INVESTIGATION OF THE CONTRIBUTION OF GENETIC VARIANTS ON THE DEVELOPMENT OF OBESITY AND METABOLIC COMPLICATIONS IN A MULTI-ETHNIC CONTEXT

by

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LAY ABSTRACT

Obesity is a complex disease with a genetic basis. Obesity is associated with several metabolic complications. Currently the genetic basis of obesity is incompletely understood and low-grade inflammation is debated as a cause of metabolic complications. This thesis aims to (1) discuss ethnic differences in the genetics of obesity, including comparing heritability estimates of body mass index (BMI), (2) examine the effects of genetic variants on type 2 diabetes-related traits, (3) investigate the contribution of genetic variants in inflammatory genes on metabolic traits and (4) determine the effects of genetic variation in the insulin sensitizing protein, adiponectin on metabolic traits. The major findings are (1) there is no difference between heritability estimates for BMI among different ethnic groups, (2) circulating lipids interact with genetic variants to modulate insulin resistance, (3) there is no association between inflammation-related genes and metabolic traits, and (4) adiponectin levels are strongly associated with metabolic traits.

ABSTRACT

Obesity is a complex, multifactorial disease associated with several metabolic complications including type 2 diabetes (T2D) and cardiovascular disease. Obesity is also characterized by a state of chronic low-grade inflammation due to dysregulated adipokine secretion and macrophage infiltration, which is believed to be the pathophysiological link between obesity and other metabolic complications. It is currently unclear if inflammation is a cause of obesity and metabolic complications, or merely a consequence of it. Moreover, some ethnic groups are disproportionately affected by obesity and its metabolic complications, suggesting underlying genetic differences in the susceptibility to obesity.

This thesis aims to (1) to provide a comprehensive discussion of the ethnic differences in the genetic architecture of obesity, including a meta-analysis of heritability estimates of body mass index (BMI) from various ethnic groups, (2) examine the effects of the *PPARγ* Pro12Ala polymorphism on T2D-related traits, (3) investigate the contribution of genetic variants in inflammation-related genes on metabolic traits, and (4) determine the effects of genetic variation in the insulin sensitizing adipokine adiponectin and cardio-metabolic traits, aims (2), (3) and (4) being investigated in a high-risk population of Mexican children.

The major findings are (1) there is no difference between heritability estimates for BMI among African, admixed American and Asian populations, relative to Europeans, and in Mexican children: (2) circulating lipids can interact with *PPARγ* Pro12Ala to modulate markers of insulin resistance, (3) there is no association between inflammation-related genes and metabolic traits, and (4) circulating adiponectin concentration is strongly associated with metabolic traits.

Together this thesis provides insight into the biological mechanisms involved in obesity and its metabolic complications. With a better understanding of the molecular mechanisms involved, more effective prediction of high-risk individuals, preventions and treatments and can be developed.

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LIST OF ABBREVIATIONS

1000G – 1000 Genomes Project

ADIPOQ - Adiponectin

ADIPOQR1 – Adiponectin Receptor 1

ADIPOQR2 - Adiponectin Receptor 2

BBS - Bardet-Biedl Syndrome

BMI - Body Mass Index

CNV - Copy Number Variants

CRP - C-Reactive Protein

DBP - Diastolic Blood Pressure

FPG - Fasting Plasma Glucose

FTO - Fat Mass and Obesity Associated Gene

G x E - Gene x Environment Interaction

G x G - Gene x Gene Interaction

GWAS - Genome-Wide Association Studies

HC - Hip Circumference

HDL - High Density Lipoprotein Cholesterol

HNF1A - Hepatocyte Nuclear Factor 1 Alpha

HOMA-B - Homeostatic Model Assessment of Beta Cell Function

HOMA-IR - Homeostatic Model Assessment of Insulin Resistance

HWE – Hardy-Weinberg Equilibrium

IFG - Impaired Fasting Glucose

IL-6 - Interleukin-6

IL-10 -Interleukin-10

IR - Insulin Resistance

LEP – Leptin

LEPR - Leptin Receptor

LD - Linkage Disequilibrium

LDL - Low Density Lipoprotein Cholesterol

MAF – Minor Allele Frequency

MC4R - Melanocortin 4 Receptor

NHANES - National Health and Nutrition Examination Survey

PCA - Principal Components Analysis

PCSK1 - Proprotein/Prohormone Convertase 1

POMC - Pro-opiomelanocortin

PPARγ - Peroxisome Proliferator-Activated Receptor-γ

PWS - Prader-Willi Syndrome

RAF - Risk Allele Frequency

RETN - Resistin

SBP - Systolic Blood Pressure

SDS - Standard Deviation Scores

sICAM-1 - Soluble Intercellular Adhesion Molecule 1

SNPs - Single Nucleotide Polymorphisms

T2D - Type 2 Diabetes

TC – Total Cholesterol

TG - Triglycerides

TNF- α - Tumor-Necrosis Factor-α

TZD - Thiazolidinedione

WC - Waist Circumference

WHR - Waist-to-Hip Ratio

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CHAPTER 1: INTRODUCTION

The burden of obesity and its comorbidities

With an estimated 650 million adults worldwide classified as obese in 2016 (Body Mass Index, BMI ≥ 30 kg/m^2, http://www.who.int/), obesity is a global health concern. Worldwide, the prevalence of overweight and obesity is higher in developed than developing countries[1]. In parallel to the rise of obesity in adults, the worldwide prevalence of childhood overweight and obesity has increased from 4.2% in 1990 to 6.7% in 2010 and is expected to reach 9.1% by 2020[2]. Two countries with an exceptionally high prevalence of obesity include the United States and Mexico with an estimated prevalence of 38.9% and 32.8%, respectively[3,4]. Within the United States 30% of Caucasians, 45% of African Americans, and 36.8% of Mexican American adults were obese, compared to 4.8% of Asian Americans, demonstrating notable differences across ethnic groups[5,6]. According to recent national representative studies, approximately 17% and 34% of children in the United States and Mexico, respectively were obese[7,8]. This is especially problematic given that childhood obesity is the main predictor of adult obesity[9].

Obesity is accompanied by several co-morbidities including depression, sleep apnea, osteoarthritis, non-alcoholic fatty liver disease, hypertension, cardiovascular disease, and some forms of cancer[10]. Obesity is also the main risk factor for type 2 diabetes (T2D), with 80% of individuals with T2D being overweight or obese at the time of diagnosis[11]. According to the American Diabetes Association, the prevalence of diagnosed T2D in 2016 was 8.6%. When the prevalence was examined by ethnicity, 7.4% of Caucasians, 8.0% of Asians, 12.1% of Hispanics, 12.7% of African Americans and 15.1% of Native Americans had diabetes in 2015 in the United States. In contrast, East and South Asians tend to develop T2D at an earlier age and at a lower

BMI than Europeans, highlighting the complex associations between obesity and its complications[12]. The prevalence of T2D in Mexico is estimated to be as high as 14.4%[13]. Ultimately, obesity in its more severe forms decreases life expectancy by 13 and 8 years for men and women, respectively[14].

Due to the high costs incurred through complications, obesity is one of the costliest and burdensome chronic diseases of our time. Treating those with obesity places a substantial burden on the health care system; in 2008, the estimated direct costs (e.g., hospitalizations, medications, physician and emergency room visits) attributable to overweight and obesity were $114 billion, representing 4.8% of the total health expenditures in the United States[15].

In order to mitigate further health and economic consequences of obesity, several treatment options have emerged including bariatric surgery, pharmacological treatments, lifestyle and behavioural interventions[16]. To date, bariatric surgery is the most effective procedure for managing weight and comorbidities in those with morbid obesity (BMI $\geq$ 40 or $\geq$ 35 kg / m^2 with comorbidities). On average, weight loss of 60-70% of excess body weight can be achieved within 1-2 years post-operatively and many comorbidities such as T2D, hypertension and sleep apnea can be improved or reversed[17]. Canada has experienced a rapid increase in the number of bariatric surgeries over the past decade. Approximately 8, 583 procedures were performed in 2015 – 2016, representing a 400% increase from 2006 – 2007[18]. However, this procedure is highly invasive and associated with serious complications including bowel obstruction, ulceration, nutrient malabsorption and the need for reoperation and approximately 25% of patients regain significant amounts of weight 1 year post-operatively[18,19]. Furthermore, only 1% of those who could qualify for bariatric surgery in the United States underwent a procedure, possibly due to insurance coverage, limited access to bariatric centres and misconceptions

surrounding the effectiveness and risks associated with bariatric surgery[18]. Access to bariatric surgery is also a challenge in Canada where distance and access to bariatric surgery centers, and limited funding, resources and patient prioritization has resulted in lengthy wait-times, with some patients seeking privately funded strategies[18]. Pharmacological treatments for obesity provide modest weight loss and are associated with serious side effects including hypertension, steatorrhea, malabsorption of fat-soluble vitamins, cognitive impairments, and cardiovascular events[20]. Due to the adverse risks of anti-obesity drugs, the Food and Drug Administration in the United States has been extremely cautious to approve pharmacological treatments for obesity and only one new anti-obesity drug has gained approval in the past decade[21,22]. In Canada, three prescription medications are available for the treatment of obesity: orlistat, liraglutide, and bupropion / naltrexone combination[23]. Orlistat inhibits lipase activity, preventing dietary fat from being absorbed in the intestines. This mechanism of action is likely responsible for the gastro-intestinal side effects including oily stools and fecal incontinence, resulting in the discontinuation of orlistat[23]. Patients taking liraglutide in addition to lifestyle interventions lose approximately 4 – 9 kg more than those taking placebo and 3 kg more than those prescribed orlistat[23]. The greater weight loss observed in those taking liraglutide may be explained by its mechanism of action; liraglutide is a glucagon-like peptide-1 receptor agonist which promotes appetite suppression and reductions in gastric emptying[23]. Burpropion and naltrexone were initially approved in Canada for depression / smoking cessation and the treatment of opioid / alcohol dependence, respectively. Bupropion / naltrexone combination results in a 8.1% total weight loss, with minor side-effects reported[23]. This combination therapy is thought to aid in weight loss by promoting the release of anorexic hormones in the hypothalamus while simultaneously inhibiting the release of hormones which counteract these effects[23].

Therefore, lifestyle interventions such as dietary changes to reduce caloric intake and increased physical activity have become valuable alternative. When coupled with caloric restriction, exercise can induce an additional 1- 1.5 kg weight loss over 1 year in addition to a dietary intervention alone[24]. Behavioral therapies including goal setting, self-monitoring (i.e. recording caloric intake, physical activity, hunger level, mood, place of eating etc.), addressing barriers, problem-solving, positive reinforcement, and ongoing support also provide favorable weight-loss results[25]. For example, the randomized DiRECT Clinical Trial involved obese individuals diagnosed with T2D who were randomized to a weight-loss intervention (total diet replacement with a formula diet for 3-5 months) or best-practice care guidelines[26]. At 12 months, 24% of participants in the weight-loss intervention group achieved a weight loss of 15 kg or more and 46% remission of T2D in addition to improvements in hepatic and / or pancreatic ectopic lipid deposition[26].

Despite these well-established interventions, less is known about how to effectively prevent weight gain and obesity and currently no country has reversed its obesity epidemic in the past 30 years, emphasizing the difficulty of treating and managing obesity[1,27]. Consequently, the treatment and prevention of obesity and its complications are important public health priorities.

The etiology of obesity and type 2 diabetes

While a detailed description of the etiology of obesity and T2D is beyond the scope of this thesis, a brief overview is warranted. Historically, obesity was thought to be the result of an energy imbalance between a sedentary lifestyle and increased caloric intake. However, it soon became apparent that this is an oversimplification as the global number of overweight and obese individuals has surpassed the number of those who are underweight[28]. Over the last 50 years, investigation into the causes and etiology of obesity has generated a large body of knowledge

and we can now appreciate that obesity is a complex, multifactorial disease[29]; traits contributing to the etiology of obesity include demographics, behaviour, energy metabolism, hormone signalling, central regulation of energy, adipose tissue biology, skeletal muscle biology, the gut microbiome, epigenetic and genetic factors[29].

While homeostatic mechanisms work to maintain energy balance close to zero, a positive energy balance results in an increase in energy storage in the adipose tissue in the form of fat (triglycerides). This is accomplished by an increase in the number to adipocytes (hyperplasia) or an enlargement in adipocyte size (hypertrophy)[30]. Obesity is characterized by both larger adipocytes and an increase number of adipocytes, relative to lean individuals[30].

Obesity is the main risk factor for the development of T2D. While the molecular and physiological link between obesity and T2D is complex and not fully understood, it can be distilled to impairments in insulin sensitivity (i.e. insulin resistance) and corresponding β-cell failure due to increased demand for insulin to compensate for the decline in insulin sensitivity[31]. T2D is a heterogenous condition where patients can range from severe insulin resistance to those who require insulin early in the course of disease progression[32]. At the cellular level, insulin resistance is largely attributed to ectopic lipid accumulation in insulin-sensitive tissues including the adipose, the muscle and liver which impairs activation of the insulin signalling cascade. T2D also associated with impaired adipocyte metabolism resulting in excessive lipolysis and increase in plasma free fatty acid levels and the secretion of pro-inflammatory cytokines[31]. Chronic hyperglycemia and hyperlipidemia exert damaging effects on β-cells[33].

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand activated transcription factor highly expressed in adipose tissue and is critical for the regulation of adipogenesis, glucose and lipid homeostasis and insulin sensitivity[34]. Polymorphisms and mutations in PPARγ

have been associated with adiposity in humans. The rare Pro115Gln gain of function substitution results in severe obesity and T2D heterozygous carriers[35]. Furthermore, rare dominant negative and heterozygous mutations are characterized by severe early onset insulin resistance, T2D, partial lipodystrophy, but a normal BMI[36]. Conversely, the common Pro12Ala substitution which will be described in subsequent chapters, is associated with a higher BMI and decreased risk of T2D[37,38]. Together these observations demonstrate the essential role of PPARγ for insulin sensitivity and body fat distribution.

Genetic Predisposition to Obesity

Once thought to be caused by an energy imbalance between a sedentary lifestyle and increased caloric intake, an ever-growing body of research now indicates that this is an oversimplification of the cause of overweight and obesity. It is now appreciated that obesity is a complex, multifactorial disease resulting from a combination of demographics, behavior, hormone signaling, central regulation of energy homeostasis, adipose tissue biology, skeletal muscle biology and the gut microbiome[29]. Although these factors play an important role in the development of obesity, only a subset of individuals will develop obesity in a shared obesogenic environment, suggesting underlying genetic differences in the susceptibility to obesity. Twin, family and adoption studies have played an important role in the understanding of the genetic epidemiology of obesity. Estimates of heritability of BMI from twin studies range between 40% - 70%, indicating that BMI is a heritable trait. Heritability estimates of twins raised apart and twins raised together found comparable estimates of heritability for BMI, providing evidence that genetic factors have a stronger influence on BMI than the environment[39].

The last two decades have seen significant advances in understanding the genetic architecture of obesity. The discovery of leptin in 1994 provided the first genetic evidence for

extreme obesity in rodents[40]. Shortly after, two severely obese children with leptin mutations were identified, demonstrating the importance of leptin in regulating energy balance in humans[41,42]. In subsequent years, family studies in those with severe forms of obesity have been highly successful in identifying other mutations in the leptin-melanocortin pathway. This pathway is expressed in the hypothalamus and is critical for regulating energy homeostasis thus homozygous/ heterozygous compound loss of function mutations in genes of the leptin-melanocortin pathway results in severe hyperphagia and fully penetrant, early-onset obesity[43]. These cases of non-syndromic monogenic obesity are exceptionally rare, with a higher proportion of obesity (~10% of obesity cases) resulting from heterozygous deleterious coding mutations in genes in the leptin-melanocortin pathway[44]. Known as oligogenic obesity, carriers display non-fully penetrant obesity[44]. Obesity is also a defining characteristic of 79 distinct syndromes, characterized by mental retardation, dysmorphic features and organ-specific abnormalities[45]. While the genetic basis for syndromic monogenic obesity has not been fully elucidated, they provide further evidence for the role of genetics in the etiology of obesity[45]. Together, monogenic/ oligogenic forms of obesity have allowed for significant advances in understanding the genetic architecture of obesity and the role of the central nervous system in the development of obesity.

The genetic predisposition to obesity for most, however, is polygenic in nature. Polygenic variants themselves have a small effect on the obese phenotype, but in combination with other predisposing variants, a sizeable effect emerges[46]. Perhaps the most significant contribution to the field of obesity genetics comes from genome-wide association studies (GWAS). This high-throughput methodology allows geneticists to scan the entire human genome in an unbiased, hypothesis-free manner to identify genetic variants associated with a trait[47]. *FTO* (fat mass and

obesity associated gene) was the first obesity-susceptibility gene identified through GWAS in 2007 by four independent groups[48-51]. Initially discovered through GWAS for T2D in Europeans, a cluster of variants in the first intron of *FTO* showed a significant association with T2D risk[48]. However, after adjusting for BMI, the association with T2D was lost, suggesting that it was mediated by *FTO*'s effect on BMI[48]. Shortly after, three independent groups confirmed the association with BMI and obesity risk[49-51]. The risk allele of *FTO* (rs9939609) is associated with a 0.36 kg/m^2 increase in BMI and a 1.20-fold increased risk for obesity[52]. While this effect size is not comparable to the rare variants associated with monogenic obesity, *FTO* is considered as the main contributor to polygenic obesity in Europeans due to the high frequency of the minor allele of rs9939609 which ranges from 38% - 44%[47]. The function of FTO and its role in obesity is still under investigation, with emerging data suggesting a role in nucleic acid demethylation, adipogenesis, browning of white adipocytes and regulating feeding behaviours[53,54]. Since 2007, large meta-analyses of GWAS in predominately European populations have identified around 1,500 polygenic loci associated with BMI, WHR (adjusted for BMI), percent body fat, and/or obesity, though these loci have considerably smaller effects than *FTO*[55,56]. Pathway analysis of these loci show support for the role of the central nervous system, insulin secretion, energy metabolism and adipogenesis[57]. While these loci provide valuable insight into the genetic architecture of obesity, they only account for 4% and 6% of interindividual variation in waist-to-hip ratio and BMI respectively, suggesting that more variants remain to be discovered[57].

Common genetic variants explain 30% of variance in BMI, suggesting a small missing heritability[58]. Missing heritability is largely due to low frequency / rare variants with medium / large effect sizes or is attributable to common variants with very small effect sizes[58]. Low frequency (~1-5%) and rare (<1%) variants contributing to obesity are not frequent enough to be

captured by current genome-wide association approaches, yet they could explain part of the missing heritability of disease risk[59-61]. For example, a rare heterozygous 16p11.2 deletion was associated with severe early-onset obesity[62]. In a recent meta-analysis, Turcot *et al* identified 14 rare and low frequency coding variants (including *MC4R* and *KSR2*) associated with BMI where the effect sizes were 10-times larger than those of common variants[63]. An investigation of the role of pathogenic mutations in MC4R on common obesity determined that the gain of function mutations Ile251Leu and Val103Ile may be responsible for 2% of the population-protective fraction against obesity, mirroring the prevalence of monogenic obesity due to MC4R hapoloinsufficiency[64]. Furthermore, a genome-wide polygenic risk score comprised of 2.1 million common variants identified those with a BMI increase similar to those with a monogenic mutation[65]. Lastly, the recently proposed 'omnigenic' model suggests that gene regulatory networks are sufficiently interconnected such that all genes expressed in disease-relevant cells are liable to affect the functions of core disease-related genes and that most of the heritability can be explained by the effects of genes outside core pathways[66].

Obesity and Inflammation

Historically, the adipose was considered as a passive, long-term energy storage organ, but it is now appreciated for its dynamic role in systemic metabolism through the secretion of numerous proteins, collectively referred to as adipokines[67]. Currently over 100 adipokines have been identified including proinflammatory mediators such as tumor-necrosis factor-α (TNF- α) and interleukin-6 (IL-6), the insulin sensitizing adipokine, adiponectin which is protective against the development of obesity-associated complications and leptin which controls appetite through the central nervous system[67].

