increments in disease risk (1.1-1.5 fold) (258). This stimulates exploration of proposed variants that could explain the 'missing heritability', including variants of low MAF (0.5% to <5%), rare variants (MAF <0.5%) and structural variations such as CNV, insertions and deletions (259). Other areas that remain equally important are investigations of potential environmental confounders, gene-gene interactions, gene-environmental interactions, and their biological implications in complex disease pathology, especially in understanding obesity phenotypes where gene x environment interactions have shown to play an important role (260).

More specifically, the results from ***Paper I*** make the fetal origins hypothesis an attractive area of research in Indian populations, considering that distinct IR and adiposity-related phenotypic characteristics are exhibited at birth in this population. These observations warrant further studies that investigate common genetic variants that associate with both phenotypes among Indians. Identification of causal variants appears to be important in all genetic studies. An example for this is that the birth weight signal marked by rs900400 maps approximately 353 kb 3-prime to the leucine, glutamate and lysine rich 1 (*LEKR1*) locus, and 67 kb 3-prime to *CCNL1*. Neither of these genes has been implicated in previous GWAS, and their functions appear to be unknown. Therefore other approaches, such as resequencing and functional studies, will be required to establish which gene (*CCNL1*, *LEKR1* or another gene) actually mediates the effect on birth weight. Also, epigenetic processes are strong candidates for mediating the above traits. This is demonstrated in animal models, in which a causal link between early nutritional status and epigenetic changes predisposing to adult T2DM and obesity risk have been demonstrated (261). Therefore, investigations of the molecular basis of fetal programming that involves DNA methylation may provide substantial new knowledge on mechanisms that link fetal growth and adult disease. Epigenetic mechanisms may also underlie the propagation of obesity from mother to child by a 'thrifty epigenotype' mechanism, and this opens opportunities for genome-wide assessment of epigenetic variation (262). An understanding of genetic and epigenetic associations will therefore illuminate the biological pathways important for fetal growth and its relationship to adult disease.

With regards to the genetic effects of *FTO* and *MC4R*, the biological mechanisms responsible for the association with obesity-related traits remain elusive. Given that these loci are highly expressed in the hypothalamus (192), and that evidence for their association with appetite regulation, energy intake and satiety has been provided from human studies (263, 264), it is expected that their obesity effects may be related to dysregulation of satiety leading to overfeeding. Studies have also shown that the effect of these variants on common obesity is attenuated with alteration in life-style, particularly physical activity (263, 265). So far, this remains unexplored in Indians, and warrants well-designed clinical trials to infer a causal relationship. Epigenetic mechanisms that underlie common obesity are still largely unknown, and there is emerging evidence that supports the role of both genetic and environmental interactions with epigenetics to perpetuate disease risk which requires further exploration (266).

The facts that the genetic variants tested here associate relatively strongly with SAT measures, and that DXA did not show any difference in VAT, warrant more detailed epidemiological and clinical studies investigating the role of specific adipose depots and skeletal muscle and its relationship cardio-metabolic disease. This would be particularly insightful in this population since most studies have highlighted the importance of VAT in Indians and very few recent evidence provide support for the role of SAT in disease risk. More functional knowledge relating to individual pathways or genes can be gained by *in vitro* studies on biopsy specimens obtained from different adipose depots and skeletal muscle.

5 SAMMANFATTNING PÅ SVENSKA

Genetiska faktorer spelar en väsentlig roll för känsligheten för vanliga kroniska sjukdomar såsom diabetes or hjärt och kärlsjukdomar (multifaktoriella eller komplexa sjukdomar). Framsteg inom identifieringen av genetiska varianter, gjorda via genotypning i stor skala, har hjälpt till att fastställa flera genetiska varianter som ger ökad risk för komplexa sjukdomar. Denna avhandling utfördes för att undersöka effekten av genetiska varianter associerade med låg födelsevikt och fetma hos indier och bedöma om dess genetiska markörer har en effekt liknande den hos europeer. Avhandlingen syftar också till att belysa skillnader i kroppssammansättning mellan de två populationerna, särskilt bukfetma som en trolig bidragande orsak till för tidig hjärt och kärlsjukdom hos indier.

För studien användes data från "Vellore birth Cohort ", vilket är en longitudinell födelsekohort som startades 1969-1973 i närheten av Vellore, Tamil Nadu, i södra Indien. Alla överlevande ensamfödda (n = 10,670) som nedkom under studieperioden inkluderades i den longitudinella uppföljningen och de undersöktes vid olika tidpunkter (födelse, barndom, tonår och vuxenliv). För de genetiska studierna, inkluderades de 2,218 personer som fullföljde alla fyra tidpunkterna. Några väletablerade genetiska varianterna för födelsevikt (*ADCY5* och nära *CCNL1*) och fetma (*FTO* och *MC4R*) testades för relevanta associationer med tillgängliga variabler såsom födelsevikt, glukos- och insulinrelaterade variabler respektive överviktsparametrar hos vuxna. Vidare studerades effekten av *FTO* och *MC4R* i en annan kohort bestående av 1,200 ungdomar i 17-års åldern. Resultat från dessa studier samt oklarheter i den tillgängliga litteraturen ledde också till en studie i syfte att gen en detaljerad kartläggnng av fettvävsfördelning hos indier i jämförelse med europeer.

Födelseviktsstudien visade inget samband mellan födelsevikt och de två genetiska varianterna för födelsevikt hos i den indiska populationener. En möjlig förklaring kan vara att det finns icke-genetiska faktorer (intrauterin miljö, moderns nutritionsstatus etc.) som dominerar över de genetiska effekterna. Däremot fann vi att den genetiska varianten *ADCY5* var associerad till förhöjda glukos- och minskade insulinnivåer hos unga vuxna, vilket talar för en generell genetisk koppling mellan födelsevikt och risk för typ 2 diabetes. I studien där sambandet mellan genetiska fetma varianter och fetma variabler undersöktes fann vi ett samband mellan *FTO* varianter och både generell och regional övervikt hos vuxna, till skillnad från de yngre åldersgrupperna, där sambandet var starkare för regional fetma. Vi bekräftade detta samband i en meta-analys på 23,982 indier.

En stark association mellan *FTO* varianter och underhudsfett gav inspiration till en mer detaljerad analys av fettvävsfördelninghos indier och europeer. Det fanns ingen skillnad i visceral fetma, men indier hade lägre muskelmassa och ökat generellt subkutant kroppsfett. Denna studie visade att det finns principiella skillnader is fettvävsfördelning och kroppsammansättning mellan indier och europeer..

Resultaten från denna avhandling visar att genetiska varianter som predisponerar för övervikt har ungefär samma effekt hos indier som hos europeer. Avsaknaden av genetisk påverkan på födelsevikt hos indier skulle kunna förklaras av icke- eller epigenetiska faktorer. Trots detta indikerar sambandet med insulin nivåer hos vuxna att gener som reglerar födelsevikten samspelar med uppkomsten av vuxendiabetes. Avsaknaden av skillnader i visceral fetma mellan de två etniska grupperna leder till slutsatsen att fettinlagringen hos indier framför allt sker subkutant. Ytterligare studier krävs för att undersöka om subkutant fett är skadligt och innebär större risk för hjärt-metabol, kärl och diabetessjukom hos indier.

6 ACKNOWLEDGEMENTS

I wish to acknowledge all the people who have been a part of my academic journey and life here in Sweden.

My main supervisor, **Kerstin Brismar**. Firstly sincere thanks for accepting me in your group. Secondly, for the constant encouragement and support that you have given me until today. I am particularly grateful for the freedom that you gave me to follow projects of my own interest. You had so much trust in me and I hope I lived to it. For everything you have done for me, Thank you!

My co-supervisor, **Tove Fall**, who have been a big pillar of strength and tremendous help. I owe my deepest gratitude for all the guidance you provide throughout my PhD. Thanks especially for being extremely tolerant to every mistake of mine right from simple mathematics to statistical modeling. It has been a lot of learning from you and many thanks for everything. I am so very grateful! I hope we would be able to do a more relaxed trip to India sometime in the future.

My co-supervisor, **Harvest F Gu**, for introducing me to the world of laboratory science. Thanks for teaching the art of being meticulous in the lab and always being considerate to my academic needs. Thank you very much for the excellent guidance and genuine interest in my academic growth.

My co-supervisor, **Nihal Thomas**, for the faith you had in me and persistently stimulating me to pursue research as a career. I know I would not have ever stepped into PhD if not for you. Thanks so much for all the support since 2002.

My external mentor, **Prathap Tharyan.** The support and care you provided have always helped me to overcome my setbacks and stay focused. You made life look all easy and worth. Thanks for being a friend to me more than a psychiatrist or mentor.