Obesity-associated systemic inflammation is believed to originate predominantly from the adipose tissue due to changes in adipokine secretion and macrophage inflitration[67]. Under the control of the master regulator of adipocyte differentiation and metabolism, peroxisome proliferator-activated receptor-γ (*PPARγ*), initially the adipose expands in size through adipocyte hypertrophy to store excess energy[68]. As adipocytes become saturated, blood flow to the adipose is compromised resulting in a hypoxia and eventual cell death[69]. In an attempt to restore blood flow, an inflammatory response is initiated by the adipose, characterized by an increased secretion of proinflammatory adipokines and macrophage recruitment to scavenge cellular debris[69]. Macrophage accumulations is proportional to both adipocyte size and adiposity and is associated with a phenotypic switch from an anti-inflammatory M2 polarization to a proinflammatory M1 polarization[70,71]. M1 macrophages have been shown to promote the secretion of proinflammatory adipokines including TNF- α and IL-6 and chemokines while M2 macrophages are associated with adipose tissue remodeling and clearing of apoptotic adipocytes[72]. Macrophage accumulation in adipose of lean mice express markers of M2 macrophages while obesity induces the expression of genes associated with the M1 macrophage phenotype[71]. In humans, macrophage accumulation is greater in visceral adipose than subcutaneous adipose tissue, characterized by a unique inflammatory profile, consistent with the associations with visceral fat and deleterious metabolic complications[73].

The dysregulation of adipokine secretion and macrophage infiltration results in a chronic state of low-grade inflammation which has been suggested as one of the pathophysiological mechanisms linking obesity to other metabolic complications. For example, obesity-induced insulin resistance is associated with increase cytokine secretion from the adipose and adipose macrophages. The expression of the proinflammatory cytokine TNF- α is increased in the

adipose of rodent models of obesity and T2D[74]. TNF- α levels are also increased in the adipose and plasma of obese individuals while weight loss is associated with decreased TNF- α expression[75]. Blood levels of TNF- α also positively correlate with markers of insulin resistance[76]. Mechanistically, TNF- α attenuates tyrosine phosphorylation of the insulin receptor in muscle and adipose tissues, thereby promoting insulin resistance[77]. The proinflammatory adipokine IL-6 also plays an important role in the development of obesity-induced insulin resistance. Plasma concentrations of IL-6 are increased in obese individuals and weight loss is associated with reduced IL-6 concentrations[78] . Elevated plasma concentrations of IL-6 are also observed in those with T2D and are predictive of the development of T2D[79]. Adiponectin is one of the most well-characterized adipokines, known for its insulin-sensitizing effects. Genetic mouse models have shown that deficiency of adiponectin contributes to insulin resistance, while its overexpression in obese mice encourages adipocyte hypertrophy and improves insulin sensitivity[80,81]. In lean humans, adiponectin has a strong anti-inflammatory effect[82]; adiponectin inhibits the activation of TLR4/ NF-κβ proinflammatory signalling pathway and promotes the production of the anti-inflammatory cytokine, IL-10 by macrophages[82]. In humans, low adiponectin concentrations have been associated with obesity, insulin resistance and T2D[83]. Consistent with this, adiponectin secretion is impaired by TNF- α, IL-6 and hypoxia[67]. Similar patterns of adipokine dysregulation are reported for other obesity-induced complications including dyslipidemia, nonalcoholic fatty liver disease, hypertension and cardiovascular disease[72].

Influence of genetic variants on obesity associated inflammation and complications

Currently it is unclear if inflammation is a cause of obesity and other metabolic complications, or merely a consequence of it. Consistent with the contribution of genetic variants

to the development of obesity, evidence from genetic epidemiology suggests a link between genetics and the development of obesity associated complications. For example, a recent GWAS identified a variant in *TLR4* associated with BMI[57]. Furthermore, the Pro12Ala (rs1801282) polymorphism in *PPARγ* is associated with a 30-50% decreased binding affinity and ability to stimulate transcription of PPARγ target genes[84]. Carriers of the Ala12 allele are reported to have a greater BMI and 12% decreased risk of T2D[63,85]. *PPARγ* Pro12Ala knock-in mice on a high fat diet display overexpression of adiponectin receptors in adipose tissue and muscle and plasma adiponectin in Ala/Ala mice, suggesting that the Ala12 allele sensitizes the transcriptional activity of *PPARγ* in adipose and muscle to adiponectin signalling[86]. Given that adiponectin expression is under the transcriptional control of *PPARγ,* it is possible that altered adiponectin signalling contributes to the improvement of insulin sensitivity in Ala/Ala mice. Candidate gene studies, and more recently GWAS studies have identified numerous common and rare variants associated with serum inflammatory markers and metabolic traits[87,88]. Because observational epidemiology is susceptible to bias, confounding and reverse causation, the question of whether chronic inflammation is a cause of obesity and other metabolic complications, or a consequence of it, cannot be answered[89]. Combining genetic epidemiology with classic observational epidemiology is one way to strengthen causality and understand the direction of these associations. For example, the common adiponectin variant, rs266729 alters adiponectin gene expression and has consistently been associated with lower serum adiponectin concentrations and increased risk of IR and T2D[19,50,71]. However, other studies employing Mendelian randomization and genetic variants in inflammatory pathways failed to show the directional link between inflammation, adiposity and T2D[90,91]. Therefore, more research is needed to understand

the contribution of systemic, low grade inflammation in the development of obesity and obesity-associated complication, particularly in at risk, non-European populations.

RATIONALE

Obesity is a multifactorial disease resulting from complex interactions between genetic, demographic, environmental and behavioral factors. Individuals with obesity are at greater risk for numerous metabolic complications including T2D and cardiovascular disease and place a substantial burden on the health care system. Moreover, some ethnic groups are disproportionately affected by obesity and its complication, suggesting underlying genetic differences in the susceptibility to obesity. Substantial evidence now points towards a link between genetics and obesity and is evidenced by loss of function mutations in the leptin-melanocortin pathway resulting in monogenic obesity. Furthermore, common variants in polygenic genes such as *FTO* have consistently been shown to increase obesity risk in predominately European population. Studying the genetic architecture of obesity and its complications in multiple ethnic groups will provide insight into the genetic causes of these conditions. Examining these associations in non-European populations will improve the generalizability of the results. Obesity is also associated with a state of chronic low-grade inflammation characterized by dysregulated adipokine secretion, which has been associated with the development of several metabolic complications. However, it is unclear if inflammation is a cause of obesity and other metabolic complications, or merely a consequence of it. It is also unknown if these effects are present early in life. As observational epidemiology is subject to bias and confounding, causality is difficult to assess. However, genetic epidemiology can strengthen causality and provide insight into the direction of these associations. Together this thesis will provide insight into the biological mechanisms involved in obesity and its metabolic complications. With a better understanding of the molecular mechanisms involved, at risk

individuals can be identified sooner and more effective treatments and preventions can be developed.

OVERALL OBJECTIVE

The overall objective of this thesis was to investigate the contribution of genetic variants on the development of obesity and its metabolic complications in a multi-ethnic context. The specific objectives were to (1) to provide a comprehensive discussion of the ethnic differences in the genetic architecture of obesity, including a meta-analysis of heritability estimates of BMI from various ethnic groups, (2) examine the effects of the *PPARγ* Pro12Ala polymorphism on T2D-related traits in at-risk individuals, (3) investigate the contribution of genetic variants in inflammation-related genes on metabolic traits in the Mexican population, and (4) determine the effects of genetic variation in the insulin sensitizing adipokine adiponectin and cardio-metabolic traits.

CHAPTER OUTLINES

The objective of Chapter 2 was to provide a comprehensive examination of the ethnic differences in the genetic architecture of obesity, characterized by BMI. A meta-analysis of heritability estimates of BMI from 19 twin and 20 family studies from various ethnic groups was performed. The debate over definitions of race and ethnicity are summarized, possible explanations for ethnic differences in the prevalence of obesity are provided and heritability and admixture studies of obesity-related traits are described. Ethnic differences in monogenic syndromic, non-syndromic and polygenic forms of obesity are outlined. A thorough discussion of the advantages and limitations of using multi-ethnic study designs to better understand ethnic differences and the genetic etiology of obesity, follows. This manuscript has been published in *Obes Rev*. 2018 Jan;19(1):62-80.

The effects of the *PPARγ* Pro12Ala polymorphism and T2D risk on at-risk individuals are further investigated in Chapter 3. Using a pediatric population from Mexico, the association between the *PPARγ* Pro12Ala polymorphism and obesity and T2D-realted traits is examined in this at-risk population. For the first time, significant gene-environment interaction between *PPARγ* Pro12Ala, circulating lipids (as an indirect estimator of high-fat diet) and markers of insulin resistance are reported. These results show that genetic predisposition can alter metabolic traits early in life in presence of an obesogenic environment presents childhood as a critical period of opportunity for prevention and intervention strategies. This manuscript has been published in *Sci Rep*. 2016 Apr 14;6:24472.

Chapters 4 and 5 moves from the genetics of obesity and T2D to the inflammatory mechanisms of obesity. Chapter 4 investigates the contribution of genetic variants in inflammation-related genes on metabolic traits in the Mexican population. Although the results

fail to show significant associations, the findings are consistent with previous work in European populations. This manuscript has been published in *PeerJ*. 2016 Jun 23;4:e2090. In chapter 5, the effects of genetic variation in the insulin sensitizing adipokine adiponectin and cardio-metabolic traits are more thoroughly investigated. Working in the Mexican population, this is the first study to investigate the association of genetic variation in adiponectin and its receptors, adiponectin concentrations and cardio-metabolic traits and provides insight into the early biological determinants of obesity and cardio-metabolic complications. This manuscript has been accepted for publication at *Scientific Reports*.

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CHAPTER 2: Ethnic and population differences in the genetic predisposition to human obesity

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ABSTRACT: Obesity rates have escalated to the point of a global pandemic with varying prevalence across ethnic groups. These differences are partially explained by lifestyle factors in addition to genetic predisposition to obesity. This review provides a comprehensive examination of the ethnic differences in the genetic architecture of obesity. Using examples from evolution, heritability, admixture, monogenic and polygenic studies of obesity, we provide explanations for ethnic differences in the prevalence of obesity. The debate over definitions of race and ethnicity, the advantages and limitations of multi-ethnic studies and future directions of research are also discussed. Multi-ethnic studies have great potential to provide a better understanding of ethnic differences in the prevalence of obesity that may result in more targeted and personalized obesity treatments.

INTRODUCTION

Obesity rates have escalated to the point of a global epidemic over the last three decades. According to the World Health Organization, approximately 600 million adults worldwide were classified as obese in 2014 (Body Mass Index, BMI $\geq$ 30 kg/m^2) while, in parallel, the worldwide prevalence of childhood overweight and obesity has increased from 4.2% in 1990 to 6.7% in 2010 and is expected to reach 9.1% by 2020[1]. Obesity is associated with several comorbidities including type 2 diabetes (T2D), cardiovascular disease and some forms of cancer[2]. Furthermore, childhood obesity is associated with more serious health outcomes later in life[3]. Ultimately, severe forms of obesity reduce life expectancy by 13 and 8 years for men and women, respectively[4].

Notable differences in the prevalence of obesity have been observed across diverse ethnic groups. In the United States alone, 21.8% of Caucasians, 34.8% of African Americans, 28.3% of Hispanics, 34.3% of Native Americans, and 33.0% of Pacific Islanders over the age of 30 were considered to be obese between 2001-2002[5]; in contrast, only 4.8% of Asian Americans (individuals of Chinese, Filipina, Asian Indian, Vietnamese, Korean, Japanese and other Asian ancestry) were found to be obese[5]. More recently, the National Health and Nutrition Examination Survey (NHANES) found 30% of Caucasians, 45% of African Americans, and 36.8% of Mexican American adults over the age of 20 to be obese in the United States between 2009-2010[6]. These data clearly demonstrate ethnic disparities in the prevalence of obesity despite living in the same country. These disparities may be due to differences in lifestyle, socioeconomic status, access to health care, social marginalization, or discrimination; however, these differences may also reflect ethnic differences in biological susceptibility for obesity[7]. The Oslo Immigrant Health Study, for example, found the highest prevalence of obesity among Turks

(51%) and the lowest prevalence among the Vietnamese (2.7%) with differences in BMI remaining despite adjusting for socio-demographic and lifestyle factors[8].

Currently a growing body of evidence demonstrates ethnic differences in the genetic predisposition to obesity however, many of the genetic variants responsible for these differences remain unidentified. This review provides a comprehensive examination of the ethnic differences in the genetics of obesity, characterized by BMI. We summarize the debate over the definitions of race and ethnicity, offer possible explanations for ethnic differences in the prevalence of obesity and describe heritability and admixture studies of obesity-related traits. We outline ethnic differences in monogenic syndromic, non-syndromic and polygenic forms of obesity followed by a discussion of the advantages and limitations of using multi-ethnic study designs to better understand ethnic differences in the prevalence of obesity and the genetic etiology of this disease. We also propose several innovative research strategies.

I. How do we define ethnicity?

"Race" and "ethnicity" are controversial and misunderstood terms within the scientific community[9]. Historically race has been used to classify populations based on shared biological characteristics such as skin color while ethnicity generally takes into account cultural characteristics[10,11]. However, both terms are complex, multifactorial concepts reflecting religion, history, and ancestral geographic origins[11,12].

Indeed, 99.9% of the human genome is identical in every individual however evolutionary forces including genetic drift, natural selection, and *de novo* mutations have led to slight genetic differences among populations[13]. With the completion of the Human Genome Project, genetic variants associated with disease susceptibility have been identified with varying frequencies across populations[14]. Genetic variants can now be used with a high degree of

precision to differentiate individuals from various ethnic groups, providing further evidence for the link between race, ethnicity and biology[15]. Yet this link is often blurry due to i) numerous non-genetic connotations of race and ethnicity, ii) the high degree of genetic diversity and the presence of population isolates within a given ethnic group, iii) the lack of defined boundaries between populations, iv) the admixed composition of certain populations (e.g. the Mexican population), and v) the fact that many people have ancestors from diverse regions of the world[12,16].

The lack of clear definitions poses serious problems for geneticists as definitions can vary between studies. To bypass the definition debate, some suggest using geographical location and ancestry rather than race as genetic variation does not support the existence of race *per se;* however, this practise is highly debated[12,13]. Researchers are in need of guidelines to properly describe diverse populations that accounts for both ethnicity and geographical location to improve generalizability and protect against spurious associations[17]. Self-reported ethnicity is a practical way to adjust for ethnicity in genetic association studies however as Serre *et al* demonstrated, it is not sufficient to protect against population stratification[15]. Instead, principal components analyses (PCA) methods including EIGENSTRAT have been developed to correct for population stratification and geographical differences between and within ethnic groups (Figure 1)[18]. PCA is so precise that Karakachoff *et al* accurately determined one's geographic origin within a few hundred kilometers by in a sample of 1,684 individuals from Western France[19].

II. Origins of the ethnic differences in the prevalence of obesity

Mankind has historically been exposed to prolonged periods of starvation where abilities to effectively store energy in times of abundance would grant one a survival and reproductive

advantages. This is the essence of the thrifty genotype hypothesis proposed by James Neel in 1962[20]. Neel suggests the human genome is enriched with metabolically thrifty genes that provide a survival advantage during times of food shortage[20]. However, these thrifty genes have been rendered detrimental by progress. Though highly controversial, this hypothesis can be applied today to explain the high and ethnic-dependent prevalence of obesity[21]. Human ingenuity has mechanized many formerly labour-intensive processes resulting in a sedentary population reliant on automation. Food is no longer scarce, resulting in increased energy consumption. Today's industrialized countries see an improved quality of life at the expense of an evolutionary disadvantageous obesogenic environment[22].

The Pima Indians of Arizona, who have the highest reported prevalence of obesity (64% and 75% in men and women, respectively) are a living example of the transition from a traditional to a modern, sedentary lifestyle[23]. The Pima Indians were traditionally farmers but today, live a rural American lifestyle[23]. It is believed that the migration of Pima Indian ancestors across the Bering land bridge and settling in the desert for 1000's of years may have selected for thrifty genes[23]. These genes however, no longer provide a survival advantage against starvation and may make this population more susceptible to obesity. In contrast, Europeans have benefited from a stable food supply and the availability of labour-saving devices for at least 300 years[24]. Because they have reached food stability for centuries, Gerstein suggests Europeans have "purged" their thrifty genes because of their negative impact on cardio-metabolic health[24]. It is possible that Europeans have been selected for an ability to thrive in a food-secure environment while other more recently exposed populations have little resistance to obesity because they have not had enough time to genetically adapt[24].

Studies of rare and common variants predisposing to obesity have been done to test the validity of the thrifty genotype hypothesis. Analysis of several validated obesity variants provide some evidence of positive natural selection at the *FTO*, *NEGR1*, *SH2B1*, and *FAIM2* loci in accordance with the thrifty genotype hypothesis[25,26]. Recently, Wang et *al.* found that nine out of 115 BMI single nucleotide polymorphisms (SNPs) were positively selected; however, five of these involved positive selection for the obesity protective allele[27]. The lack of consistent signals for positive selection does not support the notion that genetically driven adiposity provided a survival or selective advantage[27].

A recent genome wide association study (GWAS) in 3,072 Samoans discovered a private mutation in *CREBRF* (rs12513649) strongly associated with BMI[28]. The Samoans are a founder population with an extremely high prevalence of obesity. The *CREBRF* variant is common in Samoans (frequency of 0.30) but almost absent from other populations, demonstrating that rare variants can be highly prevalent in isolate populations[28]. While *CREBRF* is presented as a "thrifty" variant, the high frequency of this variant may be explained by a founder effect and a lack of natural selection pressures. Rare mutations in the melanocortin 4 receptor (*MC4R*) are the most common cause of monogenic obesity. Evolutionary analysis of non-synonymous deletions in *MC4R* in both humans and primates suggests a strong negative or purifying selection on *MC4R* to remove deleterious mutations from the population, which is in contrast to the thrifty genotype hypothesis[29]. Analysis of common variants associated with obesity indicate either an absence of positive selection, positive selection for leanness promoting variants, or positive selection for tall and slender stature among Europeans, providing further evidence that the thrifty genotype hypothesis, if true, may be context-dependent[25-27,30].

Beyond the thrifty genotype hypothesis, the "predation release," "drifty gene" and "thrifty epigenotype" hypotheses may explain ethnic differences in the prevalence of obesity[31-33]. The "drifty gene" or "predation release" hypotheses were put forward by John Speakman as an alternative to the long-standing thrifty genotype hypothesis[31,32]. Speakman argues that when ancestral humans acquired the ability to use fire and tools and form organized societies, they subsequently removed the threat of predatory danger[31,32]. In the absence of the predation selection pressure, genes promoting energy storage were allowed to drift[31,32]. The "thrifty epigenotype" hypothesis builds upon the thrifty genotype and phenotype hypotheses, arguing that all human posses a thrifty genome, but phenotypic expression can vary due to inherited epigenetic changes[33]. Ströger argues that individuals born during times of famine carry epigenetic changes allowing for more efficient energy storage[33]. Conversely, individuals born during times of food abundance will be less prone to obesity[33].

Societal conventions such as the practice of consanguineous marriages has resulted in a high prevalence of monogenic obesity in Pakistani children with 30% of the severe cases of obesity being due to genetic mutations in the genes encoding leptin and *MC4R*[34]. The practise of intra-caste marriages in India may also increase the average degree of homozygosity in the genome resulting in an increased incidence of autosomal recessive disorders including recessive forms of Mendelian obesity[35]. Assortative marriages for BMI confer a higher genetic predisposition to obesity in the offspring generation; 50% of parents of extremely obese offspring had a BMI in the top 10% themselves[36]. Consanguineous marriages and assortative marriages may therefore lead to genetic differences between countries with a divergent prevalence in obesity within a few generations.

III. Heritability and ethnic background

The familial aggregation of one's body size is not a recent concept. The strongest risk factor for childhood obesity is parental obesity where a child's risk of obesity is 2.5-4.0-fold higher if one parent is obese and 10-fold higher if both parents are obese, compared to having both parents of normal weight[37]. Knowing that familial resemblance can be explained by both shared environments and genetic factors, milestone twin and family studies have emerged over the past 35 years. Because monozygotic twins share all genetic makeup while dizygotic twins share only half, one would expect monozygotic twins to be more similar in terms of weight than dizygotic twins if body weight is influenced by genetic factors[38]. In fact, estimates of heritability (defined as the proportion of phenotypic variation of a trait attributed to genetic variation) from twin and family studies range between 40% and 70%[39]. Studies of twins reared apart and twins raised together found similar estimates of heritability for BMI, providing evidence that genetics have a stronger impact on weight than the environment[40].