My collaborators: **Prof. Fredrik Karpe,** for the enormous support and contribution to my academic career. I owe you so much for the endless efforts you took in making me understand science and quality research. Thank you for all the invaluable scientific discussions and the continual motivation you provided. I am certain that without your guidance and persistent help, this dissertation would not have been possible. Sincere gratitude for the clinical and laboratory opportunities you provided at OCDEM, Oxford. I could not be prouder of my academic roots and hope that I can in-turn pass on the research values and the dreams that you have given to me. On the personal side, special thanks for all the invitations for the wonderful moments spent with your family and at your summer house.

Prof. Erik Ingelsson, for being such a big inspiration! You have set an academic standard that I always admire. Thanks for patiently responding to all my mails and I deeply appreciated all your valuable suggestions and knowledgeable comments.

Prof. Caroline Fall. Thank you for your constructive criticisms and proof reading of the manuscripts. Your insights on interpretation of birth weight associations during the Kappa preparation were very useful. Thanks for the continual warm support and encouragement.

To the entire VBC team, especially **Prof. Antonisamy, Dr. Geethanjali, Prasanna Samuel** and **Solomon Christopher** from CMC, Vellore**.** Thank you for the trust you all had in me and generously allowing me to use the VBC for my PhD studies. I sincerely look forward to all the future work together.

I would like to acknowledge with deepest appreciation all my colleagues and friends back home at the **Department of Endocrinology, Diabetes and Metabolism, Christian Medical College and Hospital, Vellore.** Thanks everyone for all the support and help in the formative years of my physician training, and in Endocrinology. Special thanks to Prof **M.S.Seshadri**, who has always been a major source of inspiration in the Endocrine clinics and one of the best teachers I have come across in my medical career.

Thanks to all my friends at M1: **Tianwei Gu, Carol Muller, Mohammed, Norhashimah, Galena, Elizabeth Noren-Krog, Saad Alqahtani, Jun Ma, Tina Wirström, Mattias Vesterlund, Hannes Leumann, Neuza Dominguez, Jia Lidia Li and Corrina Mohr.** The numerous fikas, lab outings, laughter and all the fun together made life so enjoyable and less stressful. Special thanks to **Agneta Hilding** for all the statistical discussions and Swedish teaching. Det var allt bra. Tack! Warm hugs to two of my lovely colleagues: **Faradiana Lokman** and **Neil Portwood**, who added so much life to the office. At the end of the day, it always brings a good laugh when I reminisce the silly jokes we have shared and the super-good times we have had in the office. Fara, the intellectual talks continue to inspire me even today!! Neil, I know what you are thinking….Thanks **Julien Pelletier** for all the help during thesis writing and the photography discussions.

To the L1 crew: **Jacob Grunler, Vivek Sunkari, Ileana Botusan, Elisabete Forsberg, Ishrath Ansurudeen, Ismael Valladolid, Jing Wang, Marianna del sole, Mike Teklam, Noah Moruzzi, Teresa Pereira, Xiaowei, Charlotte Mattson, Åse Mattson, Katrin Brandt and Stina Lindberg** for all the encouragement and support. Many thanks to **Elvi Sandberg** and **Inga-Lena** for the laboratory help with IGF assays. I sincerely appreciate the feedback and the support from **Sergiu-Bogdan Catrina** and **Christina Bark** during lab meetings**.** Special thanks to **Gustav Dallner**! I hope that I could be as lively, enthusiastic, and energetic as you.

Special thanks to the administrative staff at MMK: **Ann-Brit Wikström, Kerstin Florell, Britt-Marie Witasp, Katarina Breitholtz** for all the kind assistance and the excellent IT support from **Lennart Helleday, Jan-Erik Karre** and **Thomas Westerberg**.

Thanks to everyone at OCDEM, Oxford, especially **Constantinos, Matt Neville, Kiki Marinou, Sandy Humpreys** and staff at the clinical research unit for accommodating me completely as a part of your team every time I was there.

Beyond academia, I owe my gratitude to all those people who made Sweden, a home away from home. The 5-years of my living here is something that I will cherish forever.

Many thanks to all my Indian friends and families here in Stockholm who kept me sane and going. None mentioned, none forgotten. You have all been so wonderful.

Special thanks to **Rouknuddin Qasim Ali** and **Atul Paulson**, for always being there in the most special way, be it a weekend party or the rough times. You are priceless and I greatly value your friendship.

Thanks to **Monir Mazheri** and **Catherine Ricklesford** for being such great friends. Thanks **Rona Strawbridge** for teaching me PLINK, the casual lunches, post-doc advice, and many more!

To anyone whom I have missed, I am sorry!

The study was funded by the Swedish Institute, Family Erling-Person foundation, Robert Turners fellowship and Svenska Sällskapet för Medicinsk Forskning.

Most importantly, thanks to my entire family, for your unconditional love and support.

Appa and **amma,** I owe my biggest thanks to both of you for being such wonderful people in my life. I did it once again!!

7 REFERENCES

1. Chandalia, M., Lin, P., Seenivasan, T., Livingston, E.H., Snell, P.G., Grundy, S.M., and Abate, N. 2007. Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PloS one* 2:e812.
2. Liew, C.F., Seah, E.S., Yeo, K.P., Lee, K.O., and Wise, S.D. 2003. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 27:784-789.
3. Yajnik, C.S., Lubree, H.G., Rege, S.S., Naik, S.S., Deshpande, J.A., Deshpande, S.S., Joglekar, C.V., and Yudkin, J.S. 2002. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab* 87:5575-5580.
4. Hattori, M., Fujiyama, A., Taylor, T.D., Watanabe, H., Yada, T., Park, H.S., Toyoda, A., Ishii, K., Totoki, Y., Choi, D.K., et al. 2000. The DNA sequence of human chromosome 21. *Nature* 405:311-319.
5. Dunham, I., Kundaje, A., Aldred, S.F., Collins, P.J., Davis, C.A., Doyle, F., Epstein, C.B., Frietze, S., Harrow, J., Kaul, R., et al. 2012. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489:57-74.
6. Strachan, T., Read, A. . 2004. *Human Molecular Genetics*.
7. Dunham, I., Shimizu, N., Roe, B.A., Chissoe, S., Hunt, A.R., Collins, J.E., Bruskiewich, R., Beare, D.M., Clamp, M., Smink, L.J., et al. 1999. The DNA sequence of human chromosome 22. *Nature* 402:489-495.
8. Goldstein, D.B. 2009. Common genetic variation and human traits. *N Engl J Med* 360:1696-1698.
9. Mu, X.J., Lu, Z.J., Kong, Y., Lam, H.Y., and Gerstein, M.B. 2011. Analysis of genomic variation in non-coding elements using population-scale sequencing data from the 1000 Genomes Project. *Nucleic Acids Res* 39:7058-7076.
10. Collins, F.S., Guyer, M.S., and Charkravarti, A. 1997. Variations on a theme: cataloging human DNA sequence variation. *Science* 278:1580-1581.
11. Lockhart, D.J., and Winzeler, E.A. 2000. Genomics, gene expression and DNA arrays. *Nature* 405:827-836.
12. Levy, S., Sutton, G., Ng, P.C., Feuk, L., Halpern, A.L., Walenz, B.P., Axelrod, N., Huang, J., Kirkness, E.F., Denisov, G., et al. 2007. The diploid genome sequence of an individual human. *PLoS Biol* 5:e254.
13. Falconer, D.S., Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*.
14. Weatherall, D.J. 2000. Single gene disorders or complex traits: lessons from the thalassaemias and other monogenic diseases. *BMJ* 321:1117-1120.
15. Kota, S.K., Meher, L.K., Jammula, S., and Modi, K.D. 2012. Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities. *Diabetes Metab Syndr* 6:54-58.
16. Vaxillaire, M., and Froguel, P. 2006. Genetic basis of maturity-onset diabetes of the young. *Endocrinol Metab Clin North Am* 35:371-384, x.
17. Koopman, W.J., Willems, P.H., and Smeitink, J.A. 2012. Monogenic mitochondrial disorders. *N Engl J Med* 366:1132-1141.
18. Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., and O'Rahilly, S. 2003. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med* 348:1085-1095.
19. Lander, E.S., and Schork, N.J. 1994. Genetic dissection of complex traits. *Science* 265:2037-2048.
20. Hirschhorn, J.N., Lohmueller, K., Byrne, E., and Hirschhorn, K. 2002. A comprehensive review of genetic association studies. *Genet Med* 4:45-61.
21. McCarthy, M.I., Abecasis, G.R., Cardon, L.R., Goldstein, D.B., Little, J., Ioannidis, J.P., and Hirschhorn, J.N. 2008. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* 9:356-369.
22. Hardy, J., and Singleton, A. 2009. Genomewide association studies and human disease. *N Engl J Med* 360:1759-1768.