We performed a random-effect meta-analysis of heritability estimates of BMI from 19 twin and 20 family studies from various ethnic groups. Heritability estimates were pooled on the logit scale and standard errors were derived using the delta-method (Figures 2 and 3). Our meta-analysis includes only studies involving adults as the genetic influences on BMI have been shown to increase in strength during childhood[40]. Overall, heritability estimates in twin studies (h^2= 0.72, [0.69-0.75]) were higher than those from family studies (h^2= 0.46, [0.40-0.52]). Due to the limited number of twin studies from non-European populations, we were unable to assess ethnic differences in the heritability of BMI. Heritability estimates for BMI obtained from family studies were not significantly different in African (h^2= 0.53, [0.46-0.60]), admixed (h^2= 0.49

[0.42-0.56]) and Asian (h^2= 0.41, [0.25-0.59]) populations, relative to Europeans (h^2= 0.43, [0.33-0.54]).

IV. Admixture studies and obesity-related traits.

Most American ethnic groups present today are the result of the intermixing of European, African, and Native American populations during the colonization of the New World[41]; genetic variants from previously isolated populations were brought together in new combinations to establish the contemporary European, African, Hispanic, and Native American gene pools. Consequently, populations may have inherited ethnic specific disease susceptibility genetic variants, affecting the likelihood of acquiring diseases[42,43].

Genetic admixture studies have been valuable in identifying differences in ethnicities that cannot be explained by environmental factors alone. Data from the 2003-2004 NHANES found African Americans to be 1.5 times more likely to be obese than European Americans despite homogenous socio-economic status, suggesting that differences in genetic background may account for ethnic differences in obesity risk[5]. Using genome-wide admixture mapping in 15, 280 African Americans, Cheng *et al* identified inverse negative correlation with BMI and percentage of European ancestry[44]. Similar associations with BMI and Native American admixture have been reported, suggesting that the European genome may contain fewer obesity risk alleles and/or may be enriched in obesity protective genetic factors[45].

V. Monogenic syndromic forms of obesity and ethnic diversity

Currently, obesity is a defining characteristic of 79 distinct Mendelian syndromes, providing further evidence for the role of genetics in the etiology of obesity[46]. Ethnic differences in the prevalence of these diseases and syndromic obesity are well documented for Alström

syndrome, Bardet-Biedl syndrome (BBS), Cohen syndrome and Prader-Willi syndrome (PWS) and are outlined below.

Alström Syndrome

Alström syndrome is a rare autosomal recessive disease affecting less than one in one million people in the general population[47]. Clinical symptoms of Alström syndrome include childhood obesity, severe insulin resistance, hyperinsulinemia, impaired glucose tolerance and T2D, independent of the degree of obesity[47]. Alström syndrome is the result of mutations in exons 8, 10 and 16 in the *ALMS1* gene on chromosome 2p13[47]. To date, 109 different mutations in *ALMS1* have been identified, mostly frameshift and nonsense mutations resulting in the premature truncation of ALMS1[47].

Founder mutations for Alström syndrome have been observed in families of French Acadian and English descent. Genealogical analysis of large Acadian kindred including 8 individuals with Alström syndrome confirmed that the affected individuals are from a common founder. In the early 17th century, the first Acadians migrated from France to Acadia, now known as Nova Scotia where they lived in relative isolation[48]. The ancestry of the affected individuals was traced back to a small group of 17th century Acadians who emigrated from Northern France to Acadia[48]. One ancestral pair common to both maternal and paternal lineages of all affected individuals was found, suggesting a founder effect for Alström syndrome in this population[48]. Historical records also confirm the presence of Alström syndrome in this lineage; two half-sisters were reported to have been blind, hearing impaired, obese and chronically hyperglycemic[48]. Further investigation identified a 19-base pair insertion in exon 16 of affected individuals, causing a frameshift and early termination at codon 3530[49]. Among 12 unrelated patients with Alström syndrome in the United Kingdom, a deletion in exon 16 was identified in 5

affected individuals. These individuals either resided or originated from Yorkshire, United Kingdom, suggesting the possibility of a founder effect[50]. Founder effects for Alström syndrome have also been identified in Pakistani and Turkish families[51,52].

Lastly, four novel mutations in *ALMS1* were identified among six Saudi Arabian patients with Alström syndrome. These mutations are believed to have arisen independently at a rate similar to that of other populations due to the high prevalence of consanguinity in the Saudi population. Thus, the high degree of homozygosity in this population has led to the expression of this disease and provides evidence for the powerful effect of consanguinity in shaping the genetic landscape[53].

Bardet-Biedl Syndrome

Bardet-Biedl syndrome is a rare autosomal recessive disease characterized by six cardinal manifestations: obesity, retinitis pigmentosa, renal anomalies, polydactyly, learning disabilities, and urogenital tract defects[54]. The prevalence of obesity among individuals with BBS is between 72-86%[54].

To date, 21 genes involved in BBS have been identified through various gene identification strategies[55-57]. The majority of pathogenic mutations are found in *BBS1* and *BBS10*[54]. The BBS proteins form a complex (BBSome) essential for ciliary function; this complex associates with the ciliary membrane and sorts and directs protein and vesicle trafficking[58]. Interestingly, heterozygous carriers of BBS mutations have an increased risk of developing obesity than non-carriers despite not exhibiting other BBS phenotypes[59]. Furthermore, associations between four common genetic variants in three BBS genes (*BBS2*, *BBS4*, *BBS6*) and common obesity have been identified in French-Caucasian populations suggesting that BBS genes may be associated with polygenic obesity risk[59]. The association of

SNPs at the *BBS4* locus and polygenic adult obesity has been recently confirmed by a large-scale GWAS[60].

Some BBS genes appear to have a greater ethnic specific frequency than others although no genes are found exclusively in one ethnic group. In northern Europeans, mutations in *BBS1* and *BBS10* are common while mutations in *BBS4*, *BBS5*, and *BBS8* are commonly seen in individuals of Middle Eastern and North African descent[54]. The prevalence of BBS has also been found to vary between populations from 1 in 160 000 in Northern Europe to 1 in 13 500 and 1 in 180 000 in isolated communities in Kuwait and Newfoundland respectively[61,62]. In Newfoundland, at least six BBS loci and eight different BBS mutations have been found in affected individuals, suggesting that the high prevalence of BBS cannot be due to a single founder[54]. It is possible that consanguinity, large sibship sizes and a survival advantage for heterozygotes who have an enhanced ability to store fat, may contribute to the high prevalence of BBS in Newfoundland[62]. In Tunisia, the prevalence of BBS was estimated to be 1 in 156 000 while the frequency in the North of the country was estimated to be 1 in 87 000[63]. The high prevalence of BBS in the Tunisian population may be due to the high rate of consanguinity (31%)[63].

Cohen Syndrome

Cohen syndrome is a rare autosomal recessive disorder characterized by mental retardation, motor clumsiness, microcephaly, severe myopia, distinct facial features, childhood hypotonia and joint laxity[64]. Truncal obesity is present but is not always a ubiquitous feature of this disease. Cohen syndrome is caused by a mutation in the *COH1* gene on chromosome 8q22. This gene encodes a protein of unknown function however domain structure and homologies suggest a role in vesicle-mediated sorting and intracellular protein transport[64]. A recent study

found *COH1* to code for a Golgi-associated matrix protein which is required for Golgi integrity[65].

To date, about 100 mutations in *COH1* have been identified with the majority resulting in a null allele; missense and frameshift mutations have also been identified but are less common[66]. The best characterized mutation is the c.3348_3349delCT which causes a frameshift at codon 1117, resulting in protein truncation at codon 1124[67]. This mutation is found in high frequencies in the Finnish population where Cohen syndrome is overrepresented and may explain the high levels of clinical homogeneity within this population[68]. Overrepresentation of this allele in the Finnish population provides evidence for a founder effect with a common ancestral mutation being responsible for most cases[66].

Other mutations in *COH1* in populations with a known founder effect have been identified. Cohen syndrome is frequently observed among Irish travellers (estimated 0.5 per 1000 Irish traveller children) where the c.4471G->T results in a null mutation[69]. The c.11564delA deletion results in the deletion of exons 6-16 and was identified in 14 Greek patients originating from two small neighboring islands where the incidence of Cohen syndrome is 1 in 110[70]. The c.11564delA has also been identified in two families from Central Italy and one in Southern Italy. The c.8459T->C variant among a population of Ohio Amish results in a null mutation where the prevalence of Cohen syndrome is as high as 1 in 500[67]. These findings suggest that extensive allelic heterogeneity is responsible for this disease[68]. The prevalence of obesity among affected individuals shows ethnic variation with a prevalence of 80% among Irish travellers, 53% among Greek/ Mediterranean individuals, 37.5% among the Amish, and 17% among Finnish children[67].

Prader-Willi Syndrome

Prader-Willi syndrome is characterized by short stature, small hands and feet, hypogonadism, mental retardation, obsessive-compulsive behaviours, early childhood-onset hyperphagia and obesity[71]. Most morbidities and mortalities in PWS are the result of being severely obese[72]. The majority of PWS cases (70-75%) are caused by a deletion of imprinted genes within the paternally inherited locus 15q11-q13[73]. Ten known paternally expressed loci are involved in PWS features and include *MKRN3*, *MAGEL2*, *NDN*, *NPAP1*, *SNURF-SNRPN* and 5 small nucleolar RNAs (snoRNAs)[58]. A microdeletion in the HBII-85 snoRNA cluster in a child with PWS provides conclusive evidence for the role of snoRNAs in the etiology of PWS[74]. Other case of PWS are the result of maternal uniparental disomy (~30%), imprinting defects (>5%), or balanced translocations on 15q11-q13 (>1%)[72].

PWS cases have been reported worldwide and generally occur in about 1 in 15 000 births. In the United States, the prevalence of PWS has been reported between 1 in 16, 000 to 1 in 25, 000 [75,76]. Elsewhere, the prevalence of PWS ranges from 1 in 8, 000 in rural Sweden, to 1 in 16, 000 in the San-in district of western Japan, 1 in 15, 830 in Australia and 1 in 26, 676 in Flanders Belgium [77-80]. In the United Kingdom, the proposed true prevalence of PWS is 1 in 45, 000[81]. Given that most cases of PWS (~70%) are the result of *de novo* deletions and epigenetic effects, the prevalence of PWS is not influenced by consanguinity or founder effects.

VI. Monogenic/oligogenic Non-Syndromic Forms of Obesity and Ethnic Diversity

Obesity can show Mendelian patterns of inheritance due to homozygous / heterozygous compound loss of function mutations in five genes which are part of the leptin melanocortin pathway: leptin (*LEP*), leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*), proprotein/prohormone convertase 1 (*PCSK1*), and *MC4R*[82]. This pathway is critical for

regulating food intake and body weight thus complete inactivation of these genes results in severe hyperphagia and fully penetrant early-onset obesity[82]. Loss-of-function mutations display recessive inheritance and as will be shown below, monogenic forms of obesity have been identified mainly in ethnic groups practising consanguinity and founder populations[34]. Partial inactivation of these genes results in oligogenic forms of obesity[58].

LEP and LEPR

Leptin is produced by adipose tissue and plays an essential role in regulating food intake and body weight[83]. Cases of complete leptin deficiency are very rare with only 34 cases being reported worldwide and results in severe hyperphagia and early-onset obesity[83,84]. The first cases of complete leptin deficiency were identified in Pakistani cousins from a highly consanguineous pedigree with severe obesity and since then, other cases of leptin deficiency have been identified in this population. In a cohort of Pakistani children with early-onset severe obesity, 16.1% were found to have homozygous mutations in *LEP*[85]. Of these, 9 children were homozygous for the ΔG133 frameshift mutation and one child was homozygous for a 3-base pair deletion[85]. Pakistani individuals with obesity heterozygous for the ΔG133 frameshift mutation have also been identified[83]. The ΔG133 mutation is frequently identified in the Pakistani population, suggesting a possible founder mutation[85]. Other mutations in *LEP* have been identified in individuals from Pakistan (n=27), Turkey (n=5), Turkmenistan (n=2), Egypt (n=1), Austria (n=1) and China (n=1), suggesting they are ethnic-specific[86].

Mutations in *LEPR* result in a leptin receptor lacking transmembrane and intracellular domains due to abnormal transcript splicing[87]. Like *LEP* mutations, mutations in *LEPR* cause extreme hyperphagia and early-onset obesity. The first *LEPR* mutations were identified in three Algerian siblings from a consanguineous family and to date, only 13 cases of complete LEPR

deficiency have been identified[82,87]. Among those with hyperphagia and severe early-onset obesity, the prevalence of *LEPR* mutations was 3%[87]. In a multi-ethnic cohort, homozygous frameshifts were found in Bangladeshi, Turkish, and Iranian subjects, homozygous nonsense mutations in Southern European subjects, and homozygous missense mutations were found in subjects of Turkish, Norwegian, or British descent[87]. Mutations in *LEPR* have also been identified in Pakistani, Turkmenian and Egyptian children and may be due to the high rate of consanguineous marriages[34,88,89]. Recently a *LEPR* frameshift mutation (p.P166CfsX7) was identified in six individuals with morbid obesity from Reunion Island and is suggestive of a founder effect[90].

POMC

POMC is expressed in the pituitary gland and sequential cleavage of POMC produces the melanocortin peptides adrenocorticotropin (ACTH), alpha-melanocyte-stimulating hormone (α-MSH), β-MSH and β-endorphin. Obesity is thought to be due to deficiency of α-MSH signaling at MC4R, resulting in a lack of appetite suppression[91]. Humans homozygous for loss of function mutations in *POMC* develop severe obesity, adrenal dysfunction, and red hair pigmentation[92]. Heterozygous loss-of-function mutations in *POMC* result in a non-fully penetrant/ oligogenic form of obesity[93]. The most frequent mutation in *POMC* is R236G which alters POMC processing and reduces its ability to activate MC4R[91]. The frequency of the R236G mutation was found to be 0.6% among Danish individuals, 0.76% in British individuals, and 1.65% among French individuals; R236G was also associated with early-onset obesity in British and French individuals[91]. *POMC* mutations were found in 1.5% of Italian adults with obesity but were not associated with early-onset obesity[91]. Other *POMC* mutations have been identified in children with severe obesity of German, UK Caucasian, French, Egyptian and Indian origin[94-97]. Two

children with obesity of Turkish and North African ancestry with *POMC* deficiency have been described despite lacking the characteristic red hair phenotype, suggesting an ethnic dependent clinical presentation[98,99]. It can be assumed that other genetic variants act epistatically in populations practising consanguinity to maintain pigmentation while pigmentation is more dependent on the presence of POMC-derived ligands in European populations[93].

PCSK1

PCSK1 is expressed in the brain, enteroendocrine cells, and the neuroendocrine system and is responsible for processing precursor proteins[100]. Mutations in *PCSK1* cause early-onset obesity, hyperphagia, postprandial hypoglycemia, diabetes insipidus, intestinal and endocrine dysfunctions[100]. Nineteen patients with homozygous or compound heterozygous mutations in *PCSK1* have been identified. Two carriers of homozygous *PCSK1* mutations with obesity were identified in a North-African consanguineous family and in a Turkish family with possible consanguinity[101-103]. An additional thirteen carriers of homozygous *PCSK1* mutations have been reported from a multi-ethnic cohort with consanguineous families[101,104]. Three carriers of *PCSK1* compound heterozygous mutations were identified in non-consanguineous Caucasian families[105-108]. Haploinsufficient heterozygous *PCSK1* mutations result in non-fully penetrant obesity, with an estimated prevalence of 0.83% among European children and adults with severe obesity[100].

MC4R

MC4R is expressed mainly in the central nervous system where it regulates energy metabolism[85,109]; roles of MC4R in controlling food intake and food choices have also been suggested[109]. In humans, MC4R haploinsufficiency due to loss of function mutations is the most common cause of monogenic obesity[109]. At the population level, only 20 individuals with a complete MC4R deficiency have been identified[82].

The prevalence of *MC4R* heterozygous, heterozygous compound and homozygous loss-of-function mutations reported in children and adults with obesity from various ethnic groups ranges from 0.5-5.8%[110,111]. A study from the United Kingdom reported a prevalence of 5.8% in a sample of 500 individuals with severe early onset obesity and a frequency of 4% was reported in a French study of adults with severe obesity[112,113]. In contrast, low frequencies of *MC4R* mutations have been reported in German (1.9%), Greek (0.2%) and Italian (< 0.5%) populations[114-116]. To date, only two individuals of Japanese descent with mutations in *MC4R* have been identified[117,118]. In two small studies of Chinese adults and children with obesity, mutations in *MC4R* were identified in less than 1.5% of the cohort[119,120]. A subsequent study of Chinese, Malay and Indian children and adolescents with severe obesity identified three individuals (1.3%) with *MC4R* mutations, suggesting that mutations in *MC4R* are not a major cause of obesity in Asian populations[121]. A high frequency of homozygous loss of function mutations (3.2%) has been identified among Pakistanis, likely due to the high rate of consanguineous marriages (60-70%, one the highest rates in the world) in this population[85]. Among Pima Indians, three private mutations in *MC4R* have been identified which are not found in other populations[122]. Of particular interest is the R165Q variant which was found in 3% of Pima individuals with severe obesity and is one of the highest reported frequency for any *MC4R* variant[123].

While *MC4R* loss of function homozygous / heterozygous compound mutations lead to a fully penetrant form of early-onset morbid obesity, heterozygous individuals have a milder, non-fully penetrant form[82]; among European heterozygotes the penetrance of obesity ranges from 40 - 100%[124]. In a Greek population, the penetrance of obesity was found to be 6.3% which is relatively low in relation to the high penetrance seen in other European populations. This is of

interest as the prevalence of obesity in the Greek population is 18%, one of the highest in Europe[115]. It is possible that variants in other genes directly or indirectly involved in the melanocortin pathway may antagonistically interact with the loss-of-function mutations or the Mediterranean diet can attenuate the effects of *MC4R* loss of function, minimizing the penetrance of obesity[115]. Similarly, Pakistani heterozygous carriers were found to have a normal weight, suggesting that a rural environment may mitigate the penetrance of *MC4R* mutations[85]. Together, these results suggest that the penetrance of obesity due to *MC4R* heterozygous mutations may to a certain extent be dependent on the environment and lifestyle choices.

VII. Polygenic forms of obesity and ethnic diversity

GWAS for obesity in European and non-European populations

Genetic predisposition to obesity is polygenic in nature in most cases and is attributed to the simultaneous presence of risk polymorphisms in multiple genes. Independently, polygenic variants have small to modest effects on the obese phenotype but together, give rise to a sizeable effect[82]. Until recently, the genetic determinants of obesity were largely unknown until the emergence of statistically powerful GWAS which have revolutionized the search for genetic determinants of complex traits. GWAS searches the genome for several hundred-thousand SNPs and identifies SNPs that occur more frequently in individuals with a particular disease than in those without the disease[82]. In 2007, common variation in intron 1 of *FTO* was associated with obesity in Europeans by four independent groups[125-128]. To date, *FTO* is viewed as the main contributor to polygenic obesity in Europeans. Since 2007, the association of *FTO* on obesity has been extended to diverse ethnic groups including African-American, Hispanic, Pacific Islander and East Asian populations[129]. Subsequent large meta-analyses of GWAS in predominately European populations have identified 142 polygenic loci associated with BMI and/or obesity[58]. Although these loci have considerably smaller effects than *FTO,* they provide valuable insight

into the genetic architecture of obesity. Pathway analysis of genes associated with BMI provide strong support for a role of the central nervous system, adipose tissue, the musculoskeletal system and digestive tract, highlighting the complex etiology of obesity that encompasses biological pathways in multiple organ systems[58]. Meta-analysis of GWAS of BMI in predominantly European children identified 12 loci previously associated with BMI in adults, demonstrating the shared genetic background between childhood and adult BMI[130].

In recent years, several GWAS for obesity traits have been conducted in non-European populations such as East Asians and Africans. GWAS in non-Europeans have been critical in confirming European obesity loci and identifying novel, ethnic-specific loci. In a recent meta-analysis of 86, 757 individuals of Asian ancestry, Wen *et al* confirmed seven previously reported BMI-associated loci in European populations (*FTO*, *SEC16B*, *MC4R*, *GIPR-QPCTL*, *ADCY3-DNAJC27*, *BDNF,* and *MAP2K5*) and identified three novel loci associated with BMI (*CDKAL1*, *PCSK1,* and *GP2*)[131]. Another study of East Asians also confirmed *CDKAL1* as a novel BMI-susceptibility locus, with *KLF9* as an additional locus[132]. In 2011, the first GWAS in a Filipino population (n ~ 1,7000 women) replicated GWAS signals for *MC4R, FTO* and *BDNF*[133]. Among Asian Indians, Been *et al* confirmed the association of *MC4R* (rs12970134) with BMI[134]. Genome-wide heterogeneity of variance analysis in 14, 131 Pakistani individuals identified an interaction with smoking status and a novel obesity variant in *FLJ33534* (rs140133294) on BMI[135]. Meta-analysis of 9, 881 African-Americans has demonstrated an association between *FTO* (rs3751812 and rs9941349) and obesity while two smaller GWAS with individuals of African ancestry provide some evidence of replication of the association between *MC4R* (rs6567160 and rs17782313) and BMI[136,137]. More recently, a large meta-analysis in over 30,000 individuals of African ancestry identified one new locus associated

with BMI (*GALNT10*) and five previously identified European BMI loci (*MC4R, FTO, GNPDA2, ADCY3* and *SEC16B,*) reached genome-wide significance[138]. GWAS in Samoans identified a private variant in *CREBRF* (rs12513649), common in this population (frequency: 25.9%) and strongly associated with BMI[28]. Taken together, GWAS in non-European populations are suggestive of a partial genetic overlap between obesity loci across various ethnic groups (Figure 4).

Filling in the gaps of missing heritability

Despite the surge in GWAS and meta-analyses, most of the genetic variability in obesity remains unexplained. In a large meta-analysis by the GIANT consortium, 97 BMI-associated loci only explain 2.7% of the variance in BMI, suggesting that numerous additional variants associated with obesity remain unidentified[60]. Possible explanations for this "missing" heritability include lack of power to detect common variants with subtle effects and causal variants, poor coverage of rare variants, genetic heterogeneity, structural variants, epigenetics, and gene-gene and gene-environment interactions. The majority (> 80%) of identified variants are located in non-coding regions and are thought to be non-causal[139]. GWAS index SNPs are assumed to be in linkage disequilibrium (LD) with the causal variant, making it difficult to distinguish the causal variant and elucidate its role in the development of obesity. Furthermore, if index SNPs are tested in a population with different ancestry, the LD between the index SNP and causal SNP may be weaker. This results in a weak, none, or inverse association with the trait in the replication cohort and a wrongful conclusion that the SNP does not transfer across ancestries[140]. Such is the case with *FTO* rs3751812 (a perfect surrogate for the previously reported *FTO* rs9939609) which replicates in both European and African American populations, unlike *FTO* rs9939609 which only replicates in Europeans[141].

In evolutionary terms, populations of African ancestry are the most ancestral and have experienced more generations of LD decay, relative to European- and Asian-ancestry populations. Due to the accumulation of more recombination events, the African population has smaller regions of LD. Out-of-Africa migrations and genetic bottlenecks have reduced haplotype diversity in European- and Asian-ancestry populations, resulting in larger regions of LD. The weak LD in African populations can be leveraged for fine-mapping studies to pinpoint the causal variant[136]. To date, most fine-mapping efforts have focused on the *FTO* locus due to its strong association with obesity-related traits[140]. Recent mechanistic work suggest that the *FTO* rs1421085 variant may be a functional variant involved in white adipocyte browning and thermogenesis by disrupting ARID5B repression of IRK3/5, resulting in increased lipid storage and weight gain[142,143]. However, initial attempts to fine-map the causal variant(s) in *FTO* in populations of African ancestry have yielded inconsistent results. As described above, Grant *et al* determined that the rs3751812 variant was a better surrogate for the causal variant then rs9939609 in African-American children[141]; these results were further confirmed by Hassanein *et al* who fine-mapped the association between variation at the *FTO* locus and BMI in 9, 881 African adults[136]. In a large study of over 20, 000 African Americans, Peters *et al* densely fine-mapped the entire *FTO* gene and the adjacent *RPGRIP1L* gene to narrow down the functional variant[144]. While they significantly reduced the number of functional candidates, they were unable to narrow in on the functional variant. More recently, Gong *et al* fine mapped 21 BMI-related loci in African Americans and found eight of the 21 associated with BMI in this population[145]. These examples demonstrate the utility of fine-mapping in non-European populations to narrow down on the causal variant.

Admixture mapping in recently admixed populations is a powerful way to identify

disease-causing variants and is well suited for the genetic investigation of complex diseases such as obesity[146]. Knowing that the prevalence of certain diseases and complex traits varies with ethnicity, admixture studies scan the genome for regions where the proportion of one ethnicity is significantly different than average. Admixture mapping has greater statistical power to identify variants with modest effects and have successfully reported associations between risk of obesity or increased BMI in West African and Native American populations[45]. Furthermore, individuals of mixed ethnicities (Asian/white, Hawaiian/white, Hawaiian/Asian, Latina/white, and Hawaiian/Asian/white) have been found to have an above average BMI than their parental ethnic groups, suggesting that differences in ancestral background may partially explain ethnic differences in the prevalence of obesity[45]. Admixture mapping in African Americans has identified several chromosomal regions associated with BMI, including regions on chromosomes X (Xq25 and Xq13.1)[44], 1 (1q23.2 and 1q25.1)[41], 2 (2p23.3)[42], 3 (3q29)[147], 5 (5q14 and 5q13.3)[44,147], 11 (11q23.2)[41], 12 (12p13.31)[41], and 15 (15q26)[147]. A fine mapping study of four genomic regions reported in previous admixture analyses identified an association between SNP rs631465 in *F2RL1* and BMI in an African-American population[148].

The risk allele frequencies (RAF) of obesity loci identified through GWAS are generally high (allele frequency > 10%) but RAF can vary across populations[149]. For example, the RAF of *FTO* rs3751812 in the 1000 Genomes Project demonstrates considerable ethnic differences, ranging from 0.05 in African, 0.17 in East Asian, 0.29 in South Asian and 0.41 in European populations.

Low frequency (~1-5%) and rare (<1%) variants contributing to polygenic obesity are not frequent enough to be captured by current genome-wide association approaches, nor are they penetrant enough to be identified through traditional linkage studies, yet they could explain part

of the missing heritability of disease risk[150-152]. Candidate gene approaches in populations of European descent have identified low-frequency loss-of-function coding non-synonymous variants in *GPR120* (R270H/rs116454156), and *PCSK1* (N221D/rs6232) associated with increased risk of obesity, and low-frequency coding gain-of-function non-synonymous variants in *MC4R* (V103I/rs2229616 and I251L/rs52820871) associated with protection from obesity[153-155]. No well-established association between low frequency / rare variants and obesity traits has been reported in non-European populations to date.

Genomic rearrangement due to deletions or duplications of chromosomal regions can give rise to copy number variants (CNV). Studies of CNV and obesity have been performed in predominately European populations in attempt to further explain the missing heritability of obesity. The rare heterozygous 16p11.2 deletion of at least 593 kb is well studied and is associated with severe early-onset obesity in Europeans[156]. Genome-wide association meta-analyses of individuals of European ancestry identified deletions in regions near *NEGR1*and *GPRC5B* associated with BMI[157,158]. A GWAS for early-onset extreme obesity in individuals of German ancestry identified a CNV near 11q11 associated with early-onset obesity[159]. A GWAS for BMI in a small Chinese population also identified a region near 10q11.22 associated with BMI[160]. Subsequent studies in European populations confirmed this association, demonstrating the utility of screening for CNV in non-European populations to identify novel variants implicated in obesity[161]. Currently the role of rare and common CNV in obesity remains relatively unexplored, however it is unlikely the CNV explain a significant portion of the missing heritability of obesity[162].

Convincing evidence for gene x gene (G x G) interactions has emerged for several obesity loci. A study in East Asians identified a significant G x G interaction between two new

BMI associated SNPs in the *CDKAL1* and *GDF8* loci[132]. Recent data indicate that obesity-predisposing variants interact with a variety of environmental, lifestyle and therapeutic treatments[163]. Consistent gene x environment (G x E) interactions between *FTO*, level of physical activity and BMI or obesity have been described in 16 cross-sectional and intervention studies in European, East Asian and African populations[164]; this was confirmed by a large meta-analysis of 218, 166 adults predominately of European descent where physical activity reduced the risk of obesity by 27%[165]. Using a quantitative measure of energy expenditure (Metabolic Equivalent Score) to provide a more comprehensive assessment of physical activity, Reddon *et al* demonstrated that physical activity can blunt the effects of *FTO* on adiposity (measured by BMI and body adiposity index) by 36-75% in a longitudinal multi-ethnic cohort[164]. Populations in low- and middle- income countries are undergoing rapid transitions from traditional to Western lifestyles. Taylor *et al* investigated the influence of living in rural versus urban India on the role of *FTO* on obesity related traits[166]. When genetic variants in these genes were analyzed with regards to environment, a stronger association between *FTO*, weight, and living in an urban environment was found in comparison with those living in a rural environment[166,167]. A novel interaction between smoking status and the *FLJ33534* locus on BMI has recently been reported in a Pakistani population[135]. G x G and G x E interaction remain largely unexplored due to statistical challenges associated with inadequate sample sizes but may explain some of the missing heritability[164].

Lastly, epigenetic changes may explain the missing heritability in obesity. Epigenetics is defined as changes in gene transcription and expression that do not involve changes to the underlying DNA sequence[168]. Epigenetic modifications include DNA methylation, histone post-translational modifications and chromatin remodelling or the inheritance of mRNAs that regulate

gene expression[58]. DNA methylation consists of the addition of methyl groups to cytosine residues and are typically associated with gene silencing[168]. Candidate gene approaches and more recently, epigenome-wide association studies have identified changes in DNA methylation patterns in genes associated with BMI and obesity[169,170]. After analyzing 450 million CpG sites and subsequent validation in two replication cohorts, Dick *et al* found an association with increased BMI and DNA methylation at the *HIF-3α* locus[171]. More recently, methylation within a variably methylated region in *POMC* has been strongly associated with BMI in a multi-ethnic cohort[172]. Higher methylation of sites within intron 1 of *FTO* and differential methylation of other genes has been observed, suggesting that *FTO* can influence methylation patterns of other genes[173]. Of the 52-known obesity-associated SNPs, 28 have been associated with DNA methylation levels at 107 proximal CpG sites, suggesting they affect multiple genes[174]. Using an epigenome-wide association study, Wahl *et al* recently found an association with BMI and changes in DNA methylation in 187 loci involved in lipid and lipoprotein metabolism, adipose tissue biology and insulin resistance[170]. Replication has been problematic for epigenetic studies; except for *HIF-3α* where the association between *HIF-3α* methylation and BMI has been replicated in subsequent studies, many associations have not been successfully replicated in independent cohorts[175,176]. Furthermore, it is unclear if changes in DNA methylation are a consequence of obesity rather than the cause[177]. Overall, the field of epigenetics has provided novel insight into the complex genetic architecture of obesity but is unlikely to fully explain the missing heritability of obesity. Further studies, especially in non-European populations are needed.

To conclude, GWAS and meta-analyses have made significant advances in identifying genetic variants associated with BMI and obesity, however they have explained very little in the

variance of BMI[60]. Part of the missing heritability is hypothesized to be due to rare genetic variants with large effect sizes which are not captured by GWAS, multiple common genetic variants with small effect sizes cannot be detected in very large GWAS or heritability is overestimated due to environmental effects or genetic interactions[178,179]. New methods of estimating heritability suggest that the heritability of BMI is likely 30 – 40%, therefore there is little missing heritability[180]. In order to fully understand the missing heritability, large sample sizes and new technologies are needed to discover more obesity-associated loci.

VIII. Advantages, limitations and future directions for multi-ethnic designs in obesity genetics

Advantages

While over 90% of obesity-susceptibility loci have been identified in European populations (Figure 4), a growing number of GWAS are now being performed in populations of non-European ancestry in addition to replication and transferability studies[140]. The inherent advantage of using multi-ethnic studies is identifying which genetic signals are shared across populations with distinct genetic ancestries or are ethnic specific (Table 1)[140]. Multi-ethnic studies are also advantageous for identifying ethnic specific disease predisposing variants and private mutations. Moving beyond GWAS, other methods including whole-exome and whole-genome sequencing are better suited to assess rare variants and copy number variants and can be applied to multi-ethnic cohorts to reveal novel loci implicated in obesity.

As ethnic differences are observed in obesity predisposing genes, it is essential to assemble multi-ethnic designs to assess the ethnic-specific contribution of these genes[181]. This study design is also a pre-requisite for identifying causal variations using trans-ethnic fine-mapping approaches through candidate gene/locus resequencing or genotyping of custom arrays (i.e. metabochips)[144,182]. Future large-scale trans-ethnic designs combining data from diverse

ethnic groups with differences in LD structure can provide better resolution to identify causal variants for functional follow-up studies[140]. The use of dense genome-wide SNP arrays (5 million SNPs) in combination with whole-genome sequencing and imputation in multi-ethnic populations may lead to the identification of additional independent signals within GWAS loci and likely causal obesity predisposing variants for functional follow-up studies in one step in a near future[145,183,184].

Studying genetic differences in diverse ethnic groups is critical for reconstructing the evolutive and non-evolutive forces (i.e. genetic drift, migration and founder effect) that have shaped the genetic predisposition or protection from obesity in modern human populations[185]. Interestingly, it may be advantageous to use small and historically isolated founder populations in genetic association studies due to their increased statistical power and high allele frequency of deleterious variants[28].

Multi-ethnic designs aid in the understanding of the impact of societal practises (i.e. intra-caste marriages, preferred consanguineous marriages, polygamy, and assortative marriages) on the present and future genetic susceptibility to obesity[35]. To overcome problems of limited statistical power, populations practising consanguinity can be used as this practise leads to an exceptionally high prevalence of rare homozygous mutations[85].

Multi-ethnic populations are also highly relevant for identifying G x G and G x E interactions. G x G interactions may be identified in certain ethnic groups due to the frequent co-occurrence of the interacting genetic variants[186]. Similarly, novel G x E interactions may emerge when ethnic groups are exposed to unique combinations of lifestyle and environment[115].

Limitations

Early replication efforts aimed to directly replicate European candidate SNPs in independent cohorts reveal significant challenges as some disease-associated SNPs reaching genome-wide significance do not directly replicate in populations of different ancestries. Lack of replication across multiple ethnic groups can be attributed to several factors[187].

Limited statistical power due to relatively small sample sizes of non-European cohorts is a major challenge for replication studies (Table 1). Statistical power to detect an association is a function of sample size, the effect size and minor allele frequency (MAF). Often, replication cohorts are substantially smaller than the discovery population and ethnic minorities are under-represented in multi-ethnic studies. Variants identified in European GWAS generally have larger effect sizes and/or MAF, making them easier to discover[140]. It is often unclear if the lack of significance in the replication cohort is the result of limited power/ sample size or truly an absence of genetic association, thus authors need to report power calculations when discussing the presence/ absence of an association[188]. The formation of large, international genomic consortia for replication in various ethnic groups may alleviate the problem of inadequate sample size[189].

Transferability may also be limited due to differences in genetic architecture. If the GWAS index SNP is tested in a population with different ancestry, the LD between the index SNP and causal SNP may be weaker than in the discovery population. The result is a weak, no or inverse (flip-flop) association with the trait in the replication cohort and a wrongful conclusion that the SNP does not transfer across ancestries[140]. New methods are needed to assess differences in population allele frequencies and LD in order to determine which SNPs are expected (or not) to be replicated in other populations to avoid conducting replication studies in populations where

the variant is too rare[188]. For example, transferability studies in other ethnic groups may use a dense set of variants rather than testing the GWAS hit alone[141].

G x G and G x E interactions also challenge replication and transferability. Adequate statistical power is critical for interaction studies and meta-analyses are recommended to reach sufficient power. Care must also be taken when selecting study design to adequately capture interactions with population based or nested case-control studies having a greater ability to detect interactions[190].

Population stratification and admixture must also be considered as polymorphism frequency may vary by ancestral origin. Using self-reported ethnicity is the simplest and most economical approach but does not adequately control for population stratification[15]. Genetic classification of ancestry through ancestry informative markers provides a more objective and accurate method of defining ethnicity. Other methods including PCA using EIGENSOFT can precisely identify national and local ancestry[19]. These corrections are required in genetic association studies to account for population stratification to minimize spurious associations without compromising power to detect true associations[191].

Lastly, BMI thresholds for obesity (BMI $\geq$ 30 kg/m^2) have been derived from European populations and correspond with an elevated risk for morbidity and mortality[192]. The use of a single, universal threshold for obesity for non-European populations has been questioned with evidence suggesting that Asian populations suffer from a greater risk of type 2 diabetes, hypertension and dyslipidemia despite a low BMI (< 25 kg/m^2)[193]. Despite no formal recommendation for ethnic-specific thresholds by the World Health Organization, the use of ethnic-specific thresholds has been proposed[193,194]. Care must be taken when using multi-ethnic

cohorts to avoid misclassifying non-European individuals for accurate estimates of the prevalence of obesity.

VIX. Conclusions

This review demonstrates the importance of a multi-ethnic perspective in the genetic elucidation of obesity. Identifying obesity predisposing genes in European populations has been undeniably successful but non-European and multi-ethnic populations have been under-investigated so far. Multi-ethnic study designs have great potential to reconstruct the evolutionary history of genetic predisposition to obesity, isolate disease-causing variants, and distinguish global from local G x G and G x E interactions. Novel epidemiological approaches including Mendelian randomization have been conducted predominately in European populations to determine the causal role of obesity loci in the pathology of obesity[195]. While the results from Mendelian randomization studies in Europeans are considered universally valid, it is uncertain if these results hold true in different ethnic groups. Funding initiatives expanding gene identification efforts in non-European or isolated populations should be encouraged, especially in populations at high or low risk for obesity. Undoubtedly such studies will enhance our understanding of the biological bases of obesity susceptibility and protection and encourage innovative prevention and treatment strategies. The observed unique ethnic patterns of genetic predisposition to obesity stress the limitations of a 'one size fits all' approach and emphasize the importance of ethnicity as we transition from big genetic data to precision medicine for all[196].

Table 1: Summary of advantages and limitations of using multi-ethnic study designs in obesity genetics.

Advantages	Limitations
Identify genetic variants shared across multiple ancestries, ethnic specific variants and private mutations	Definitions of ethnicity can vary between studies, limiting the generalizability of results
Differences in LD structure across diverse ethnic groups can be leveraged to pin-point causal variants	Weak LD structure in ancestral populations may result in weak, null or inverse associations, limiting transferability and replication
Reconstruct evolutive and non-evolutive forces (i.e. founder effect) that have shaped the genetic architecture of obesity susceptibility	Spurious results arise when population stratification and admixture are not considered and accounted for
Increased statistical power due to high frequencies of rare and deleterious variants in isolated populations or populations practising consanguinity	Small sample size of non-European replication cohorts and under-representation of ethnic minorities in multi-ethnic studies limits statistical power and the ability to detect associations
Identify novel G x G and G x E interactions due to the co-occurrence of interacting variants and unique environments	Achieving adequate statistical power to detect G x G and G x E interactions is difficult in multi-ethnic studies
Understanding of how societal practises (i.e. consanguineous marriages) influence genetic susceptibility for obesity	Unique societal practises can be population specific, limiting generalizability of results

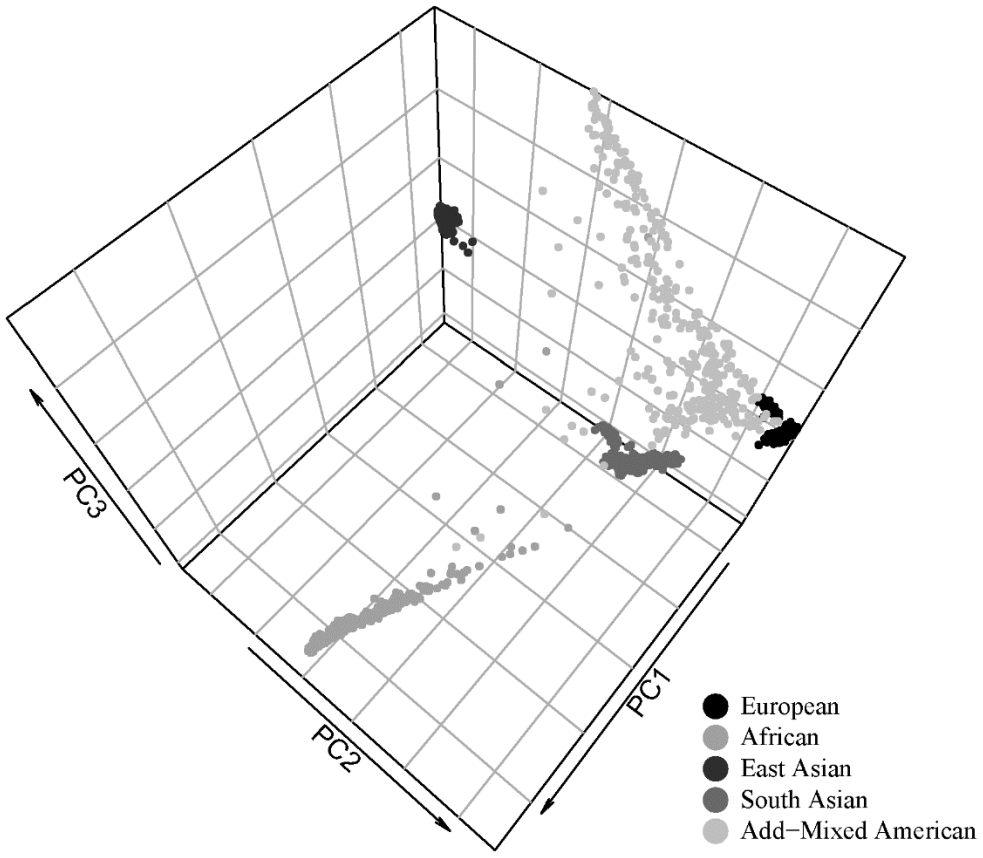

Figure 1: 3D principle component analysis of different ethnic groups of the 1000 Genomes Project.