23. Risch, N.J. 2000. Searching for genetic determinants in the new millennium. *Nature* 405:847-856.
24. Benke, K.S., and Fallin, M.D. 2010. Methods: genetic epidemiology. *Psychiatr Clin North Am* 33:15-34.
25. Cardon, L.R., and Bell, J.I. 2001. Association study designs for complex diseases. *Nat Rev Genet* 2:91-99.
26. Tabor, H.K., Risch, N.J., and Myers, R.M. 2002. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 3:391-397.
27. Schork, N.J., Fallin, D., and Lanchbury, J.S. 2000. Single nucleotide polymorphisms and the future of genetic epidemiology. *Clin Genet* 58:250-264.
28. Altshuler, D., Kruglyak, L., and Lander, E. 1998. Genetic polymorphisms and disease. *N Engl J Med* 338:1626.
29. Ioannidis, J.P., Ntzani, E.E., Trikalinos, T.A., and Contopoulos-Ioannidis, D.G. 2001. Replication validity of genetic association studies. *Nat Genet* 29:306-309.
30. Lohmueller, K.E., Pearce, C.L., Pike, M., Lander, E.S., and Hirschhorn, J.N. 2003. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177-182.
31. Zondervan, K.T., and Cardon, L.R. 2004. The complex interplay among factors that influence allelic association. *Nat Rev Genet* 5:89-100.
32. Manolio, T.A. 2010. Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 363:166-176.
33. Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. 2001. The sequence of the human genome. *Science* 291:1304-1351.
34. Panagiotou, O.A., and Ioannidis, J.P. 2012. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. *Int J Epidemiol* 41:273-286.
35. Chambers, J.C., Elliott, P., Zabaneh, D., Zhang, W., Li, Y., Froguel, P., Balding, D., Scott, J., and Kooner, J.S. 2008. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. *Nat Genet* 40:716-718.
36. Dorajoo, R., Blakemore, A.I., Sim, X., Ong, R.T., Ng, D.P., Seielstad, M., Wong, T.Y., Saw, S.M., Froguel, P., Liu, J., et al. 2012. Replication of 13 obesity loci among Singaporean Chinese, Malay and Asian-Indian populations. *Int J of obesity* 36:159-163.
37. Kilpelainen, T.O., Zillikens, M.C., Stancakova, A., Finucane, F.M., Ried, J.S., Langenberg, C., Zhang, W., Beckmann, J.S., Luan, J., Vandenput, L., et al. 2011. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. *Nat Genet* 43:753-760.
38. Guilherme, A., Virbasius, J.V., Puri, V., and Czech, M.P. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 9:367-377.
39. Rosen, E.D., and Spiegelman, B.M. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444:847-853.
40. Organization, W.H. 2008. Obesity and overweight.
41. Hossain, P., Kawar, B., and El Nahas, M. 2007. Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* 356:213-215.
42. Abate, N., and Chandalia, M. 2001. Ethnicity and type 2 diabetes: focus on Asian Indians. *Journal of diabetes and its complications* 15:320-327.
43. Yajnik, C.S., and Ganpule-Rao, A.V. 2010. The obesity-diabetes association: what is different in indians? *The international journal of lower extremity wounds* 9:113-115.
44. Kopelman, P.G. 2000. Obesity as a medical problem. *Nature* 404:635-643.
45. Mokdad, A.H., Ford, E.S., Bowman, B.A., Dietz, W.H., Vinicor, F., Bales, V.S., and Marks, J.S. 2003. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 289:76-79.
46. Haslam, D.W., and James, W.P. 2005. Obesity. *Lancet* 366:1197-1209.
47. Basen-Engquist, K., and Chang, M. 2011. Obesity and cancer risk: recent review and evidence. *Curr Oncol Rep* 13:71-76.

48. Powell, K. 2007. Obesity: the two faces of fat. *Nature* 447:525-527.
49. de Ferranti, S., and Mozaffarian, D. 2008. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem* 54:945-955.
50. Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Naslund, E., Britton, T., et al. 2008. Dynamics of fat cell turnover in humans. *Nature* 453:783-787.
51. Kahn, B.B., and Flier, J.S. 2000. Obesity and insulin resistance. *J Clin Invest* 106:473-481.
52. Kahn, S.E., Hull, R.L., and Utzschneider, K.M. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444:840-846.
53. Lancerotto, L., Stecco, C., Macchi, V., Porzionato, A., Stecco, A., and De Caro, R. 2011. Layers of the abdominal wall: anatomical investigation of subcutaneous tissue and superficial fascia. *Surg Radiol Anat* 33:835-842.
54. Smith, S.R., Lovejoy, J.C., Greenway, F., Ryan, D., deJonge, L., de la Bretonne, J., Volafova, J., and Bray, G.A. 2001. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism* 50:425-435.
55. Despres, J.P., Nadeau, A., Tremblay, A., Ferland, M., Moorjani, S., Lupien, P.J., Theriault, G., Pinault, S., and Bouchard, C. 1989. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. *Diabetes* 38:304-309.
56. Pouliot, M.C., Despres, J.P., Nadeau, A., Moorjani, S., Prud'Homme, D., Lupien, P.J., Tremblay, A., and Bouchard, C. 1992. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes* 41:826-834.
57. Ross, R., Aru, J., Freeman, J., Hudson, R., and Janssen, I. 2002. Abdominal adiposity and insulin resistance in obese men. *Am J Physiol Endocrinol Metab* 282:E657-663.
58. Ross, R., Freeman, J., Hudson, R., and Janssen, I. 2002. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *J Clin Endocrinol Metab* 87:5044-5051.
59. Abate, N., Garg, A., Peshock, R.M., Stray-Gundersen, J., Adams-Huet, B., and Grundy, S.M. 1996. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. *Diabetes* 45:1684-1693.
60. Abate, N., Garg, A., Peshock, R.M., Stray-Gundersen, J., and Grundy, S.M. 1995. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest* 96:88-98.
61. Goodpaster, B.H., Thaete, F.L., Simoneau, J.A., and Kelley, D.E. 1997. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* 46:1579-1585.
62. Chowdhury, B., Lantz, H., and Sjostrom, L. 1996. Computed tomography-determined body composition in relation to cardiovascular risk factors in Indian and matched Swedish males. *Metabolism* 45:634-644.
63. Shen, W., Wang, Z., Punyanita, M., Lei, J., Sinav, A., Kral, J.G., Imielinska, C., Ross, R., and Heymsfield, S.B. 2003. Adipose tissue quantification by imaging methods: a proposed classification. *Obes Res* 11:5-16.
64. Kaul, S., Rothney, M.P., Peters, D.M., Wacker, W.K., Davis, C.E., Shapiro, M.D., and Ergun, D.L. 2012. Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity (Silver Spring)* 20:1313-1318.
65. 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363:157-163.
66. Misra, A., Chowbey, P., Makkar, B.M., Vikram, N.K., Wasir, J.S., Chadha, D., Joshi, S.R., Sadikot, S., Gupta, R., Gulati, S., et al. 2009. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assn of Physicians of India* 57:163-170.
67. Deurenberg, P., Yap, M., and van Staveren, W.A. 1998. Body mass index and percent body fat: a meta analysis among different ethnic groups. *Int J of obesity rltd met disorders* 22:1164-1171.