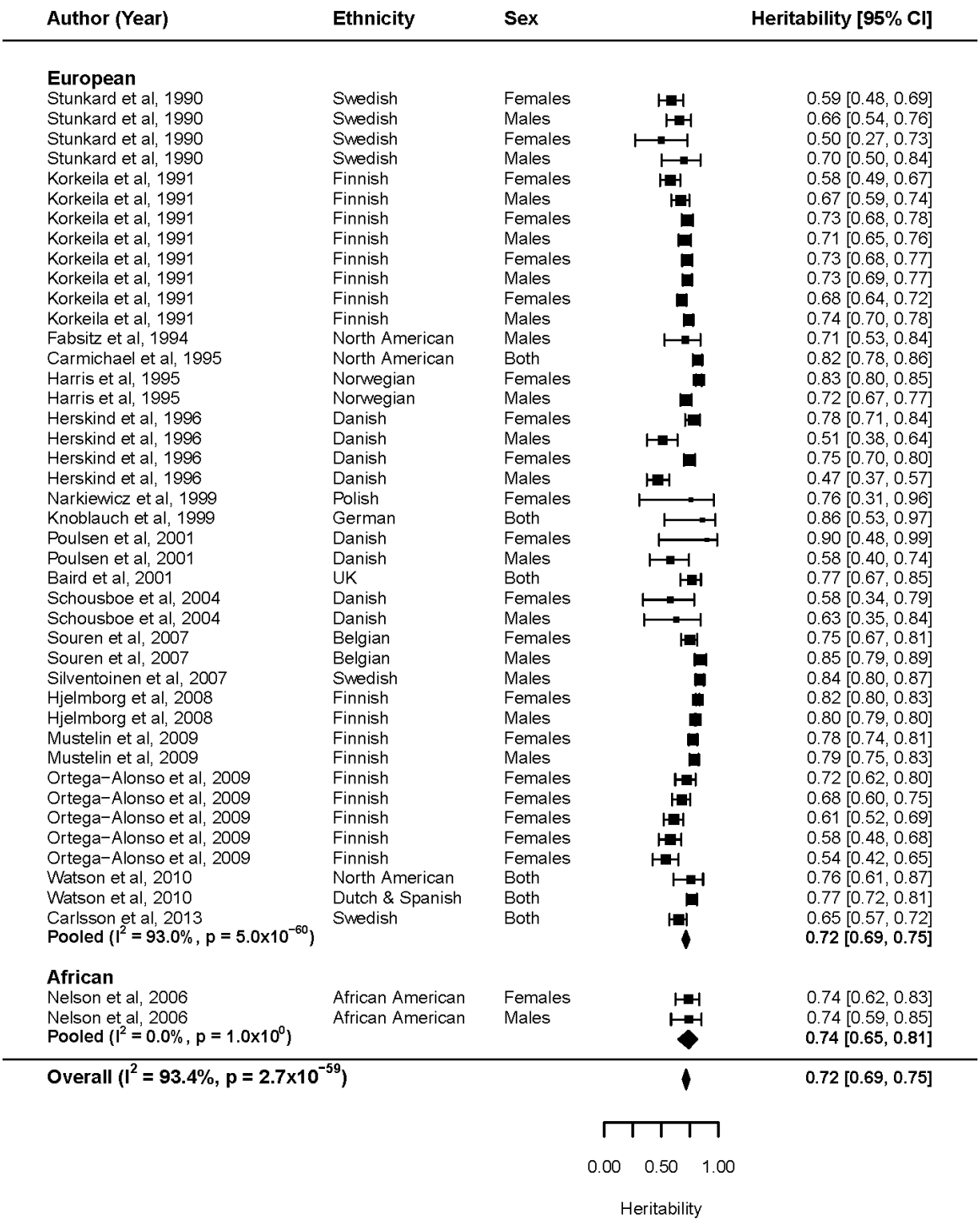

Figure 2: Meta-analysis of BMI heritability estimates in twin studies.

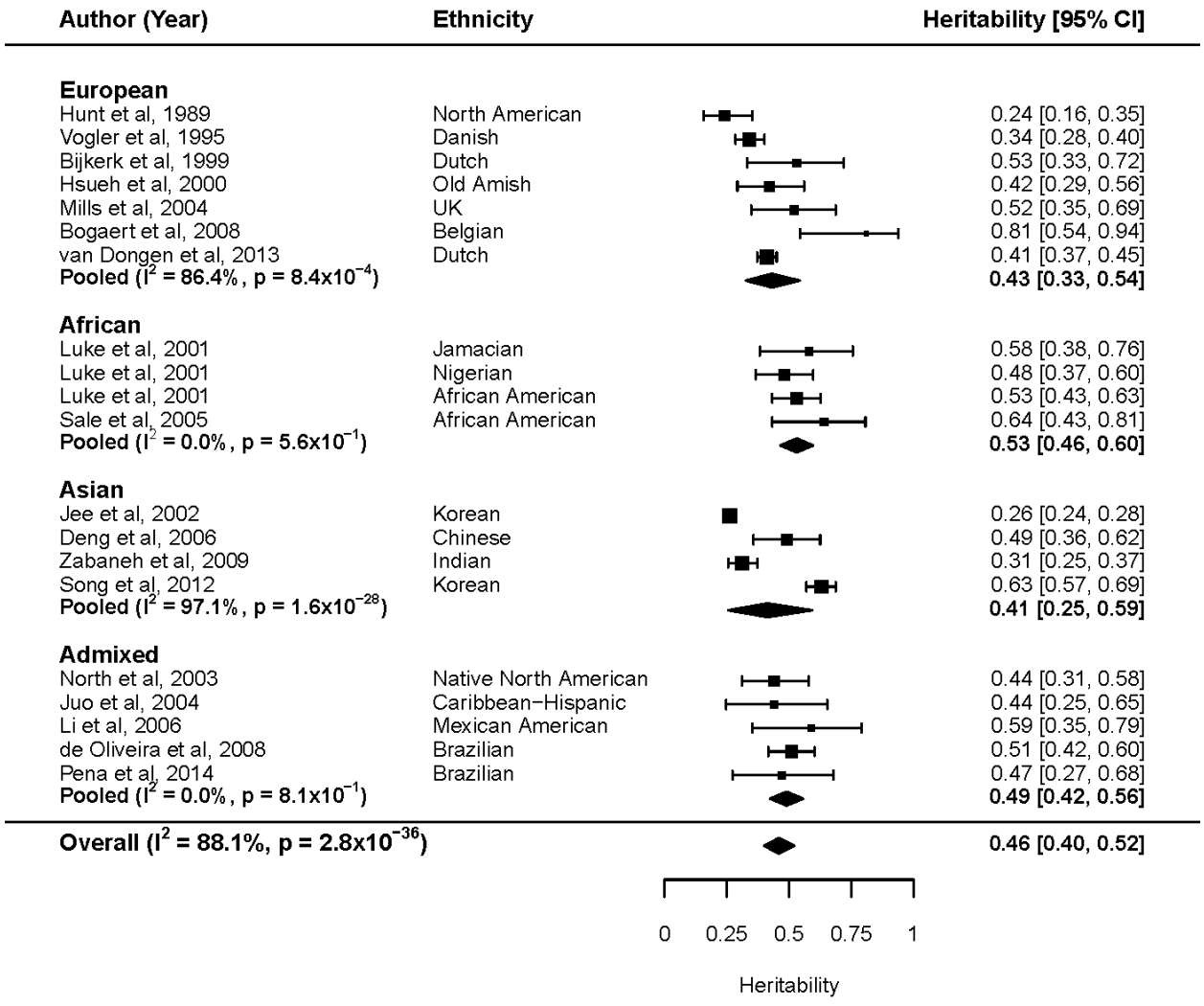

Figure 3: Meta- analysis of BMI heritability estimates in family studies.

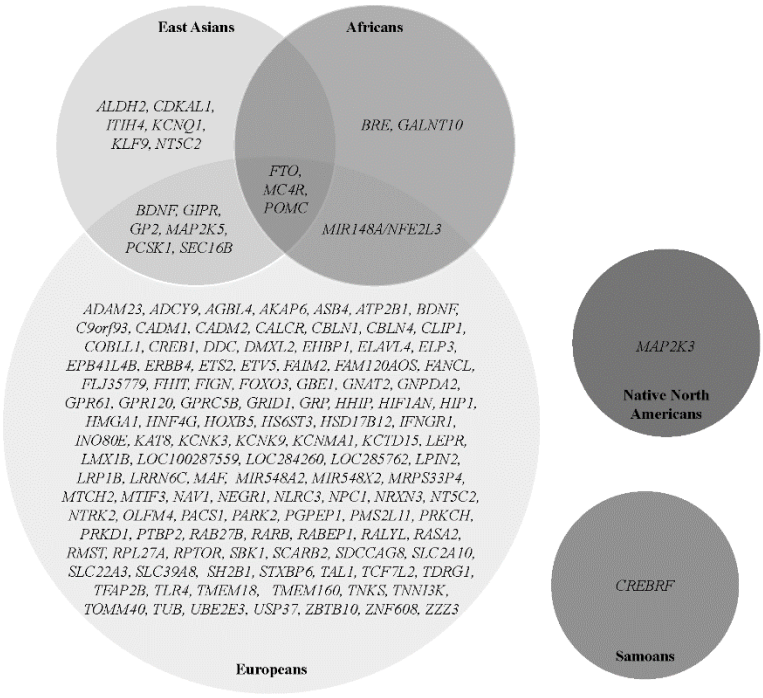

Figure 4: Obesity loci discovered through genome-wide association studies in European and non-European populations.

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CHAPTER 3: Association between *PPARγ* Pro12Ala genotype and insulin resistance is modified by circulating lipids in Mexican children

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ABSTRACT: The Pro12Ala (rs1801282) polymorphism in peroxisome proliferator-activated receptor-γ (PPARγ) has been convincingly associated with insulin resistance (IR) and type 2 diabetes (T2D) among Europeans, in interaction with a high-fat diet. Mexico is disproportionally affected by obesity and T2D however, whether the Pro12Ala polymorphism is associated with early metabolic complications in this population is unknown. We assessed the association of *PPARγ* Pro12Ala with metabolic traits in 1457 Mexican children using linear regression models. Interactions between *PPARγ* Pro12Ala and circulating lipids on metabolic traits were determined by adding an interaction term to regression models. We observed a high prevalence of overweight/obesity (49.2%), dyslipidemia (34.9%) and IR (11.1%). We detected nominally significant/significant interactions between lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol), the *PPARγ* Pro12Ala genotype and waist-to-hip ratio, fasting insulin, HOMA-IR and IR ($9.30 \times 10(-4) \leq P_{interaction} \leq 0.04$). Post-hoc subgroup analyses evidenced that the association between the *PPARγ* Pro12Ala genotype and fasting insulin, HOMA-IR and IR was restricted to children with total cholesterol or LDL-cholesterol values higher than the median ($0.02 \leq P \leq 0.03$). Our data support an association of the Pro12Ala polymorphism with IR in Mexican children and suggest that this relationship is modified by dyslipidemia.

INTRODUCTION

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand activated transcription factor highly expressed in adipose tissue and is intimately involved in the regulation of adipogenesis, glucose and lipid homeostasis and insulin sensitivity[1]. PPARγ is the molecular target of the anti-diabetic drug thiazolidinedione (TZD)[1]. A missense coding variant in *PPARγ* resulting in a proline to alanine substitution (Pro12Ala, rs1801282) has been associated with a 30-50% decrease in ligand-induced activity[2].

The association of *PPARγ* Pro12Ala polymorphism with type 2 diabetes (T2D) is well established. A recent literature-based candidate gene meta-analysis by Gouda *et al* in 32,849 T2D cases and 47,456 controls from Europe, North America and East Asia determined that the deleterious Pro allele is associated with a 16% increased risk of T2D[3]. More recently, a large-scale association study combining the data from GWAS and from the custom array Metabochip in 34,840 T2D cases and 114,981 controls predominantly of European descent confirmed that the deleterious Pro12 allele was associated with a 13% increased risk of T2D[4]. The association of *PPARγ* Pro12Ala polymorphism with body mass index (BMI) has been long debated in literature, but a recent meta-analysis of 49,092 subjects from diverse ethnic backgrounds demonstrated that the *PPARγ* Pro12 allele was associated with a lower BMI[5]. The authors also evidence a trend for a stronger effect of the Pro12 allele in Caucasians[5].

Dietary fats are known ligands for PPARγ and have been shown to interact with the Pro12Ala polymorphism to modulate obesity-related traits in six independent studies[6-11]. Similar gene x diet interactions have been described between dietary fat intake and Pro12Ala polymorphism for insulin resistance (IR) and T2D-related traits[12,13]. These results are suggestive of a diet-dependent interaction between the Pro12Ala polymorphism, body weight and T2D that

can possibly explain the conflicting results regarding the influence of this variant on metabolic traits in individual studies.

The Mexican population is disproportionately affected by both obesity and T2D. In 2008, the United Nations Food and Agricultural Organization estimated the prevalence of obesity in Mexico to be 32.8%, surpassing that of the United States[14]; the prevalence of T2D in Mexico is estimated to be as high as 14.4%[15]. According to the Mexican National Institute of Public Health, 34.4% of children between 5 and 11 years of age were overweight or obese in 2011[16]. This is especially problematic given that childhood obesity is the main predictor of adult obesity[17].

Despite the well-established association between the *PPARγ* Pro12Ala variant, obesity and T2D in populations of European ancestry and the high prevalence of these conditions in Mexicans, only a few studies have examined these associations in a Mexican population. The *PPARγ* Ala12 allele has been associated with a higher risk of overweight / obesity in adult Mexican Mestizo subjects and in five Mexican Amerindian groups[18]. This trend was confirmed in 921 Mexican-American adults from the San Antonio Family Heart Study, where carriers of at least one Ala allele had a higher BMI and waist circumference[19]. No associations between the *PPARγ* Pro12Ala polymorphism and T2D were observed in three modestly powered studies of Mexican adults[20-22]. In 473 adult individuals from 89 Mexican-American families, the *PPARγ* Pro12Ala polymorphism was not associated with IR measured by oral and intravenous glucose tolerance tests[23]. To our knowledge, the association of Pro12Ala with obesity / T2D related traits has never been examined in Mexican children. Thus, we aimed to determine the association between the *PPARγ* Pro12Ala variant and metabolic parameters in 1457 Mexican children and its interaction with circulating lipids used as stable surrogate of a high-fat diet.

METHODS

Study population

A total of 1457 unrelated children aged 6-14 were randomly selected to participate in a cross-sectional study from four areas in Mexico City at the Primary Care Unit of the National Mexican Social Security Institute (Cuauhtémoc West, Independencia South, Nezahualcóyotl Est and Morelos North area). Recruitment was done in collaboration with local public schools. The study started in July 2011 and is still ongoing. Children who had diagnosis of infectious disease, gastrointestinal disorders, administration of antimicrobial agents (within 6 months previous to study), incomplete questionnaires or biological samples were excluded. The study protocol was approved by the Mexican Social Security Institute National Committee and the Ethical Committee Board and informed consent was obtained from both parents and the child, in accordance with the Declaration of Helsinki.

Phenotyping

All participants were weighed using a digital scale (Seca, Hamburg, Germany) and height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Waist circumference was measured at the midpoint between the lowest rib and the iliac crest after a normal exhalation with children in the standing position. Hip circumference was measured at the level of the greater trochanters. Body mass index was calculated as weight (kg) / height $(m)^2$ and classified (underweight, normal weight, overweight, obese) according to the Centers for Disease Control and Prevention CDC 2000 references. Blood samples were obtained following an 8-12 hour fast and were analyzed for fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) using the ILab 350 Clinical Chemistry System (Instrumentation Laboratory IL. Barcelona

Spain). Insulin (IU) was measured by chemiluminescence (IMMULITE, Siemens, USA) and homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the equation by Matthews *et al* [24]. Due to the risk of blood hemolysis, fasting insulin values < 1 μU/mL were discarded from the study. Insulin resistance was defined as HOMA-IR ≥ 3.4 (the 90th percentile of HOMA-IR in a population of healthy Mexican children)[25]. Hypertension was defined as average measured blood pressure above the American Heart Association's recommendations (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg). Dyslipidemia was defined as fasting TG ≥ 100 mg/dL (0-9 years of age) or TG ≥ 130 mg/dL (10-19 years of age) and/or HDL-C < 35 mg/dL and/or LDL-C ≥ 130 mg/dL, according to current recommendations[26,27].

Genotyping

Genomic DNA was isolated from peripheral blood using a standard extraction protocol on an Autogen FLEX STAR (Holliston, Massachusetts USA). Genotyping of the Pro12Ala polymorphism was performed using the TaqMan Open Array Real-Time PCR System (Life Technologies, Carlsbad, USA), following the manufacturer's instructions. The Open Array experiment involved 64 polymorphisms. From the initial sample of 1559 participants, 102 were excluded from the current analysis because i) no blood sample was collected for DNA extraction; ii) DNA extraction was unsuccessful; iii) the genotyping success rate of the Open Array experiment based on the 64 polymorphisms was < 90.6% (≥ 6 genotypes missing). The current analysis included 1457 children. The Pro12Ala genotyping call rate was 99.1%. Deviation from Hardy-Weinberg equilibrium (HWE) for Pro12Ala was tested using a chi-square test and no deviation from HWE was observed ($p = 0.30$).

Statistical analysis

The normal distribution of continuous variables was tested using the Shapiro-Wilk test. All traits of interest deviated significantly from normality. Inverse normal transformations

corrected the lack of normality for BMI, WHR, insulin, HOMA-IR, and TG (Supplementary Figure S1). Non-biological outlier data were discarded. The effect of the rs1801282 variant on metabolic traits (BMI, WHR, fasting glucose, fasting insulin, HOMA-IR, TC, TG, HDL-C and LDL-C) was determined under an additive genetic model using linear regression adjusted for age, sex and recruitment center. The minor allele Ala12 was considered as the effect allele. Interactions between plasma lipids (as continuous traits) and Pro12Ala on metabolic traits were investigated by adding an interaction term to the linear regression model. To investigate further significant interactions, genetic association tests in subgroups were performed using the median of the interacting factor to classify the population into high and low groups. Differences between recruitment centers were determined using a one-way ANOVA and a Tukey post-hoc test. After adjusting for multiple testing using Bonferroni correction (6 metabolic traits in interaction with 4 lipid traits), a p-value below 2.08×10^{-3} (0.05/24) was considered statistically significant and a p-value between 0.05 and 2.08×10^{-3} was considered nominally significant. All statistical analyses were performed using SPSS software (version 20.0). We assessed the power of our sample using QUANTO software version 1.2.4 (University of Southern California, Los Angeles, CA, USA).

RESULTS

Phenotypic characteristics of the studied population

Anthropometric and biochemical characteristics of the study population are presented in Table 1. Of the 1457 children sampled (between 6 and 14 years old, average age 9.24 ± 2.07), 1.4% of the children in the population were underweight, 49.4% were a normal weight, 21.3% were overweight and 27.9% were obese. Insulin resistance was identified in 11.1% of children. 3.1% of children had IFG and only one child was diabetic. Hypertension was present in 22

children (1.5%). Dyslipidemia was identified in 34.9% of the population. Children displayed a significantly higher BMI in the Cuauhtémoc area (20.71 ± 4.34) than in the other areas (Independencia: 19.60 ± 4.15; Nezahualcóyotl: 19.15 ± 4.07; Morelos: 19.32 ± 4.11) using a one-way ANOVA and a Tukey post-hoc test (P between 2.1 x 10^{-6} and 4.3 x 10^{-3}, data not shown). The genotype distribution of *PPARγ* Pro12Ala in the study population was 73.9% (n = 1067), 24.5% (n = 354) and 1.6% (n = 23) for the Pro/Pro, Pro/Ala and Ala/Ala genotypes, respectively. Thirteen individuals were not successfully genotyped (Pro12Ala genotyping call rate: 99.1%.).

Associations / interactions between PPARγ Pro12Ala and metabolic quantitative traits

Knowing that previous reports provide evidence for interactions between *PPARγ* Pro12Ala and dietary exposures to alter metabolic traits and that *PPARγ* is activated by dietary lipids, we tested the interaction between *PPARγ* Pro12Ala and fasting plasma lipid concentrations on metabolic traits[28,29]. Circulating plasma lipids were used as stable surrogate of a high-fat diet[30]. A nominally significant interaction between *PPARγ* Pro12Ala and HDL-C was found to modulate WHR (main genotype effect: β = -0.57 ± 0.20, p = 4.89 x 10^{-3}; interaction: β = 1.14 x 10^{-2} ± 3.81 x 10^{-3}, p = 2.91 x 10^{-3}) (Table 3). Nominally significant interactions between *PPARγ* Pro12Ala and TC on fasting insulin levels (main genotype effect: β = 0.55 ± 0.26, p = 0.04; interaction: β = -3.79 x 10^{-3} ± 1.62 x 10^{-3}, p = 0.02) and HOMA-IR (main genotype effect: β = 0.49 ± 0.26, p = 0.06; interaction: β = -3.38 x 10^{-3} ± 1.61 x 10^{-3}, p = 0.04) were also identified. Given the interactions between plasma lipids, insulin and HOMA-IR, we subsequently tested the interaction between circulating lipids on the presence of IR (Table 4). Both TC and plasma LDL-C concentrations were found to interact with *PPARγ* Pro12Ala to influence the presence of IR ($OR_{\text{main genetic effect}}$ = 18.39, 95% CI 2.57 – 131.79, $OR_{\text{interaction}}$ = 0.98, 95% CI

0.97 - 0.99, $p_{\text{main genetic effect}} = 9.54 \times 10^{-4}$, $p_{\text{interaction}} = 9.30 \times 10^{-4}$; $OR_{\text{main genetic effect}} = 8.70$, 95% CI 1.62 – 46.87, $OR_{\text{interaction}} = 0.98$, 95% CI 0.96 - 0.99, $p_{\text{main genetic effect}} = 0.01$, $p_{\text{interaction}} = 8.09 \times 10^{-3}$, respectively).

We further investigated the direction of the genetic effects of the *PPARγ* Pro12Ala polymorphism on adiposity and insulin resistance parameters showing interaction with lipids. Genetic association tests in subgroups were performed using the median of plasma lipids to classify the population into high and low groups (Table 5). Despite a nominally significant interaction between *PPARγ* Pro12Ala and HDL-C on WHR, the results failed to reach significance in the subgroup analyses. In the high TC subgroup, the carriers of Ala12 displayed nominally significant lower fasting insulin levels / HOMA-IR values ($\beta = -0.19 \pm 0.08$, $p = 0.02$ and $\beta = -0.17 \pm 0.08$, $p = 0.03$, respectively). No evidence of association between *PPARγ* Pro12Ala, fasting insulin levels and HOMA-IR was observed in the low TC subgroup ($p = 0.24$ for both). When LDL-C and TC levels were high, Ala12 carriers were also found to have a nominally significant reduced risk of developing IR (OR = 0.44, 95% CI 0.27 – 0.87, $p = 0.02$ and OR = 0.41, 95% 0.20 – 0.84, $p = 0.02$, respectively). No association between *PPARγ* Pro12Ala and IR was found in the low LDL-C and TC groups ($p = 0.07$ for both).

DISCUSSION

In the present study we examined the association of the Pro12Ala variant in *PPARγ* with metabolic traits and identified nominally significant or significant evidence for gene-environment interactions involving *PPARγ* genotype and high circulating concentrations of TC, HDL-C and LDL-C influencing WHR, plasma insulin, HOMA-IR and IR.

We observed a high prevalence of obesity, IR and dyslipidemia in our sample of 1457 Mexican children. Mexico is experiencing significant epidemiological transitions. Reduced physical activity due to urbanization and technological innovations and shifts in dietary patterns away from traditional high-fiber foods to the increased consumption of processed foods laden with fat, refined carbohydrates and added sugar have resulted in a rise in non-communicable chronic diseases among all age groups[31]. Indeed, the prevalence of overweight and obesity in Mexican children reached 34.4% in 2011, representing one of the highest rates of pediatric obesity in the world[16]. Our sample exceeds the national average with a prevalence of overweight / obesity of 49.2%, which may be partly explained by our strategy to recruit children within an urban setting.

Pediatric obesity is accompanied by an early onset of a number of co-morbidities including T2D, hypertension, dyslipidemia, and non-alcoholic fatty liver disease[32]. The prevalence of dyslipidemia in our sample was an outstanding 34.9%, much higher than previously reported. The high prevalence of dyslipidemia may be attributed to a diet rich in refined carbohydrates and animal fats but limited in fiber[33]. Furthermore, we cannot exclude the possibility that the prevalence of dyslipidemia reported in this study may stem from the employed definition. Abnormal concentrations of one or two lipids are routinely used to identify dyslipidemia. However, the use of three lipids in our study may have artificially increased the prevalence of dyslipidemia in our sample. The prevalence of IR in our sample (11%) is lower than previously reported. In a cross-sectional study of Mexican children aged 7-18, the prevalence of IR was estimated at 20.3% while the National Health and Nutrition Examination Survey found 52.1% of obese Mexican-Americans aged 12-19 to have IR (compared to 23.4% of obese children in our sample, data not shown)[34,35]. This discrepancy may be attributed to the

younger age of our sample given that insulin and glucose concentrations gradually increase with age[36]. We also observed a very low prevalence of hypertension in our sample (1.5%). Previous reports show the prevalence of hypertension among Mexican children varying from 4.7% to 14%[37-39]. These studies however classified hypertension using percentiles rather than a threshold, making comparisons challenging.

Since its discovery, the *PPARγ* Pro12Ala polymorphism has garnered considerable interest due to its ability to modulate both T2D and obesity risk. Results from GWAS in diverse ethnic groups have established the protective role of the Ala12 allele against T2D despite it being an obesity-risk allele, as suggested by a recent large-scale meta-analysis[4,5]. Allele frequencies of the Pro12Ala polymorphism vary among ethnic groups with the highest Ala12 allele frequencies generally reported in Caucasian, South Asian and South American (all 12%) populations in the 1000 Genomes Project. The lowest frequencies are found among East Asian (3%) or African (0.5%) populations. In our sample, the frequency of the Ala12 allele was similar to the allele frequencies reported in the 1000 Genomes Project for Mexican-American adults (14% vs 13%, respectively). In addition to many other genetic variants, the varying frequency of the Pro12Ala polymorphism among ethnic groups contributes to the contrasting patterns of predisposition to obesity and T2D among populations.

Fatty acids, in particular unsaturated fatty acids, serve as ligands for *PPARγ*. Therefore, we examined the interaction between circulating lipids as a surrogate for a high-fat diet and *PPARγ* genotype on metabolic traits[40]. Previous studies have shown diet-gene interactions between total, saturated or polyunsaturated fat intake on obesity and T2D related traits, however to our knowledge, ours is the first study to report significant interactions between *PPARγ* genotype and circulating lipids on IR. IR is driven by dyslipidemia (elevated concentrations of

TC and LDL-C and decreased concentrations of HDL-C) and is a strong predictor of T2D[41]. A nominal association towards lower fasting insulin concentration and lower HOMA-IR was observed among carriers of the Ala12 allele when TC levels were high. Carriers of the Ala12 allele were found to have a decreased risk for IR despite high circulating LDL-C, further suggesting the protective role of the Ala12 allele against the development of IR amid dyslipidemia[42]. In our population, a nominally significant interaction between *PPARγ* genotype and HDL-C on WHR was identified with a trend towards low WHR in carriers of the Ala12 allele. The well-established inverse relationship between circulating HDL-C and abdominal obesity was not observed in the subgroup of carriers of the Ala12 allele with high HDL-C concentrations[43]. This finding warrants further replication in another independent population of Mexican children.

These results must be interpreted with consideration for the acknowledged limitations. Firstly, our population cannot be considered representative of the Mexican pediatric population as a whole as the prevalence of overweight and obesity in Mexico is higher in urban areas with greater economic development (i.e. northern Mexico and Mexico City)[44]. Therefore, our population is representative of the urban population of central Mexico as the recruitment was random. The Mexican population is admixed with Native American (65%), European (30%), and West African ancestries (5%) with proportions being affected by geographic, demographic and historical factors[45]. As such, genetic heterogeneity exists between and within different regions of Mexico. Although all of the children in our study reside in Mexico City, we did not have access to ancestry-informative markers and thus could not adjust for genetic admixture Circulating lipid levels were used as a surrogate for a high- fat diet, however this assumption could not be confirmed as dietary intake was not directly measured. We acknowledge that our power was

modest, especially considering the Ala12Ala genotype (N = 23) and therefore our findings deserve further investigation. Due to our modest sample size, most of our results did not reach statistical significance after adjusting for multiple testing with Bonferroni correction ($P < 2.08 \times 10^{-3}$) and warrant replication in independent Mexican pediatric populations (Supplementary Table 1). Lastly, due to the cross-sectional nature of this study, causality cannot be inferred.

The results of the current study are noteworthy because the association between *PPARγ* Pro12Ala and obesity and T2D-related traits has never been examined in Mexican children. This is the first study to our knowledge to report a significant gene-environment interaction between *PPARγ* Pro12Ala, circulating lipids and markers of IR in a pediatric Mexican population. Mexican children are a high-risk population for obesity and metabolic complications and the prevalence of these conditions will likely dramatically increase in this population as they age. Our results also show that genetic predisposition can alter metabolic traits early in life in presence of an obesogenic environment. Taken together, the present study demonstrates the urgency of preventing and treating obesity and T2D and presents childhood as a critical period of opportunity for prevention and intervention strategies. These results also highlight the need for a comprehensive understanding of the genetics of obesity and T2D in diverse ethnic groups in order to establish personalized/ stratified intervention strategies.

In conclusion, the present results show an association of the Pro12Ala allele with IR in a sample of 1457 Mexican children. Our results also suggest an interaction between *PPARγ* Pro12Ala genotype and circulating lipids on IR. Knowing that Mexican children are at high risk for obesity and T2D, *PPARγ* genotype could be used in conjunction with other known obesity and T2D genes to guide early prevention strategies in the management of these diseases.

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Author Contributions

CS, JPR, MC and DM designed the experiment. JPR, RKA and MC contributed to the recruitment of participants and the clinical aspects of the study. JPR, FS and JGZ performed the DNA extraction and genotyping experiments. CS, ABG and DM prepared the dataset for analysis. CS and DM conducted statistical analyses. CS and DM wrote the manuscript and prepared all tables. JPR, RKA, FS, JGZ, ABG and MC critically reviewed the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Table 2: General characteristics of the studied population of Mexican children by *PPARγ* Pro12Ala genotype.

Characteristics	N=1457	Pro12Pro (N=1067)	Pro12Ala (N=354)	Ala12Ala (N=23)
Male/ Female, N	771/686	565/502	190/164	8/15
Age	9.24 ± 2.07	9.27 ± 2.05	9.19 ± 2.15	8.91 ± 1.73
Waist Circumference (cm)	66.47 ± 11.78	66.57 ± 11.71	66.67 ± 12.11	62.51 ± 8.84
WHR	0.85 ± 0.06	0.85 ± 0.06	0.85 ± 0.06	0.85 ± 0.05
BMI (kg/m^2)	19.65 ± 4.20	19.67 ± 4.17	19.76 ± 4.34	18.33 ± 3.37
Systolic blood pressure (mmHg)	98.57 ± 10.86	98.45 ± 11.03	99.00 ± 10.51	97.70 ± 8.83
Diastolic blood pressure (mmHg)	66.24 ± 8.80	66.03 ± 8.96	66.96 ± 8.23	65.09 ± 9.53
Glucose (mmol/L)	4.57 ± 0.53	4.56 ± 0.53	4.57 ± 0.51	4.65 ± 0.61
Insulin (μU/mL)	8.68 ± 7.10	9.15 ± 7.06	9.12 ± 7.18	7.08 ± 4.05
HOMA-IR	1.87 ± 1.52	1.88 ± 1.52	1.88 ± 1.55	1.42 ± 0.78
TG (mg/dL)	93.62 ± 49.70	94.77 ± 50.37	90.72 ± 48.00	90.78 ± 45.62
TC (mg/dL)	157.25 ± 33.56	157.21 ± 33.98	156.91 ± 31.87	166.43 ± 42.27
HDL-C (mg/dL)	50.60 ± 12.82	50.25 ± 12.59	51.41 ± 13.42	52.65 ± 15.25
LDL-C (mg/dL)	102.39 ± 26.42	102.78 ± 27.22	101.35 ± 23.93	102.30 ± 28.51
Insulin Resistance, N (%)	127 (11.1%)	97 (11.4%)	28 (10.5%)	1 (6.7%)
Dyslipidemia, N (%)	509 (34.9%)	385 (36.1%)	113 (31.9%)	9 (39.1%)
Hypertension, N (%)	22 (1.5%)	19 (1.8%)	3 (0.9%)	0 (0%)
Hyperglycemia, N (%)	45 (3.1%)	35 (3.3%)	8 (2.3%)	1 (4.3%)

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; T2D, type 2 diabetes; WHR, waist-to-hip ratio

Data are means ± standard deviation.

Table 3: Interactions between circulating lipids, *PPARγ* Pro12Ala and metabolic quantitative traits.

	Pro12Ala x TC		Pro12Ala x TG[a]		Pro12Ala x HDL-C		Pro12Ala x LDL-C	
Outcome	**Main Genetic Effect**	**Interaction**	**Main Genetic Effect**	**Interaction**	**Main Genetic Effect**	**Interaction**	**Main Genetic Effect**	**Interaction**
BMI[a]	0.16 ± 0.23 (0.49)	$-1.13 \times 10^{-3} \pm 1.42 \times 10^{-3}$ (0.42)	0.01 ± 0.04 (0.85)	0.01 ± 0.05 (0.85)	-0.21 ± 0.18 (0.27)	$4.10 \times 10^{-3} \pm 3.48 \times 10^{-3}$ (0.24)	0.08 ± 0.20 (0.68)	$-8.94 \times 10^{-4} \pm 1.90 \times 10^{-3}$ (0.64)
WHR[a]	-0.05 ± 0.24 (0.85)	$2.77 \times 10^{-4} \pm 1.50 \times 10^{-3}$ (0.85)	0.02 ± 0.05 (0.70)	0.01 ± 0.05 (0.84)	**-0.57 ± 0.20 (4.89×10^{-3})**	**$1.14 \times 10^{-2} \pm 3.81 \times 10^{-3}$ (2.91×10^{-3})**	0.08 ± 0.21 (0.69)	$-7.22 \times 10^{-4} \pm 2.03 \times 10^{-3}$ (0.72)
Glucose	-0.03 ± 0.12 (0.83)	$2.51 \times 10^{-4} \pm 7.44 \times 10^{-4}$ (0.74)	0.02 ± 0.03 (0.37)	0.03 ± 0.03 (0.20)	0.03 ± 0.10 (0.78)	$-3.85 \times 10^{-4} \pm 1.97 \times 10^{-3}$ (0.84)	0.02 ± 0.11 (0.86)	$3.65 \times 10^{-5} \pm 1.03 \times 10^{-3}$ (0.97)
Insulin[a]	**0.55 ± 0.26 (0.04)**	**$-3.79 \times 10^{-3} \pm 1.62 \times 10^{-3}$ (0.02)**	-0.04 ± 0.05 (0.45)	-0.05 ± 0.05 (0.36)	-0.06 ± 0.21 (0.77)	$6.51 \times 10^{-4} \pm 3.91 \times 10^{-3}$ (0.77)	0.25 ± 0.23 (0.27)	$-2.93 \times 10^{-3} \pm 2.16 \times 10^{-3}$ (0.18)
HOMA-IR[a]	0.49 ± 0.26 (0.06)	**$-3.38 \times 10^{-3} \pm 1.61 \times 10^{-3}$ (0.04)**	-0.03 ± 0.05 (0.58)	-0.04 ± 0.05 (0.50)	-0.04 ± 0.21 (0.86)	$3.59 \times 10^{-4} \pm 3.94 \times 10^{-3}$ (0.93)	0.21 ± 0.23 (0.36)	$-2.40 \times 10^{-3} \pm 2.15 \times 10^{-3}$ (0.26)

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; SE, standard error; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio

[a]inverse normal transformed variables

Data presented are β ± SE (p_{value}). All models were adjusted for age, sex, and recruitment center. Values in bold indicate nominally significant or significant main genetic effects and interactions ($p < 0.05$).

Table 4: Interactions between circulating lipids, *PPARγ* Pro12Ala and the presence of insulin resistance.

	OR $_{interaction}$ (95% CI)	**P $_{interaction}$**	**OR $_{main\ genetic\ effect}$ (95% CI)**	**P $_{main\ genetic\ effect}$**
PPARγ x TC	**0.98 (0.97 – 0.99)**	**9.30×10^{-4}**	**18.39 (2.57 – 131.79)**	**9.54×10^{-4}**
PPARγ x TG[a]	1.06 (0.61 – 1.85)	0.84	0.87 (0.47 – 1.61)	0.66
PPARγ x HDL-C	0.98 (0.94 – 1.02)	0.28	2.52 (0.39 – 16.43)	0.33
PPARγ x LDL-C	**0.98 (0.96 – 0.99)**	**8.09×10^{-3}**	**8.70 (1.62 – 46.87)**	**0.01**

Abbreviations: CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglycerides

[a]inverse normal transformed variables

Data presented are OR (95% CI). All models were adjusted for age, sex, and recruitment center. Values in bold indicate nominally significant or significant main genetic effects and interactions ($p < 0.05$).

Table 5: Circulating lipid subgroup analysis for significant interactions between *PPARγ* Pro12Ala and metabolic traits

	WHR[a]	
	β ± SE	**p value**
Low HDL-C	-0.10 ± 0.08	0.17
High HDL-C	0.09 ± 0.07	0.20
	Insulin[a]	
Low TC	0.09 ± 0.08	0.24
High TC	-0.19 ± 0.08	**0.02**
	HOMA-IR[a]	
Low TC	0.10 ± 0.08	0.24
High TC	-0.17 ± 0.08	**0.03**
	Insulin Resistance	
	OR (95% CI)	**p value**
Low TC	1.69 (0.92 – 2.96)	0.07
High TC	0.41 (0.20 – 0.84)	**0.02**
Low LDL-C	1.72 (0.97 – 3.04)	0.07
High LDL-C	0.44 (0.27 – 0.87)	**0.02**

Abbreviations: CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; WHR, waist-to-hip ratio

[a]inverse normal transformed variables

Data presented are β ± SE (p_{value}) or OR (95% CI). All models were adjusted for age, sex, and recruitment center. Values in bold indicate nominally significant or significant interactions ($p < 0.05$).

Supplementary Table 1: Power calculation for the main effect of PPARγ rs1801282 on BMI

MAF	Beta	Sample Size (unadjusted)[a]	Sample Size (adjusted)[b]	Interaction Sample Size (unadjusted)[a]	Interaction Sample Size (adjusted)[b]
0.14	0.50	2296	4495	118	230
	0.60	1596	3119	79	155
	0.70	1169	2290	56	109
	0.80	894	1751	41	80
	0.90	706	1382	31	60

Abbreviations: MAF, minor allele frequency

[a] power calculation unadjusted for multiple testing (2 sided p-value = 0.05, 80% power)

[b] power calculation adjusted for multiple testing (2 sided p-value = 2.08×10^{-3}, 80% power)

CHAPTER 4: Genetic markers of inflammation may not contribute to metabolic traits in Mexican children

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ABSTRACT:

BACKGROUND: Low-grade chronic inflammation is a common feature of obesity and its cardio-metabolic complications. However, little is known about a possible causal role of inflammation in metabolic disorders. Mexico is among the countries with the highest obesity rates in the world and the admixed Mexican population is a relevant sample due to high levels of genetic diversity.

METHODS: Here, we studied 1,462 Mexican children recruited from Mexico City. Six genetic variants in five inflammation-related genes were genotyped: rs1137101 (leptin receptor (*LEPR*)), rs7305618 (hepatocyte nuclear factor 1 alpha (*HNF1A*)), rs1800629 (tumor necrosis factor alpha (*TNFA*)), rs1800896, rs1800871 (interleukin-10 (*IL-10*)), rs1862513 (resistin (*RETN*)). Ten continuous and eight binary traits were assessed. Linear and logistic regression models were used adjusting for age, sex, and recruitment centre.