68. Dudeja, V., Misra, A., Pandey, R.M., Devina, G., Kumar, G., and Vikram, N.K. 2001. BMI does not accurately predict overweight in Asian Indians in northern India. *Br J Nutr* 86:105-112.
69. de Koning, L., Merchant, A.T., Pogue, J., and Anand, S.S. 2007. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *European heart journal* 28:850-856.
70. Janssen, I., Katzmarzyk, P.T., and Ross, R. 2004. Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr* 79:379-384.
71. Jackson, A., Pallock, M.L. 1985. Practical assessment of body composition. *Physician Sports Medicine* 13:76-90.
72. Durnin, J.V., and Womersley, J. 1974. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32:77-97.
73. Jackson, A.S., and Pollock, M.L. 1978. Generalized equations for predicting body density of men. *Br J Nutr* 40:497-504.
74. Stunkard, A.J., Foch, T.T., and Hrubec, Z. 1986. A twin study of human obesity. *JAMA* 256:51-54.
75. Stunkard, A.J., Harris, J.R., Pedersen, N.L., and McClearn, G.E. 1990. The body-mass index of twins who have been reared apart. *N Engl J Med* 322:1483-1487.
76. Poulsen, P., Kyvik, K.O., Vaag, A., and Beck-Nielsen, H. 1999. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. *Diabetologia* 42:139-145.
77. Rose, K.M., Newman, B., Mayer-Davis, E.J., and Selby, J.V. 1998. Genetic and behavioral determinants of waist-hip ratio and waist circumference in women twins. *Obes Res* 6:383-392.
78. Selby, J.V., Newman, B., Quesenberry, C.P., Jr., Fabsitz, R.R., Carmelli, D., Meaney, F.J., and Slemenda, C. 1990. Genetic and behavioral influences on body fat distribution. *Int J Obes* 14:593-602.
79. Loos, R.J., Lindgren, C.M., Li, S., Wheeler, E., Zhao, J.H., Prokopenko, I., Inouye, M., Freathy, R.M., Attwood, A.P., Beckmann, J.S., et al. 2008. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet* 40:768-775.
80. Ooi, C.L., Kennedy, J.L., and Levitan, R.D. 2012. A putative model of overeating and obesity based on brain-derived neurotrophic factor: direct and indirect effects. *Behav Neurosci* 126:505-514.
81. Herbert, A., Gerry, N.P., McQueen, M.B., Heid, I.M., Pfeufer, A., Illig, T., Wichmann, H.E., Meitinger, T., Hunter, D., Hu, F.B., et al. 2006. A common genetic variant is associated with adult and childhood obesity. *Science* 312:279-283.
82. Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., Perry, J.R., Elliott, K.S., Lango, H., Rayner, N.W., et al. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889-894.
83. Rankinen, T., Zuberi, A., Chagnon, Y.C., Weisnagel, S.J., Argyropoulos, G., Walts, B., Perusse, L., and Bouchard, C. 2006. The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* 14:529-644.
84. Walley, A.J., Asher, J.E., and Froguel, P. 2009. The genetic contribution to non-syndromic human obesity. *Nat rev. Genet* 10:431-442.
85. Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U., Allen, H.L., Lindgren, C.M., Luan, J., Magi, R., et al. 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 42:937-948.
86. Heitmann, B.L., Westerterp, K.R., Loos, R.J., Sorensen, T.I., O'Dea, K., McLean, P., Jensen, T.K., Eisenmann, J., Speakman, J.R., Simpson, S.J., et al. 2012. Obesity: lessons from evolution and the environment. *Obes Rev* 13:910-922.

87. Wang, K., Li, W.D., Glessner, J.T., Grant, S.F., Hakonarson, H., and Price, R.A. 2010. Large copy-number variations are enriched in cases with moderate to extreme obesity. *Diabetes* 59:2690-2694.
88. Hebebrand, J., Volckmar, A.L., Knoll, N., and Hinney, A. 2010. Chipping away the 'missing heritability': GIANT steps forward in the molecular elucidation of obesity - but still lots to go. *Obes Facts* 3:294-303.
89. Köbberling, J.T., H. 1982. Empirical risk figures for first degree relatives of non-insulin dependent diabetics: London: Academic Press. 201-209 pp.
90. Lyssenko, V., Almgren, P., Anevski, D., Perfekt, R., Lahti, K., Nissen, M., Isomaa, B., Forsen, B., Homstrom, N., Saloranta, C., et al. 2005. Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. *Diabetes* 54:166-174.
91. Kaprio, J., Tuomilehto, J., Koskenvuo, M., Romanov, K., Reunanen, A., Eriksson, J., Stengard, J., and Kesaniemi, Y.A. 1992. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 35:1060-1067.
92. Newman, B., Selby, J.V., King, M.C., Slemenda, C., Fabsitz, R., and Friedman, G.D. 1987. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. *Diabetologia* 30:763-768.
93. Shaw, J.E., Sicree, R.A., and Zimmet, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 87:4-14.
94. Chan, J.C., Malik, V., Jia, W., Kadowaki, T., Yajnik, C.S., Yoon, K.H., and Hu, F.B. 2009. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA* 301:2129-2140.
95. Yoon, K.H., Lee, J.H., Kim, J.W., Cho, J.H., Choi, Y.H., Ko, S.H., Zimmet, P., and Son, H.Y. 2006. Epidemic obesity and type 2 diabetes in Asia. *Lancet* 368:1681-1688.
96. Stumvoll, M., Goldstein, B.J., and van Haeften, T.W. 2005. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365:1333-1346.
97. Mills, G.W., Avery, P.J., McCarthy, M.I., Hattersley, A.T., Levy, J.C., Hitman, G.A., Sampson, M., and Walker, M. 2004. Heritability estimates for beta cell function and features of the insulin resistance syndrome in UK families with an increased susceptibility to type 2 diabetes. *Diabetologia* 47:732-738.
98. Muniyappa, R., Lee, S., Chen, H., and Quon, M.J. 2008. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 294:E15-26.
99. Permutt, M.A., Wasson, J., and Cox, N. 2005. Genetic epidemiology of diabetes. *J Clin Invest* 115:1431-1439.
100. Horikawa, Y., Oda, N., Cox, N.J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T.H., Mashima, H., Schwarz, P.E., et al. 2000. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163-175.
101. Grant, S.F., Thorleifsson, G., Reynisdottir, I., Benediktsson, R., Manolescu, A., Sainz, J., Helgason, A., Stefansson, H., Emilsson, V., Helgadottir, A., et al. 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320-323.
102. Deeb, S.S., Fajas, L., Nemoto, M., Pihlajamaki, J., Mykkanen, L., Kuusisto, J., Laakso, M., Fujimoto, W., and Auwerx, J. 1998. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284-287.
103. Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., Zeggini, E., Huth, C., Aulchenko, Y.S., Thorleifsson, G., et al. 2010. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 42:579-589.
104. Saxena, R., Hivert, M.F., Langenberg, C., Tanaka, T., Pankow, J.S., Vollenweider, P., Lyssenko, V., Bouatia-Naji, N., Dupuis, J., Jackson, A.U., et al. 2010. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet* 42:142-148.
105. Sandhu, M.S., Weedon, M.N., Fawcett, K.A., Wasson, J., Debenham, S.L., Daly, A., Lango, H., Frayling, T.M., Neumann, R.J., Sherva, R., et al. 2007.

Common variants in WFS1 confer risk of type 2 diabetes. *Nat Genet* 39:951-953.
106. Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., Daly, M.J., et al. 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331-1336.
107. Scott, L.J., Mohlke, K.L., Bonnycastle, L.L., Willer, C.J., Li, Y., Duren, W.L., Erdos, M.R., Stringham, H.M., Chines, P.S., Jackson, A.U., et al. 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341-1345.
108. Sladek, R., Rocheleau, G., Rung, J., Dina, C., Shen, L., Serre, D., Boutin, P., Vincent, D., Belisle, A., Hadjadj, S., et al. 2007. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881-885.
109. Zeggini, E., Weedon, M.N., Lindgren, C.M., Frayling, T.M., Elliott, K.S., Lango, H., Timpson, N.J., Perry, J.R., Rayner, N.W., Freathy, R.M., et al. 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336-1341.
110. Saxena, R., Saleheen, D., Been, L.F., Garavito, M.L., Braun, T., Bjonnes, A., Young, R., Ho, W.K., Rasheed, A., Frossard, P., et al. 2013. Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in sikhs of punjabi origin from India. *Diabetes* 62:1746-1755.
111. Tabassum, R., Chauhan, G., Dwivedi, O.P., Mahajan, A., Jaiswal, A., Kaur, I., Bandesh, K., Singh, T., Mathai, B.J., Pandey, Y., et al. 2013. Genome-wide association study for type 2 diabetes in Indians identifies a new susceptibility locus at 2q21. *Diabetes* 62:977-986.
112. Zeggini, E., Scott, L.J., Saxena, R., Voight, B.F., Marchini, J.L., Hu, T., de Bakker, P.I., Abecasis, G.R., Almgren, P., Andersen, G., et al. 2008. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40:638-645.
113. Zeggini, E., and McCarthy, M.I. 2007. TCF7L2: the biggest story in diabetes genetics since HLA? *Diabetologia* 50:1-4.
114. Wellen, K.E., and Hotamisligil, G.S. 2005. Inflammation, stress, and diabetes. *J Clin Invest* 115:1111-1119.
115. Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H., and Hotamisligil, G.S. 2004. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306:457-461.
116. Lowell, B.B., and Shulman, G.I. 2005. Mitochondrial dysfunction and type 2 diabetes. *Science* 307:384-387.
117. Boden, G., and Shulman, G.I. 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 32 Suppl 3:14-23.
118. Patel, P.S., Buras, E.D., and Balasubramanyam, A. 2013. The role of the immune system in obesity and insulin resistance. *J Obes* 2013:616193.
119. Thorleifsson, G., Walters, G.B., Gudbjartsson, D.F., Steinthorsdottir, V., Sulem, P., Helgadottir, A., Styrkarsdottir, U., Gretarsdottir, S., Thorlacius, S., Jonsdottir, I., et al. 2009. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 41:18-24.
120. Gluckman, P.D., Hanson, M.A., and Pinal, C. 2005. The developmental origins of adult disease. *Matern Child Nutr* 1:130-141.
121. Martin-Gronert, M.S., and Ozanne, S.E. 2012. Mechanisms underlying the developmental origins of disease. *Rev Endocr Metab Disord* 13:85-92.
122. Barker, D.J. 2007. The origins of the developmental origins theory. *J Int Medicine* 261:412-417.
123. Barker, D.J., and Osmond, C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1:1077-1081.
124. Whincup, P.H., Kaye, S.J., Owen, C.G., Huxley, R., Cook, D.G., Anazawa, S., Barrett-Connor, E., Bhargava, S.K., Birgisdottir, B.E., Carlsson, S., et al. 2008.

Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 300:2886-2897.
125. Wilkin, T.J., Metcalf, B.S., Murphy, M.J., Kirkby, J., Jeffery, A.N., and Voss, L.D. 2002. The relative contributions of birth weight, weight change, and current weight to insulin resistance in contemporary 5-year-olds: the EarlyBird Study. *Diabetes* 51:3468-3472.
126. Yajnik, C.S., Fall, C.H., Coyaji, K.J., Hirve, S.S., Rao, S., Barker, D.J., Joglekar, C., and Kellingray, S. 2003. Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. *Int J Obes Relat Metab Disord* 27:173-180.
127. Newsome, C.A., Shiell, A.W., Fall, C.H., Phillips, D.I., Shier, R., and Law, C.M. 2003. Is birth weight related to later glucose and insulin metabolism?--A systematic review. *Diabet Med* 20:339-348.
128. Hattersley, A.T., and Tooke, J.E. 1999. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 353:1789-1792.
129. Weedon, M.N., Frayling, T.M., Shields, B., Knight, B., Turner, T., Metcalf, B.S., Voss, L., Wilkin, T.J., McCarthy, A., Ben-Shlomo, Y., et al. 2005. Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene. *Diabetes* 54:576-581.
130. Vaessen, N., Janssen, J.A., Heutink, P., Hofman, A., Lamberts, S.W., Oostra, B.A., Pols, H.A., and van Duijn, C.M. 2002. Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight. *Lancet* 359:1036-1037.
131. Andersson, E.A., Pilgaard, K., Pisinger, C., Harder, M.N., Grarup, N., Faerch, K., Poulsen, P., Witte, D.R., Jorgensen, T., Vaag, A., et al. 2010. Type 2 diabetes risk alleles near ADCY5, CDKAL1 and HHEX-IDE are associated with reduced birthweight. *Diabetologia* 53:1908-1916.
132. Freathy, R.M., Bennett, A.J., Ring, S.M., Shields, B., Groves, C.J., Timpson, N.J., Weedon, M.N., Zeggini, E., Lindgren, C.M., Lango, H., et al. 2009. Type 2 diabetes risk alleles are associated with reduced size at birth. *Diabetes* 58:1428-1433.
133. Lunde, A., Melve, K.K., Gjessing, H.K., Skjaerven, R., and Irgens, L.M. 2007. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. *Am J Epidemiol* 165:734-741.
134. Freathy, R.M., Mook-Kanamori, D.O., Sovio, U., Prokopenko, I., Timpson, N.J., Berry, D.J., Warrington, N.M., Widen, E., Hottenga, J.J., Kaakinen, M., et al. 2010. Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. *Nat Genet* 42:430-435.
135. Hattersley, A.T., Beards, F., Ballantyne, E., Appleton, M., Harvey, R., and Ellard, S. 1998. Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat Genet* 19:268-270.
136. Horikoshi, M., Yaghootkar, H., Mook-Kanamori, D.O., Sovio, U., Taal, H.R., Hennig, B.J., Bradfield, J.P., St Pourcain, B., Evans, D.M., Charoen, P., et al. 2013. New loci associated with birth weight identify genetic links between intrauterine growth and adult height and metabolism. *Nat Genet* 45:76-82.
137. Berends, L.M., and Ozanne, S.E. 2012. Early determinants of type-2 diabetes. *Best Pract Res Clin Endocrinol Metab* 26:569-580.
138. Santos, M.S., and Joles, J.A. 2012. Early determinants of cardiovascular disease. *Best Pract Res Clin Endocrinol Metab* 26:581-597.
139. Yajnik, C.S., Deshpande, S.S., Jackson, A.A., Refsum, H., Rao, S., Fisher, D.J., Bhat, D.S., Naik, S.S., Coyaji, K.J., Joglekar, C.V., et al. 2008. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 51:29-38.
140. Waterland, R.A., and Jirtle, R.L. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23:5293-5300.
141. Lillycrop, K.A., Phillips, E.S., Jackson, A.A., Hanson, M.A., and Burdge, G.C. 2005. Dietary protein restriction of pregnant rats induces and folic acid

supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 135:1382-1386.
142. Sandovici, I., Smith, N.H., Nitert, M.D., Ackers-Johnson, M., Uribe-Lewis, S., Ito, Y., Jones, R.H., Marquez, V.E., Cairns, W., Tadayyon, M., et al. 2011. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. *Proc Natl Acad Sci U S A* 108:5449-5454.
143. Heijmans, B.T., Tobi, E.W., Stein, A.D., Putter, H., Blauw, G.J., Susser, E.S., Slagboom, P.E., and Lumey, L.H. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A* 105:17046-17049.
144. Tobi, E.W., Lumey, L.H., Talens, R.P., Kremer, D., Putter, H., Stein, A.D., Slagboom, P.E., and Heijmans, B.T. 2009. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* 18:4046-4053.
145. McKeigue, P.M., Shah, B., and Marmot, M.G. 1991. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 337:382-386.
146. McKeigue, P.M., Pierpoint, T., Ferrie, J.E., and Marmot, M.G. 1992. Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in south Asians and Europeans. *Diabetologia* 35:785-791.
147. Chandalia, M., Abate, N., Garg, A., Stray-Gundersen, J., and Grundy, S.M. 1999. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 84:2329-2335.
148. Lear, S.A., Kohli, S., Bondy, G.P., Tchernof, A., and Sniderman, A.D. 2009. Ethnic variation in fat and lean body mass and the association with insulin resistance. *J Clin Endocrinol Metab* 94:4696-4702.
149. Raji, A., Seely, E.W., Arky, R.A., and Simonson, D.C. 2001. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *The J Clin Endocrinol Metab* 86:5366-5371.
150. Stein, C.E., Fall, C.H., Kumaran, K., Osmond, C., Cox, V., and Barker, D.J. 1996. Fetal growth and coronary heart disease in south India. *Lancet* 348:1269-1273.
151. Zhang, F.F., Cardarelli, R., Carroll, J., Fulda, K.G., Kaur, M., Gonzalez, K., Vishwanatha, J.K., Santella, R.M., and Morabia, A. 2011. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics* 6:623-629.
152. Adeyemo, A., and Rotimi, C. 2010. Genetic variants associated with complex human diseases show wide variation across multiple populations. *Public Health Genomics* 13:72-79.
153. Hancock, A.M., Witonsky, D.B., Ehler, E., Alkorta-Aranburu, G., Beall, C., Gebremedhin, A., Sukernik, R., Utermann, G., Pritchard, J., Coop, G., et al. 2010. Colloquium paper: human adaptations to diet, subsistence, and ecoregion are due to subtle shifts in allele frequency. *Proc Natl Acad Sci U S A* 107 Suppl 2:8924-8930.
154. Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., DeFelice, M., Lochner, A., Faggart, M., et al. 2002. The structure of haplotype blocks in the human genome. *Science* 296:2225-2229.
155. Li, H., Wu, Y., Loos, R.J., Hu, F.B., Liu, Y., Wang, J., Yu, Z., and Lin, X. 2008. Variants in the fat mass- and obesity-associated (FTO) gene are not associated with obesity in a Chinese Han population. *Diabetes* 57:264-268.
156. Tabassum, R., Mahajan, A., Chauhan, G., Dwivedi, O.P., Dubey, H., Sharma, V., Kundu, B., Ghosh, S., Tandon, N., and Bharadwaj, D. 2011. No association of TNFRSF1B variants with type 2 diabetes in Indians of Indo-European origin. *BMC Med Genet* 12:110.
157. 2005. The Indian Genome Variation database (IGVdb): a project overview. *Human genetics* 118:1-11.
158. Hardy, B.J., Seguin, B., Singer, P.A., Mukerji, M., Brahmachari, S.K., and Daar, A.S. 2008. From diversity to delivery: the case of the Indian Genome Variation initiative. *Nat Rev Genet* 9 Suppl 1:S9-14.

159. Yajnik, C.S. 2002. The lifecycle effects of nutrition and body size on adult adiposity, diabetes and cardiovascular disease. *Obesity reviews 3*:217-224.
160. Fall, C.H., Stein, C.E., Kumaran, K., Cox, V., Osmond, C., Barker, D.J., and Hales, C.N. 1998. Size at birth, maternal weight, and type 2 diabetes in South India. *Diabetic medicine* 15:220-227.
161. Rao, S., Yajnik, C.S., Kanade, A., Fall, C.H., Margetts, B.M., Jackson, A.A., Shier, R., Joshi, S., Rege, S., Lubree, H., et al. 2001. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. *J Nutr* 131:1217-1224.
162. Wells, J.C. 2012. Ethnic variability in adiposity, thrifty phenotypes and cardiometabolic risk: addressing the full range of ethnicity, including those of mixed ethnicity. *Obes Rev* 13 Suppl 2:14-29.
163. Deurenberg-Yap, M., Chew, S.K., and Deurenberg, P. 2002. Elevated body fat percentage and cardiovascular risks at low body mass index levels among Singaporean Chinese, Malays and Indians. *Obesity reviews* 3:209-215.
164. Wulan, S.N., Westerterp, K.R., and Plasqui, G. 2010. Ethnic differences in body composition and the associated metabolic profile: a comparative study between Asians and Caucasians. *Maturitas* 65:315-319.
165. Banerji, M.A., Faridi, N., Atluri, R., Chaiken, R.L., and Lebovitz, H.E. 1999. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 84:137-144.
166. Indulekha, K., Anjana, R.M., Surendar, J., and Mohan, V. 2011. Association of visceral and subcutaneous fat with glucose intolerance, insulin resistance, adipocytokines and inflammatory markers in Asian Indians (CURES-113). *Clin Biochem* 44:281-287.
167. Misra, A., Garg, A., Abate, N., Peshock, R.M., Stray-Gundersen, J., and Grundy, S.M. 1997. Relationship of anterior and posterior subcutaneous abdominal fat to insulin sensitivity in nondiabetic men. *Obes Res* 5:93-99.
168. Misra, A., and Khurana, L. 2009. The metabolic syndrome in South Asians: epidemiology, determinants, and prevention. *Metabolic syndrome and related disorders* 7:497-514.
169. Rao, K.R., Padmavathi, I.J., and Raghunath, M. 2012. Maternal micronutrient restriction programs the body adiposity, adipocyte function and lipid metabolism in offspring: a review. *Rev Endocr Metab Disord* 13:103-108.
170. Forouhi, N.G., Jenkinson, G., Thomas, E.L., Mullick, S., Mierisova, S., Bhonsle, U., McKeigue, P.M., and Bell, J.D. 1999. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 42:932-935.
171. Been, L.F., Nath, S.K., Ralhan, S.K., Wander, G.S., Mehra, N.K., Singh, J., Mulvihill, J.J., and Sanghera, D.K. 2010. Replication of association between a common variant near melanocortin-4 receptor gene and obesity-related traits in Asian Sikhs. *Obesity* 18:425-429.
172. Dwivedi, O.P., Tabassum, R., Chauhan, G., Kaur, I., Ghosh, S., Marwaha, R.K., Tandon, N., and Bharadwaj, D. 2013. Strong influence of variants near MC4R on adiposity in children and adults: a cross-sectional study in Indian population. *J Hum Genet* 58:27-32.
173. Xi, B., Chandak, G.R., Shen, Y., Wang, Q., and Zhou, D. 2012. Association between common polymorphism near the MC4R gene and obesity risk: a systematic review and meta-analysis. *PLoS One* 7:e45731.
174. Janipalli, C.S., Kumar, M.V., Vinay, D.G., Sandeep, M.N., Bhaskar, S., Kulkarni, S.R., Aruna, M., Joglekar, C.V., Priyadharshini, S., Maheshwari, N., et al. 2012. Analysis of 32 common susceptibility genetic variants and their combined effect in predicting risk of Type 2 diabetes and related traits in Indians. *Diabetic medicine* 29:121-127.
175. Prakash, J., Srivastava, N., Awasthi, S., Agarwal, C.G., Natu, S.M., Rajpal, N., and Mittal, B. 2011. Association of FTO rs17817449 SNP with obesity and associated physiological parameters in a north Indian population. *Annals of human biology* 38:760-763.
176. Sanghera, D.K., Ortega, L., Han, S., Singh, J., Ralhan, S.K., Wander, G.S., Mehra, N.K., Mulvihill, J.J., Ferrell, R.E., Nath, S.K., et al. 2008. Impact of

nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Med Genet* 9:59.
177. Taylor, A.E., Sandeep, M.N., Janipalli, C.S., Giambartolomei, C., Evans, D.M., Kranthi Kumar, M.V., Vinay, D.G., Smitha, P., Gupta, V., Aruna, M., et al. 2011. Associations of FTO and MC4R Variants with Obesity Traits in Indians and the Role of Rural/Urban Environment as a Possible Effect Modifier. *Journal of obesity* 2011:307542.
178. Yajnik, C.S., Janipalli, C.S., Bhaskar, S., Kulkarni, S.R., Freathy, R.M., Prakash, S., Mani, K.R., Weedon, M.N., Kale, S.D., Deshpande, J., et al. 2009. FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. *Diabetologia* 52:247-252.
179. Li, H., Kilpelainen, T.O., Liu, C., Zhu, J., Liu, Y., Hu, C., Yang, Z., Zhang, W., Bao, W., Cha, S., et al. 2012. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. *Diabetologia* 55:981-995.
180. Viswanathan, M., McCarthy, M.I., Snehalatha, C., Hitman, G.A., and Ramachandran, A. 1996. Familial aggregation of type 2 (non-insulin-dependent) diabetes mellitus in south India; absence of excess maternal transmission. *Diabet Med* 13:232-237.
181. Radha, V., Vimaleswaran, K.S., Babu, H.N., Abate, N., Chandalia, M., Satija, P., Grundy, S.M., Ghosh, S., Majumder, P.P., Deepa, R., et al. 2006. Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: Evidence for heterogeneity. *Diabetes Care* 29:1046-1051.
182. Sanghera, D.K., Demirci, F.Y., Been, L., Ortega, L., Ralhan, S., Wander, G.S., Mehra, N.K., Singh, J., Aston, C.E., Mulvihill, J.J., et al. 2010. PPARG and ADIPOQ gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor. *Metabolism* 59:492-501.
183. Been, L.F., Ralhan, S., Wander, G.S., Mehra, N.K., Singh, J., Mulvihill, J.J., Aston, C.E., and Sanghera, D.K. 2011. Variants in KCNQ1 increase type II diabetes susceptibility in South Asians: a study of 3,310 subjects from India and the US. *BMC Med Genet* 12:18.
184. Chauhan, G., Spurgeon, C.J., Tabassum, R., Bhaskar, S., Kulkarni, S.R., Mahajan, A., Chavali, S., Kumar, M.V., Prakash, S., Dwivedi, O.P., et al. 2010. Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5,164 Indians. *Diabetes* 59:2068-2074.
185. Chidambaram, M., Radha, V., and Mohan, V. 2010. Replication of recently described type 2 diabetes gene variants in a South Indian population. *Metabolism* 59:1760-1766.
186. Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., Glazer, N.L., Bouatia-Naji, N., Gloyn, A.L., et al. 2010. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 42:105-116.
187. Ryckman, K.K., Feenstra, B., Shaffer, J.R., Bream, E.N., Geller, F., Feingold, E., Weeks, D.E., Gadow, E., Cosentino, V., Saleme, C., et al. 2012. Replication of a genome-wide association study of birth weight in preterm neonates. *J Pediatr* 160:19-24 e14.
188. Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., Glazer, N.L., Bouatia-Naji, N., Gloyn, A.L., et al. 2010. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 42:105-116.
189. Chen, H.H., Wang, Y.C., and Fann, M.J. 2006. Identification and characterization of the CDK12/cyclin L1 complex involved in alternative splicing regulation. *Mol Cell Biol* 26:2736-2745.
190. Herrmann, A., Fleischer, K., Czajkowska, H., Muller-Newen, G., and Becker, W. 2007. Characterization of cyclin L1 as an immobile component of the splicing factor compartment. *FASEB J* 21:3142-3152.