RESULTS: We found that one SNP displayed a nominal evidence of association with a continuous trait: rs1800871 (*IL-10*) with LDL (beta = -0.068 ± 1.006, P = 0.01). Subsequently, we found one nominal association with a binary trait: rs7305618 (*HNF1A*) with family history of hypertension (odds-ratio = 1.389 [1.054-1.829], P = 0.02). However, no P-value passed the Bonferroni correction for multiple testing.

DISCUSSION: Our data in a Mexican children population are consistent with previous reports in European adults in failing to demonstrate an association between inflammation-associated single nucleotide polymorphisms (SNPs) and metabolic traits.

INTRODUCTION

Obesity has increased rapidly in prevalence over the last 30 years causing a growing public health burden at the worldwide level[1]. Obesity is no longer only a concern for high income countries, but is escalating in developing countries as well[2]. Even more concerning are the increasing rates of childhood obesity which have tripled over the last 30 years[1]. In 2011-2012, the age-adjusted prevalence of obesity in adults from the United States of America was 47.8%, 42.5%, 32.6% and 10.8% in non-Hispanic Blacks, Hispanics, non-Hispanic White Americans, and non-Hispanic Asians, respectively[3]. These discrepancies may be due to differences in diet, lifestyle, socioeconomic status and access to health care across ethnic groups. However they may also reflect differences in the genetic susceptibility to obesity and metabolic disorders as evidenced by admixture studies[4]. Twin studies have reported heritability estimates between 47-90% for body mass index (BMI)[5]. Eleven monogenic genes and more than 140 polygenic loci have been identified to date, accounting for a modest fraction of the heritability of obesity[6,7]. Obesity is associated with cardio-metabolic complications (insulin resistance, type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease) that cluster into the so-called metabolic syndrome[8]. However, the relationship between obesity and associated complications is complex as obesity does not always convert into a metabolic syndrome [9,10]. Consistent with the phenotypic correlations seen in observational epidemiology, shared genetic contributions between the components of the metabolic syndrome suggest that shared molecular roots may be involved in the development of the metabolic syndrome[11-13].

Inflammation has recently been advocated as one of the pathophysiological mechanisms linking obesity to other metabolic complications[14]. Inflammation can be defined as a protective response of an organism to infection and injury. This operates through initiating a healing process of pathogen killing and tissue repair to restore homeostasis at the infected and/or

damaged sites[14]. Normally, the inflammatory response to harmful stimuli is short-lived and once the damage is removed or neutralized, the inflammation is resolved through negative feedback mechanisms[14]. However, inflammatory response that fails to regulate itself becomes chronic and is believed to set the stage for a broad range of diseases[14]. Obesity and its cardio-metabolic complications are associated with low-grade chronic inflammation, characterized by abnormal cytokine production, activation of a network of inflammatory signal pathways, and new connective tissue formation[15].

Genome-wide association and in a lesser extent candidate gene studies identified around fifty common genetic variants associated with serum inflammatory biomarker levels (e.g. C-reactive protein (CRP), soluble Intercellular Adhesion Molecule 1 (sICAM-1), interleukin-6 (IL-6) or soluble P-selectin)[16]. Researchers then used these recently discovered genetic variants to determine whether this chronic inflammation is a cause of obesity and other metabolic disorders, or a consequence of it. Overall, Mendelian randomization experiments including gene variants in inflammation pathways did not evidence a causal role of inflammation in obesity or type 2 diabetes[17-19] and conflicting results about a causal link between inflammation and cardiovascular disease have been reported[16,19-21]. At this stage, more research is needed to understand the role of inflammation in the development of obesity and cardio-metabolic complications, particularly in non-European populations.

Metabolic syndrome is observed in childhood obesity, but can also develop in lean children, suggesting that obesity is a marker for the syndrome, not a cause[22]. Since obesity and its complications are associated with atherogenesis starting in childhood and early adulthood[23,24], a better understanding of the molecular mechanisms involved in the clustering of cardio-

metabolic factors early in life may help to develop more efficient programs to prevent the development of metabolic syndrome.

The Mexican population is characterized by a high prevalence of obesity and metabolic complications. The 2012 National Health and Nutrition Survey indicates that 34.4% and 71.2% of the Mexican children and adults respectively are overweight or obese[24,25]. This ranks Mexico among the countries with the highest obesity rates in the world[25,26]. The prevalence of metabolic syndrome (ATP III criteria) in children and adolescents living in Mexico was estimated to be 20% in 2006[26,27]. Depending on the definitions used (American Heart Association/ National Heart, Lung, and Blood Institute or the International Diabetes Federation), the prevalence of metabolic syndrome among Mexican adults ranges from 59.7 to 68.7%[27]. This exceptionally high burden of obesity and metabolic syndrome in the Mexican population is largely due to the rapid transition towards an 'obesogenic' environment characterized by a sedentary lifestyle, an increase in the consumption of sugar-sweetened beverages coupled with the recent proliferation of fast food restaurants[28]. However, the tremendous genetic variety and unique genetic architecture of the admixed Mexican population may partly account for a higher susceptibility to obesity and metabolic disturbances than in other populations[29]. Mexican populations consist of Native individuals as well as individuals of European or African descent[29]. The distributions and proportions of these three groups vary with the region studied however evidence shows very few true Natives remain as virtually all native groups show some degree of admixture, mainly with Europeans[30]. Thus studying the Mexican population gives insight into the disease mechanisms of a variety of races due to the genetic diversity present in the population[30,31].

In this study, we assessed the association of 6 common genetic single nucleotide polymorphisms (SNPs) in 5 inflammation-related genes with 10 continuous and 8 binary

metabolic traits in 1462 children from the Mexican population. Our data do not favor an association between inflammatory processes and the development of metabolic complications.

METHODS

Study Participants

A total of 1462 unrelated children aged 6-14 having both genetic and phenotypic data available were included in this study. Children were randomly selected to participate in a cross-sectional study from four schools in Mexico City between July 2011 and July 2012. Anthropometric traits were assessed by a trained pediatrician. Blood samples were collected for biochemical measurements and DNA extraction. Information regarding family history of type 2 diabetes, obesity and hypertension was obtained via questionnaires. The study protocol was approved by the Mexican Social Security Institute National Committee and the Ethical Committee Board and all experiments were performed in accordance with relevant guidelines and regulations. Informed consent was obtained from both parents and the child.

Genotyping

Genomic DNA was extracted from peripheral blood using the FLEX STAR Autogen platform (Holliston, Massachusetts US). The genotyping was performed using the TaqMan OpenArray Real-Time PCR System (Life Technologies, Carlsbad, US), following the manufacturer's instructions. We selected 6 SNPs in or near 5 genes that displayed redundant associations with inflammation-related traits in literature: rs1137101 (leptin receptor (*LEPR)*), rs7305618 (hepatocyte nuclear factor 1 alpha (*HNF1A)*), rs1800629 (tumor necrosis factor alpha (*TNFA)*), rs1800896, rs1800871 (interleukin-10 (*IL-10)*), rs1862513 (resistin (*RETN)*)[16,32-34]. The 6 SNPs showed no deviation from Hardy-Weinberg Equilibrium ($0.22 \leq P \leq 0.76$). The call rate

for each of the 6 SNPs was comprised between 94.6 and 100 % (Supplementary Table 2). The two SNPs rs1800896 and rs1800871 in *IL-10* display modest linkage disequilibrium in the Mexican children study sample (r^2 value = 0.239).

Phenotyping

All participants were weighed using a digital scale (Seca, Hamburg, Germany). Height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Body mass index was calculated as weight (kg) / (height $(m)^2$) and classified as underweight, normal weight, overweight, obese according to the Centers for Disease Control and Prevention CDC 2000 references. Waist circumference (WC) and hip circumference (HC) were measured at the midpoint between the lowest rib and the iliac crest at the top of the iliac crest respectively, after a normal exhalation with children in the standing position. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan). Blood pressure readings were taken for each participant twice on the right arm in a sitting position with a 5 minute rest between each measurement and the mean of the two readings was determined. Hypertension was defined as average measured blood pressure above the American Heart Association's recommendations (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg). Blood samples were obtained following a 12 hour fast and were analyzed for fasting glucose, total cholesterol (TC), HDL-cholesterol (HDL), LDL-cholesterol (LDL) and triglycerides (TG) using the ILab 350 Clinical Chemistry System (Instrumentation Laboratory IL. Barcelona Spain). Insulin (IU) was measured by chemiluminescence (IMMULITE, Siemens, USA). The 2003 ADA criteria for fasting plasma glucose (FPG) were used to classify children as normal (FPG < 5.6 mmol/L), as having impaired fasting glucose (IFG; FPG 5.6- 6.9 mmol/L), or as having T2D (FPG > 7.0 mmol/L)[35]. Subjects with IFG or T2D were considered as having hyperglycemia. Dyslipidemia was defined as fasting triglycerides ≥ 100 mg/dL (0-9 years of age) or triglycerides

≥ 130 mg/dL (10-19 years of age) and/or HDL-C < 35 mg/dL and/or LDL-C ≥ 130 mg/dL, according to current recommendations[36]. Information regarding family history of type 2 diabetes, overweight / obesity, and hypertension was obtained via questionnaires.

Statistical Analyses

Statistical analyses were performed using SPSS (version 20). We assessed the power of our sample using QUANTO software version 1.2.4 (University of Southern California, Los Angeles, CA, USA). Non-biological outlier data were discarded. Due to the risk of blood hemolysis, fasting insulin values < 1 mIU/l were discarded from the study. The normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. All traits of interest deviated significantly from normality. Logarithmic transformations corrected the lack of normality for fasting insulin, improved the distribution of six traits (BMI, waist and hip circumference, waist to hip ratio, total cholesterol, triglycerides,) despite still deviating from normality, and did not improve the distribution of fasting glucose, HDL and LDL cholesterol. Linear regression models were used to examine the association between the SNPs and metabolic traits. These tests were adjusted for sex, age and the recruitment centre. Genetic association studies were performed under an additive mode of inheritance for 5 out of 6 SNPs and the effect allele was the minor allele. Because only one AA homozygous carrier was identified for rs1800629 (*TNFA*), we used a dominant model instead. Two-sided P <0.05 before Bonferroni correction were considered as nominally significant. After applying a Bonferroni's correction for multiple testing (18 binary / continuous traits x 6 SNPs), P-values < 4.6×10^{-4} (0.05/108) was considered as significant.

RESULTS

Characteristics of the Mexican children population

The main anthropometric and biological characteristics of the 1462 Mexican children are summarized in Table 6. Fifty-three percent of the population were males. Children exhibited an average age and BMI of 9.24 ± 2.07 years and 19.65 ± 4.20 kg/m^2, respectively. Using the Centers for Disease Control and Prevention 2000 references, 1.4% of the children were underweight, 49.4% were normal weight, 21.3% were overweight and 27.9% were obese. Additionally, 1.5, 3.1 and 34.9% of children displayed hypertension, hyperglycemia, and dyslipidemia, respectively. A family history of overweight / obesity, type 2 diabetes or hypertension was reported for 53.0, 12.0 and 16.3 % of children, respectively (Table 6). The sample size was similar for all traits except fasting insulin (data available in 78.5% of subjects) due to the phenomenon of blood hemolysis.

Association between genetic markers of inflammation and continuous metabolic traits

The associations between the 6 genetic variants of inflammation and 10 continuous metabolic traits are reported in Table 7. Only one SNP displayed a nominal evidence of association: rs1800871 (*IL-10*) with LDL (β = -0.068 ± 1.006, P = 0.010).

Association between genetic markers of inflammation and binary metabolic traits

The associations between the 6 genetic markers of inflammation and 8 binary metabolic traits are reported in Table 8. One nominally significant association was found: rs7305618 (*HNF1A*) with family history of hypertension (1.389 [1.054-1.829] p=0.020). No P-value was significant after Bonferroni correction for multiple testing.

DISCUSSION

In the present study, we assessed the association of 6 common genetic variants in 5 inflammation-related genes with 10 continuous and 8 binary metabolic traits in 1462 children from the Mexican population. We found one nominal associations between a genetic variant and the continuous traits. Subsequently, we only found two nominal associations between genetic variants and continuous / binary metabolic traits. No P-value resisted to a Bonferroni correction for multiple testing ($P < 4.6 \times 10^{-4}$). The number of significant P-values obtained in this experiment at the 0.05 alpha level was less than the number of associations expected by chance (~ 5). Overall, our negative results do not suggest an association between inflammation-associated SNPs and metabolic traits in Mexican children. This is in line with previous reports from literature, that at best suggest a possible association between inflammation and cardiovascular events[16-18,20,22,37,38]. Our findings are also supported by the discoveries of hypothesis-free genome-wide association studies for metabolic traits that show a limited overlap with genetic markers of inflammation to date[16,39-42].

Power calculations on the standard trait BMI indicate that we only have a fair likelihood to identify associations at the nominal and Bonferroni corrected levels (Supplementary Figures 1 and 2). Therefore, we cannot totally exclude that the nominal associations reported here are actually true subtle positive results. For instance, we found that the rs7305618 SNP near *HNF1A* was nominally associated with a family history of hypertension. The *HNF1A* gene encodes hepatic nuclear factor 1 alpha (HNF1a), a transcription factor expressed in the liver, pancreas, gut and kidney[43]. Mutations in the *HNF1A* gene account for approximately 70% of cases of maturity onset diabetes of the young (MODY)[44]. *HNF1A* mutation carriers display a distinct hypertension status[45]. HNF-1a is an essential transcription factor in nephron development and

rare coding loss-of-function mutations in *HNF1A* lead to renal malformations and renal dysfunction in mice and humans[46-48]. Testing the associations of the *HNF1A* rs7305618 SNP with adult hypertension in independent studies may therefore be relevant. Similarly, the association of rs1800871 (*IL-10*) with LDL is indirectly supported by previous reports in literature. While the adenovirus-mediated gene transfer of interleukin-10 in an hyperlipidemic LDLr knock-out mouse model results in lowering of cholesterol levels and attenuation of atherogenesis, interleukin-10 deficiency in a distinct hyperlipidemic apolipoprotein E knock-out mouse model leads to an increase of LDL and atherosclerosis[49,50]. However, further studies in independent Mexican children populations are needed to confirm these nominal associations. No study in children has assessed the association of genetic markers of inflammation with metabolic traits, making any comparisons to our data difficult.

Our study has several strengths. It is the first to explore the associations of a representative list of genetic variants related to inflammation with metabolic traits in children and in the Mexican population. Additionally, we assessed diverse metabolic traits including both continuous and binary variables. Limitations of the study include an under-optimal statistical power to identify even substantial genetic effects, especially after corrections for multiple tests (Supplementary Figures 1 and 2). Additionally, the list of SNPs related to inflammation that we assessed did not include the more recent GWAS discoveries for inflammation traits[16]. We did not assess the association of these SNPs with intermediate inflammatory serum markers (e.g. CRP, sICAM-1, IL-6, soluble P-selectin). Finally, using ancestry informative markers to adjust for potential population stratification was not performed in this study.

In conclusion, the association study of 6 SNPs in 5 inflammation-related genes with 10 continuous and 8 binary cardio-metabolic traits in 1462 Mexican children does not suggest an

association between inflammation-associated SNPs, obesity and its metabolic complications. Additional studies with larger sample sizes, a more exhaustive panel of SNPs and the availability of both inflammatory serum biomarkers and clinical traits in Mexican and other populations will provide a more definitive answer to this important research topic.

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Table 6: Characteristics of the Mexican children population.

Trait	Mean ± Standard Deviation	Sample Size
Sex (% Male/Female)	53.0/47.0	775/687
Age (Years)	9.24 ± 2.07	1462
BMI (Kg/m2)	19.65 ± 4.20	1461
Waist to hip ratio	0.85 ± 0.06	1417
Systolic blood pressure (mmHg)	98.58 ± 10.88	1457
Diastolic blood pressure (mmHg)	66.25 ± 8.80	1458
Low density lipoprotein-cholesterol (mg/dl)	102.43 ± 26.43	1462
High density lipoprotein-cholesterol (mg/dl)	50.58 ± 12.82	1462
Total cholesterol (mg/dl)	157.27 ± 33.53	1462
Triglycerides (mg/dl)	93.67 ± 49.69	1462
Fasting glucose (mmol/l)	4.57 ± 0.53	1461
Fasting insulin (mIU/l)	9.10 ± 7.05	1148
Underweight (%)	1.40	1462
Normal weight (%)	49.40	1462
Overweight (%)	21.30	1462
Obese (%)	27.90	1462
Hypertension (%)	1.50	1452
Hyperglycemia (%)	3.10	1456
Dyslipidemia (%)	34.90	1457
Type 2 diabetes family history (%)	11.98	1461
Hypertension family history (%)	16.29	1461
Overweight / obesity family history (%)	53.05	1461

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Table 7: Characteristics of the Mexican children population.

Trait	Mean ± Standard Deviation	Sample Size
Sex (% Male/Female)	53.0/47.0	775/687
Age (Years)	9.24 ± 2.07	1462
BMI (Kg/m2)	19.65 ± 4.20	1461
Waist to hip ratio	0.85 ± 0.06	1417
Systolic blood pressure (mmHg)	98.58 ± 10.88	1457
Diastolic blood pressure (mmHg)	66.25 ± 8.80	1458
Low density lipoprotein-cholesterol (mg/dl)	102.43 ± 26.43	1462
High density lipoprotein-cholesterol (mg/dl)	50.58 ± 12.82	1462
Total cholesterol (mg/dl)	157.27 ± 33.53	1462
Triglycerides (mg/dl)	93.67 ± 49.69	1462
Fasting glucose (mmol/l)	4.57 ± 0.53	1461
Fasting insulin (mIU/l)	9.10 ± 7.05	1148
Underweight (%)	1.40	1462
Normal weight (%)	49.40	1462
Overweight (%)	21.30	1462
Obese (%)	27.90	1462
Hypertension (%)	1.50	1452
Hyperglycemia (%)	3.10	1456
Dyslipidemia (%)	34.90	1457
Type 2 diabetes family history (%)	11.98	1461
Hypertension family history (%)	16.29	1461
Overweight / obesity family history (%)	53.05	1461

Table 8: Association between 6 genetic markers of inflammation and 10 continuous metabolic traits.

	BMI[a]	WHR[a]	SBP[a]	DBP[a]	LDL	HDL	TC[a]	TG[a]	FG	FI[a]
rs1137101 (*LEPR*)	0.002 ± 0.007 (0.83)	-0.003 ± 0.002 (0.19)	-0.001 ± 0.004 (0.83)	0.003 ± 0.005 (0.50)	-0.023 ± 0.980 (0.38)	-0.009 ± 0.477 (0.73)	-0.008 ± 0.007 (0.27)	-0.007 ± 0.017 (0.66)	-0.019 ± 0.020 (0.47)	-0.008 ± 0.026 (0.78)
rs7305618 (*HNF1A*)	-0.003 ± 0.010 (0.76)	-0.004 ± 0.004 (0.319)	-0.006 ± 0.006 (0.31)	-0.010 ± 0.007 (0.15)	0.011 ± 1.478 (0.69)	0.006 ± 0.717 (0.83)	-0.006 ± 0.011 (0.57)	-0.016 ± 0.025 (0.53)	0.010 ± 0.029 (0.71)	0.029 ± 0.040 (0.46)
rs1800629 (*TNFA*)[b]	0.002 ± 0.017 (0.93)	0.008 ± 0.006 (0.75)	0.022 ± 0.010 (0.37)	0.005 ± 0.012 (0.85)	0.002 ± 2.394 (0.93)	0.037 ± 1.156 (0.14)	0.006 ± 0.019 (0.81)	0.021 ± 0.043 (0.42)	0.031 ± 4.619 (0.24)	0.000 ± 0.066 (0.99)
rs1800896 (*IL-10*)	-0.001 ± 0.008 (0.94)	-0.003 ± 0.003 (0.38)	0.005 ± 0.004 (0.28)	0.004 ± 0.006 (0.53)	-0.006 ± 1.158 (0.84)	0.012 ± 0.566 (0.65)	-0.001 ± 0.009 (0.92)	-0.018 ± 0.020 (0.37)	0.034 ± 0.023 (0.21)	3.89×10^{-5} ± 0.031 (0.99)
rs1800871 (*IL-10*)	-0.005 ± 0.007 (0.49)	-0.001 ± 0.003 (0.57)	-0.004 ± 0.004 (0.33)	-0.005 ± 0.005 (0.31)	**-0.068 ± 1.006 (0.01)**	-0.011 ± 0.489 (0.67)	-0.010 ± 0.008 (0.19)	0.010 ± 0.017 (0.56)	-0.006 ± 0.020 (0.82)	-0.024 ± 0.027 (0.37)
rs1862513 (*RETN*)	-0.020 ± 0.011 (0.08)	-0.005 ± 0.004 (0.17)	-0.005 ± 0.006 (0.39)	0.002 ± 0.008 (0.82)	0.022 ± 1.570 (0.41)	0.35 ± 0.765 (0.19)	0.014 ± 0.012 (0.24)	-0.017 ± 0.027 (0.54)	0.010 ± 0.032 (0.71)	-0.070 ± 0.042 (0.09)

Values in bold indicate P value < 0.05; data are presented as beta ± standard error (P-value). [a] Natural logarithmic transformation applied. [b] SNP analyzed under the dominant model.