191. Mook-Kanamori, D.O., Marsh, J.A., Warrington, N.M., Taal, H.R., Newnham, J.P., Beilin, L.J., Lye, S.J., Palmer, L.J., Hofman, A., Steegers, E.A., et al. 2011. Variants near CCNL1/LEKR1 and in ADCY5 and fetal growth characteristics in different trimesters. *J Clin Endocrinol Metab* 96:E810-815.
192. Gerken, T., Girard, C.A., Tung, Y.C., Webby, C.J., Saudek, V., Hewitson, K.S., Yeo, G.S., McDonough, M.A., Cunliffe, S., McNeill, L.A., et al. 2007. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 318:1469-1472.
193. Peters, T., Ausmeier, K., and Ruther, U. 1999. Cloning of Fatso (Fto), a novel gene deleted by the Fused toes (Ft) mouse mutation. *Mamm Genome* 10:983-986.
194. Dina, C., Meyre, D., Gallina, S., Durand, E., Korner, A., Jacobson, P., Carlsson, L.M., Kiess, W., Vatin, V., Lecoeur, C., et al. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724-726.
195. Scuteri, A., Sanna, S., Chen, W.M., Uda, M., Albai, G., Strait, J., Najjar, S., Nagaraja, R., Orru, M., Usala, G., et al. 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 3:e115.
196. Kring, S.I., Holst, C., Zimmermann, E., Jess, T., Berentzen, T., Toubro, S., Hansen, T., Astrup, A., Pedersen, O., and Sorensen, T.I. 2008. FTO gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. *PloS one* 3:e2958.
197. Willer, C.J., Speliotes, E.K., Loos, R.J., Li, S., Lindgren, C.M., Heid, I.M., Berndt, S.I., Elliott, A.L., Jackson, A.U., Lamina, C., et al. 2009. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41:25-34.
198. Adeyemo, A., Chen, G., Zhou, J., Shriner, D., Doumatey, A., Huang, H., and Rotimi, C. 2010. FTO genetic variation and association with obesity in West Africans and African Americans. *Diabetes* 59:1549-1554.
199. Hassanein, M.T., Lyon, H.N., Nguyen, T.T., Akylbekova, E.L., Waters, K., Lettre, G., Tayo, B., Forrester, T., Sarpong, D.F., Stram, D.O., et al. 2010. Fine mapping of the association with obesity at the FTO locus in African-derived populations. *Hum Mol Genet* 19:2907-2916.
200. Tan, J.T., Dorajoo, R., Seielstad, M., Sim, X.L., Ong, R.T., Chia, K.S., Wong, T.Y., Saw, S.M., Chew, S.K., Aung, T., et al. 2008. FTO variants are associated with obesity in the Chinese and Malay populations in Singapore. *Diabetes* 57:2851-2857.
201. Xi, B., Shen, Y., Zhang, M., Liu, X., Zhao, X., Wu, L., Cheng, H., Hou, D., Lindpaintner, K., Liu, L., et al. 2010. The common rs9939609 variant of the fat mass and obesity-associated gene is associated with obesity risk in children and adolescents of Beijing, China. *BMC medical genetics* 11:107.
202. Tung, Y.C., and Yeo, G.S. 2011. From GWAS to biology: lessons from FTO. *Ann N Y Acad Sci* 1220:162-171.
203. Cecil, J.E., Tavendale, R., Watt, P., Hetherington, M.M., and Palmer, C.N. 2008. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med* 359:2558-2566.
204. Jonsson, A., and Franks, P.W. 2009. Obesity, FTO gene variant, and energy intake in children. *N Engl J Med* 360:1571-1572.
205. Jonsson, A., Renstrom, F., Lyssenko, V., Brito, E.C., Isomaa, B., Berglund, G., Nilsson, P.M., Groop, L., and Franks, P.W. 2009. Assessing the effect of interaction between an FTO variant (rs9939609) and physical activity on obesity in 15,925 Swedish and 2,511 Finnish adults. *Diabetologia* 52:1334-1338.
206. Tanofsky-Kraff, M., Han, J.C., Anandalingam, K., Shomaker, L.B., Columbo, K.M., Wolkoff, L.E., Kozlosky, M., Elliott, C., Ranzenhofer, L.M., Roza, C.A., et al. 2009. The FTO gene rs9939609 obesity-risk allele and loss of control over eating. *Am J Clin Nutr* 90:1483-1488.
207. Wardle, J., Llewellyn, C., Sanderson, S., and Plomin, R. 2009. The FTO gene and measured food intake in children. *Int J Obes (Lond)* 33:42-45.

208. den Hoed, M., Westerterp-Plantenga, M.S., Bouwman, F.G., Mariman, E.C., and Westerterp, K.R. 2009. Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in FTO. *Am J Clin Nutr* 90:1426-1432.
209. Balthasar, N., Dalgaard, L.T., Lee, C.E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., McGovern, R.A., Kenny, C.D., et al. 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123:493-505.
210. Cone, R.D. 2005. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 8:571-578.
211. Tao, Y.X. 2010. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr Rev* 31:506-543.
212. Xi, B., Takeuchi, F., Chandak, G.R., Kato, N., Pan, H.W., Zhou, D.H., Pan, H.Y., and Mi, J. 2012. Common polymorphism near the MC4R gene is associated with type 2 diabetes: data from a meta-analysis of 123,373 individuals. *Diabetologia* 55:2660-2666.
213. Rao, P.S., and Inbaraj, S.G. 1973. Some aspects of pregnancy terminations in a South Indian population. *Indian J of Med research* 61:1247-1255.
214. Antonisamy, B., Raghupathy, P., Christopher, S., Richard, J., Rao, P.S., Barker, D.J., and Fall, C.H. 2009. Cohort Profile: the 1969-73 Vellore birth cohort study in South India. *Int J of epidemiology* 38:663-669.
215. Vasan, S.K., Thomas, N., Christopher, S., Geethanjali, F.S., Paul, T.V., Sanjeevi, C.B. 2011. Anthropometric measurements for the prediction of the metabolic syndrome: a cross-sectional study on adolescents and young adults from southern india *Heart Asia* 3:2-7.
216. Tan, G.D., Neville, M.J., Liverani, E., Humphreys, S.M., Currie, J.M., Dennis, L., Fielding, B.A., and Karpe, F. 2006. The in vivo effects of the Pro12Ala PPARgamma2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank. *Diabetologia* 49:158-168.
217. Bartoli, E., Fra, G.P., and Carnevale Schianca, G.P. 2011. The oral glucose tolerance test (OGTT) revisited. *Eur J Intern Med* 22:8-12.
218. Reaven, G.M., Brand, R.J., Chen, Y.D., Mathur, A.K., and Goldfine, I. 1993. Insulin resistance and insulin secretion are determinants of oral glucose tolerance in normal individuals. *Diabetes* 42:1324-1332.
219. Hollenbeck, C.B., Chen, N., Chen, Y.D., and Reaven, G.M. 1984. Relationship between the plasma insulin response to oral glucose and insulin-stimulated glucose utilization in normal subjects. *Diabetes* 33:460-463.
220. Warram, J.H., Martin, B.C., Krolewski, A.S., Soeldner, J.S., and Kahn, C.R. 1990. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 113:909-915.
221. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419.
222. Wallace, T.M., Levy, J.C., and Matthews, D.R. 2004. Use and abuse of HOMA modeling. *Diabetes Care* 27:1487-1495.
223. Matsuda, M., and DeFronzo, R.A. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462-1470.
224. Stumvoll, M., Mitrakou, A., Pimenta, W., Jenssen, T., Yki-Jarvinen, H., Van Haeften, T., Haring, H., Fritsche, A., and Gerich, J. 2000. Assessment of insulin secretion from the oral glucose tolerance test in white patients with type 2 diabetes. *Diabetes Care* 23:1440-1441.
225. Stumvoll, M., Mitrakou, A., Pimenta, W., Jenssen, T., Yki-Jarvinen, H., Van Haeften, T., Renn, W., and Gerich, J. 2000. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 23:295-301.
226. Phillips, D.I., Clark, P.M., Hales, C.N., and Osmond, C. 1994. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during

the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabet Med* 11:286-292.
227. Tschritter, O., Fritsche, A., Shirkavand, F., Machicao, F., Haring, H., and Stumvoll, M. 2003. Assessing the shape of the glucose curve during an oral glucose tolerance test. *Diabetes Care* 26:1026-1033.
228. Hill, W.G., Goddard, M.E., and Visscher, P.M. 2008. Data and theory point to mainly additive genetic variance for complex traits. *PLoS Genet* 4:e1000008.
229. Shoemaker, J., Painter, I., and Weir, B.S. 1998. A Bayesian characterization of Hardy-Weinberg disequilibrium. *Genetics* 149:2079-2088.
230. Brockwell, S.E., and Gordon, I.R. 2001. A comparison of statistical methods for meta-analysis. *Stat Med* 20:825-840.
231. Egger, M., Davey Smith, G., Schneider, M., and Minder, C. 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315:629-634.
232. Duval, S., and Tweedie, R. 2000. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56:455-463.
233. Nakaoka, H., and Inoue, I. 2009. Meta-analysis of genetic association studies: methodologies, between-study heterogeneity and winner's curse. *J Hum Genet* 54:615-623.
234. Higgins, J.P., and Thompson, S.G. 2002. Quantifying heterogeneity in a meta-analysis. *Statistics in medicine* 21:1539-1558.
235. Higgins, J.P., Thompson, S.G., Deeks, J.J., and Altman, D.G. 2003. Measuring inconsistency in meta-analyses. *BMJ* 327:557-560.
236. Vasan, S.K., Neville, M.J., Antonisamy, B., Samuel, P., Fall, C.H., Geethanjali, F.S., Thomas, N., Raghupathy, P., Brismar, K., and Karpe, F. 2011. Absence of birth-weight lowering effect of ADCY5 and near CCNL, but association of impaired glucose-insulin homeostasis with ADCY5 in Asian Indians. *PloS one* 6:e21331.
237. Vasan, S.K., Fall, T., Neville, M.J., Antonisamy, B., Fall, C.H., Geethanjali, F.S., Gu, H.F., Raghupathy, P., Samuel, P., Thomas, N., et al. 2012. Associations of variants in FTO and near MC4R with obesity traits in South Asian Indians. *Obesity*.
238. Vasan, S.K., Fall, T., Job, V., Gu, H.F., Ingelsson, E., Brismar, K., Karpe, F., and Thomas, N. 2013. A common variant in the FTO locus is associated with waist-hip ratio in Indian adolescents. *Pediatr Obes*.
239. Klebanoff, M.A., Mednick, B.R., Schulsinger, C., Secher, N.J., and Shiono, P.H. 1998. Father's effect on infant birth weight. *Am J Obstet Gynecol* 178:1022-1026.
240. Knight, B., Shields, B.M., Turner, M., Powell, R.J., Yajnik, C.S., and Hattersley, A.T. 2005. Evidence of genetic regulation of fetal longitudinal growth. *Early Hum Dev* 81:823-831.
241. Walther, F.J., and Ramaekers, L.H. 1982. The ponderal index as a measure of the nutritional status at birth and its relation to some aspects of neonatal morbidity. *J Perinat Med* 10:42-47.
242. Andersson, E.A., Harder, M.N., Pilgaard, K., Pisinger, C., Stancakova, A., Kuusisto, J., Grarup, N., Faerch, K., Poulsen, P., Witte, D.R., et al. 2011. The birth weight lowering C-allele of rs900400 near LEKR1 and CCNL1 associates with elevated insulin release following an oral glucose challenge. *PLoS One* 6:e27096.
243. Urbanek, M., Hayes, M.G., Armstrong, L.L., Morrison, J., Lowe, L.P., Badon, S.E., Scheftner, D., Pluzhnikov, A., Levine, D., Laurie, C.C., et al. 2013. The chromosome 3q25 genomic region is associated with measures of adiposity in newborns in a multi-ethnic genome-wide association study. *Hum Mol Genet*.
244. Yajnik, C.S. 2005. Size and body composition at birth and risk of type-2 diabetes. *Nestle Nutr Workshop Ser Pediatr Program* 55:169-178; discussion 178-182.
245. Yajnik, C.S., Lubree, H.G., Rege, S.S., Naik, S.S., Deshpande, J.A., Deshpande, S.S., Joglekar, C.V., and Yudkin, J.S. 2002. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab* 87:5575-5580.

246. Misra, A., and Khurana, L. 2011. Obesity-related non-communicable diseases: South Asians vs White Caucasians. *Int Jof obes* 35:167-187.
247. Zhao, J., Bradfield, J.P., Zhang, H., Sleiman, P.M., Kim, C.E., Glessner, J.T., Deliard, S., Thomas, K.A., Frackelton, E.C., Li, M., et al. 2011. Role of BMI-associated loci identified in GWAS meta-analyses in the context of common childhood obesity in European Americans. *Obesity* 19:2436-2439.
248. Hardy, R., Wills, A.K., Wong, A., Elks, C.E., Wareham, N.J., Loos, R.J., Kuh, D., and Ong, K.K. 2010. Life course variations in the associations between FTO and MC4R gene variants and body size. *Hum mol gen* 19:545-552.
249. Kaakinen, M., Laara, E., Pouta, A., Hartikainen, A.L., Laitinen, J., Tammelin, T.H., Herzig, K.H., Sovio, U., Bennett, A.J., Peltonen, L., et al. 2010. Life-course analysis of a fat mass and obesity-associated (FTO) gene variant and body mass index in the Northern Finland Birth Cohort 1966 using structural equation modeling. *Am J Epidemiol* 172:653-665.
250. Jess, T., Zimmermann, E., Kring, S.I., Berentzen, T., Holst, C., Toubro, S., Astrup, A., Hansen, T., Pedersen, O., and Sorensen, T.I. 2008. Impact on weight dynamics and general growth of the common FTO rs9939609: a longitudinal Danish cohort study. *Int J of obesity* 32:1388-1394.
251. Heid, I.M., Jackson, A.U., Randall, J.C., Winkler, T.W., Qi, L., Steinthorsdottir, V., Thorleifsson, G., Zillikens, M.C., Speliotes, E.K., Magi, R., et al. 2010. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet* 42:949-960.
252. Moore, S.C., Gunter, M.J., Daniel, C.R., Reddy, K.S., George, P.S., Yurgalevitch, S., Devasenapathy, N., Ramakrishnan, L., Chatterjee, N., Chanock, S.J., et al. 2011. Common Genetic Variants and Central Adiposity Among Asian-Indians. *Obesity*.
253. Sandeep, S., Gokulakrishnan, K., Velmurugan, K., Deepa, M., and Mohan, V. 2010. Visceral & subcutaneous abdominal fat in relation to insulin resistance & metabolic syndrome in non-diabetic south Indians. *Indian J Med Res* 131:629-635.
254. Kraegen, E.W., and Cooney, G.J. 2008. Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol* 19:235-241.
255. Unni, U.S., Ramakrishnan, G., Raj, T., Kishore, R.P., Thomas, T., Vaz, M., and Kurpad, A.V. 2009. Muscle mass and functional correlates of insulin sensitivity in lean young Indian men. *Eur J Clin Nutr* 63:1206-1212.
256. Hall, L.M., Moran, C.N., Milne, G.R., Wilson, J., MacFarlane, N.G., Forouhi, N.G., Hariharan, N., Salt, I.P., Sattar, N., and Gill, J.M. 2010. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 5:e14197.
257. Nair, K.S., Bigelow, M.L., Asmann, Y.W., Chow, L.S., Coenen-Schimke, J.M., Klaus, K.A., Guo, Z.K., Sreekumar, R., and Irving, B.A. 2008. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 57:1166-1175.
258. Hindorff, L.A., Sethupathy, P., Junkins, H.A., Ramos, E.M., Mehta, J.P., Collins, F.S., and Manolio, T.A. 2009. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A* 106:9362-9367.
259. Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., et al. 2009. Finding the missing heritability of complex diseases. *Nature* 461:747-753.
260. Franks, P.W., and Poveda, A. 2011. Gene-lifestyle and gene-pharmacotherapy interactions in obesity and its cardiovascular consequences. *Curr Vasc Pharmacol* 9:401-456.
261. Seki, Y., Williams, L., Vuguin, P.M., and Charron, M.J. 2012. Minireview: Epigenetic programming of diabetes and obesity: animal models. *Endocrinology* 153:1031-1038.
262. Stoger, R. 2008. The thrifty epigenotype: an acquired and heritable predisposition for obesity and diabetes? *Bioessays* 30:156-166.

263. Andreasen, C.H., Stender-Petersen, K.L., Mogensen, M.S., Torekov, S.S., Wegner, L., Andersen, G., Nielsen, A.L., Albrechtsen, A., Borch-Johnsen, K., Rasmussen, S.S., et al. 2008. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 57:95-101.
264. Speakman, J.R., Rance, K.A., and Johnstone, A.M. 2008. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. *Obesity (Silver Spring)* 16:1961-1965.
265. Kilpelainen, T.O., Qi, L., Brage, S., Sharp, S.J., Sonestedt, E., Demerath, E., Ahmad, T., Mora, S., Kaakinen, M., Sandholt, C.H., et al. 2011. Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. *PLoS medicine* 8:e1001116.
266. Feil, R., and Fraga, M.F. 2011. Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet* 13:97-109.