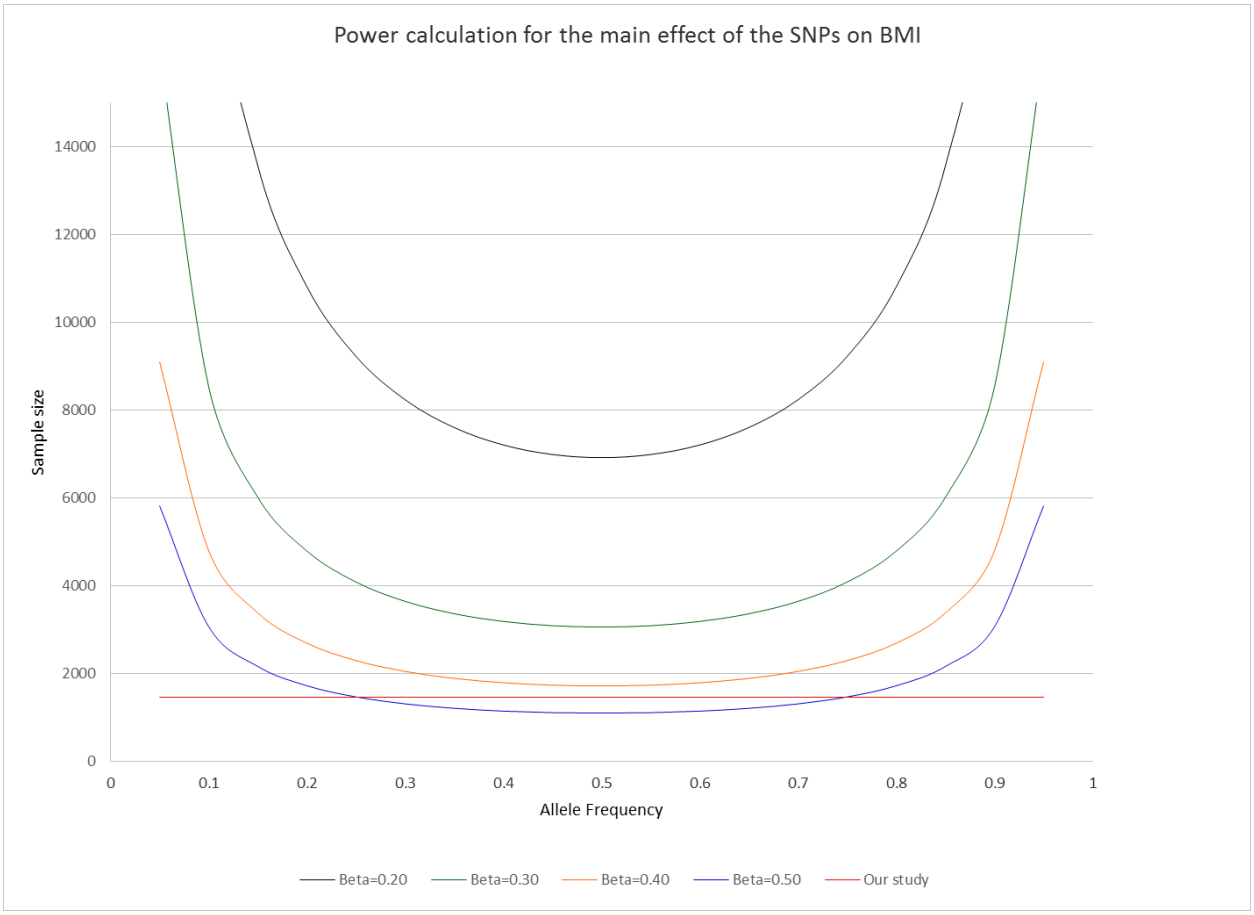
Table 9: Association between 6 genetic markers of inflammation and 8 binary metabolic traits

	Normal weight vs. obese	Normal weight vs. overweight and obese	Hypertension	Hyperglycemia	Dyslipidemia	Type 2 diabetes family history	Hypertension family history	Overweight / obesity family history
rs1137101 (*LEPR*)	0.052 [0.886-1.253] (0.56)	1.004 [0.867-1.162] (0.96)	1.415 [0.782-2.562] (0.25)	0.774 [0.505-1.186] (0.24)	0.995 [0.853-1.161] (0.95)	0.883 [0.706-1.105] (0.28)	0.861 [0.707-1.049] (0.14)	0.925 [0.799-1.072] (0.30)
rs7305618 (*HNF1A*)	0.849 [0.652-1.106] (0.23)	0.879 [0.704-1.097] (0.25)	0.732 [0.263-2.038] (0.55)	0.938 [0.485-1.816] (0.85)	0.871 [0.688-1.102] (0.25)	1.347 [0.988-1.837] (0.06)	**1.389 [1.054-1.829] (0.02)**	1.050 [0.841-1.311] (0.67)
rs1800629 (*TNFA*)[a]	1.175 [0.767-1.799] (0.46)	1.111 [0.767-1.609] (0.58)	1.110 [0.260-4.736] (0.89)	0.793 [0.244-2.576] (0.70)	1.084 [0.738-1.592] (0.68)	0.652 [0.336-1.266] (0.21)	0.553 [0.300-1.018] (0.06)	1.020 [0.702-1.482] (0.92)
rs1800896 *(IL-10)*	1.011 [0.825-1.239] (0.92)	1.050 [0.883-1.249] (0.58)	0.827 [0.393-1.743] (0.62)	1.348 [0.834-2.180] (0.22)	1.041 [0.868-1.238] (0.67)	1.060 [0.813-1.383] (0.67)	0.991 [0.785-1.253] (0.94)	1.104 [0.927-1.314] (0.27)
rs1800871 (*IL-10)*	0.929 [0.778-1.108] (0.41)	0.962 [0.827-1.118] (0.61)	0.851 [0.457-1.584] (0.61)	1.152 [0.750-1.769] (0.52)	0.944 [0.805-1.107] (0.48)	1.015 [0.807-1.277] (0.90)	0.876 [0.714-1.075] (0.20)	0.906 [0.779-1.054] (0.20)
rs1862513 (*RETN*)	0.785 [0.585-1.052] (0.11)	0.932 [0.736-1.180] (0.56)	1.065 [0.416-2.726] (0.90)	0.677 [0.310-1.477] (0.33)	0.941 [0.733-1.209] (0.64)	1.135 [0.801-1.609] (0.48)	0.981 [0.713-1.350] (0.91)	0.984 [0.777-1.246] (0.89)

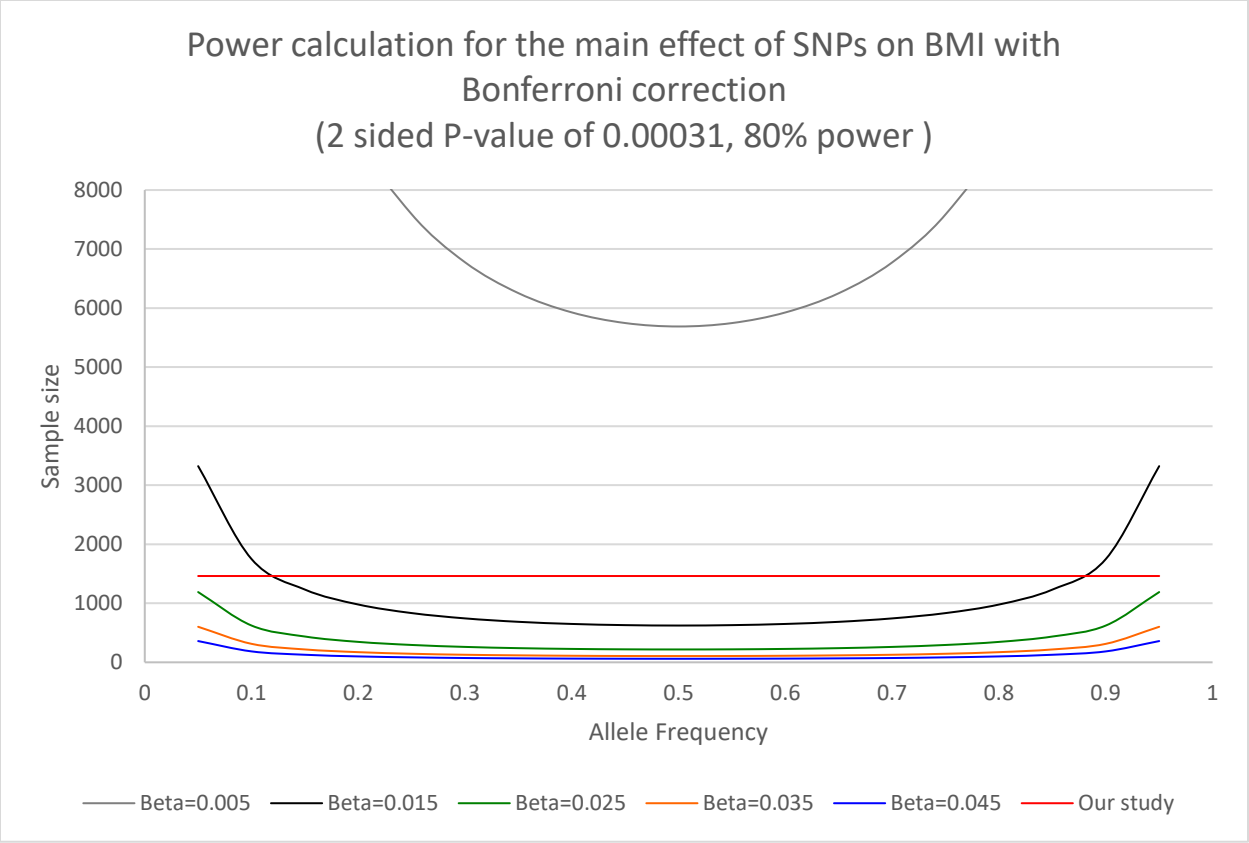
Values in bold indicate P value < 0.05; data are presented as beta [confidence interval] (P-value). [a] SNP analyzed under the dominant mode.

Supplementary Table 2: Description of the 6 SNPs studied.

Gene Name	Chromosomal Physical Location	SNP	Major Allele	Minor Allele	Genotype Count	Call Rate (%)	HWE P-value
LEPR	1q31.3	rs1137101	A	G	423 712 312	98.97	0.70
HNF1A	12q24.31	rs7305618	C	T	1083 300 27	96.44	0.25
TNFA	6p21.3	rs1800629	G	A	1343 118 1	100	0.33
IL-10	1q31-32	rs1800896	T	C	791 527 84	95.90	0.76
		rs1800871	G	A	473 732 248	99.38	0.22
RETN	19p13.2	rs1862513	C	G	1089 275 19	94.60	0.73



Supplementary Figure 1: Power calculation for the main effect of the SNPs on BMI, two-sided P-value of 0.05, 80%.



Supplementary Figure 2: Power calculation for the main effect of SNPs on BMI at a significant level (P=3.1 x 10-4).

CHAPTER 5: Adiponectin is associated with cardio-metabolic traits in Mexican children

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ABSTRACT: The adipocyte-derived adiponectin hormone bridges obesity and its cardio-metabolic complications. Genetic variants at the *ADIPOQ* locus, in *ADIPOR1*, and *ADIPOR2* have been associated with adiponectin concentrations and cardio-metabolic complications in diverse ethnicities. However, no studies have examined these associations in Mexican children. We recruited 1 457 Mexican children from Mexico City. Six genetic variants in or near *ADIPOQ* (rs182052, rs2241766, rs266729, rs822393), *ADIPOR1* (rs10920533), and *ADIPOR2* (rs11061971) were genotyped. Associations between serum adiponectin, genetic variants, and cardio-metabolic traits were assessed using linear and logistic regressions adjusted for age, sex, and recruitment center. Serum adiponectin concentration was negatively associated with body mass index, waist to hip ratio, low-density lipoprotein cholesterol, total cholesterol, triglycerides, fasting glucose, fasting insulin, homeostatic model assessment of insulin resistance, dyslipidemia and overweight/obesity status ($7.76 \times 10^{-40} \leq p \leq 3.00 \times 10^{-3}$). No significant associations between genetic variants in *ADIPOQ, ADIPOR1, and ADIPOR2* and serum adiponectin concentration were identified (all $p \geq 0.30$). No significant associations between the six genetic variants and cardio-metabolic traits were observed after Bonferroni correction (all $p < 6.9 \times 10$-4). Our study suggests strong associations between circulating adiponectin concentration and cardio-metabolic traits in Mexican children.

INTRODUCTION

In 2016, the World Health Organization reported that 1.9 billion adults and 381 million children were overweight/obese, resulting in an important global health concern. Obesity is associated with the development of comorbidities (insulin resistance (IR), type 2 diabetes (T2D), dyslipidemia, hypertension), collectively known as the metabolic syndrome[1]. Several therapeutic options are available, however controlling the development of obesity and its resulting complications has proven challenging[2]. Chronic obesity in its more severe forms leads to major reductions in life expectancy, with most of the excess deaths due to heart disease, cancer, and T2D[3]. As a result, obesity imposes a heavy socio-economic burden in both high-income and developing countries[4].

The Mexican population is a group at high risk for developing obesity and the metabolic syndrome, especially in childhood[5]. The prevalence of obesity in Mexican school-aged children was 11.8% in girls and 17.4% in boys in 2012[5]. The metabolic syndrome prevalence was 9.4% in Mexican adolescents in 2010[6]. The rise of childhood obesity in Mexico is largely explained by a 'nutritional transition' which reflects changes in dietary patterns characterized by increased consumption of foods that are high in fat and/or sugar, as well as reduced physical activity[7]. Beyond modifiable factors, the elucidation of biological determinants of obesity and its cardio-metabolic complications is expected to improve prediction, prevention and care, including novel treatments adapted to genetic profiles of high-risk populations[8].

Adiponectin, an adipocyte-derived secretagogue, may be considered as one of the key hormones bridging obesity and its cardio-metabolic complications[9]. Genetic mouse models have shown that deficiency of adiponectin contributes to IR, while its overexpression in leptin-deficient obese mice promotes adipose tissue expansion and improves insulin sensitivity[10,11].

Adiponectin acts on two receptors (adiponectin receptors 1 and 2) encoded by *ADIPOR1* and *ADIPOR2* genes, both of which appear to show functional redundancy[12]. Simultaneous disruption of both AdipoR1 and AdipoR2 in the liver of leptin-deficient obese mice leads to IR and marked glucose intolerance[12]. In humans, adiponectin is abundantly found in the bloodstream where it makes up 0.01-0.05% of total plasma protein[13]. Low serum adiponectin has been associated with obesity, IR, T2D, dyslipidemia, hypertension and coronary heart disease in cross-sectional studies[9]. Adiponectin level was also negatively associated with incident development of insulin resistance, T2D, dyslipidemia, hypertension, and coronary artery disease[9]. The relationship between adiponectin level and subsequent weight gain has been a topic of interest due to its paradoxical nature, where levels of adiponectin decrease with the development of obesity[14]. Adiponectin was positively associated with weight gain in children, but not in adults in prospective studies[15,16].

If serum adiponectin levels are influenced by modifiable factors such as physical activity and diet, genetic factors account for 30-93% of variation in adiponectin levels in humans[17,18]. Encoded by the *ADIPOQ* locus and found on chromosome 3q27, adiponectin is a 30 kDA protein with both a collagenous N and a globular C-terminus[18]. Candidate gene studies, and more recently genome-wide association studies (GWAS), fine-mapping or resequencing experiments have identified numerous common and rare variants at the *ADIPOQ* locus associated with serum adiponectin level and metabolic traits[18-24]. If common variants in the *ADIPOR1* and *ADIPOR2* genes have not been associated with serum adiponectin levels, they contribute to IR, T2D and cardiovascular disease risk[18,22,25,26].

While serum adiponectin levels negatively correlate with obesity, T2D and the components of the metabolic syndrome in Mexican children, high adiponectin concentrations are

associated with a metabolically healthy but obese profile in Mexican adults[27-29]. A few studies investigated the association of SNPs in *ADIPOQ ADIPOR1*, and *ADIPOR2* with serum adiponectin levels and cardio-metabolic traits in Mexican and Mexican-American adults[30-33]. However, to date, no study has investigated these genetic associations in Mexican children. This prompted us to analyze in 1 457 Mexican children 1) the association of adiponectin levels with cardio-metabolic traits, 2) the association of six SNPs in *ADIPOQ*, *ADIPOR1*, *ADIPOR2*, and serum adiponectin levels, and 3) the association of the same SNPs with cardio-metabolic traits.

METHODS

Study population

A total of 1, 559 children between the ages of 5 and 17 were randomly selected to participate in a cross-sectional study from four areas in Mexico City at the Primary Care Unit of the National Mexican Social Security Institute (Cuauhtémoc West, Independencia South, Nezahualcóyotl Est and Morelos North area). Recruitment was done in collaboration with local public schools. The study started in July 2011 and is still ongoing. A trained pediatrician performed all the anthropometric measurements. Blood samples were collected for biochemical measurements and DNA extraction. Children who had diagnosis of infectious disease, gastrointestinal disorders, administration of antimicrobial agents (within 6 months prior to study), incomplete questionnaires or biological samples were excluded. The child's assent and written informed consent from the parents/guardians was obtained prior to enrolment into the study. The study protocol was approved by the Mexican Social Security Institute National Committee and the Ethical Committee Board. All procedures were conducted in accordance with the relevant guidelines and regulations of the Declaration of Helsinki[34].

Phenotyping

All participants were weighed using a digital scale (Seca, Hamburg, Germany) and height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Height, weight and body mass index (BMI), calculated as weight (kg) / height $(m)^2$, were converted to age- and gender- adjusted standard deviation scores (SDS-Height, SDS-Weight and SDS-BMI, respectively) using the LMS method according to guidelines from the Centers for Disease Control (CDC)[35,36]. Waist circumference (WC) was measured at the midpoint between the lowest rib and the iliac crest after a normal exhalation with children in the standing position. Hip circumference was measured at the level of the greater trochanters. The waist to hip ratio (WHR) was also converted to age- and gender- adjusted standard deviation scores (SDS-WHR) using the LMS method and growth charts based on US National Health and Nutrition Survey, cycle III (NHANES III)[37] . BMI was used to classify children as underweight, normal weight, overweight, or obese, according to the Centers for Disease Control and Prevention CDC 2000 references. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan). Blood pressure readings were taken for each participant twice on the right arm in a sitting position with 5 minutes rest between each measurement and the mean of the two readings was determined. Age- and gender- adjusted standard deviations scores for SBP and DBP (SDS-SBP and SDS-DBP) were calculated using methods specified by the fourth report from the National High Blood Pressure Education Program (NHBPEP) in children and adolescents[38]. Hypertension was defined as average measured blood pressure above the American Heart Association's recommendations (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg). Blood samples were obtained following a 12 hour fast and were analyzed for glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) using the ILab 350 Clinical

Chemistry System (Instrumentation Laboratory IL. Barcelona Spain). Dyslipidemia was defined as fasting TG ≥ 100 mg/dL (0-9 years of age) or TG ≥ 130 mg/dL (10-19 years of age) and/or HDL-C < 35 mg/dL and/or LDL-C ≥ 130 mg/dL, according to current recommendations[39,40]. Insulin (IU) was measured by chemiluminescence (IMMULITE, Siemens, USA) and homeostatic model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) were calculated using the equation by Matthews *et al* [41]. Due to the risk of blood hemolysis, fasting insulin values < 1 μU/mL were discarded from the study. Insulin resistance was defined as HOMA-IR ≥ 3.4 (the 90th percentile of HOMA-IR in a population of healthy Mexican children)[42]. The 2003 ADA criteria for FPG were used to classify participants as having normal glucose tolerance (NGT), impaired fasting glucose (IFG), or T2D. In absence of oral glucose tolerance test (OGTT) 2-hour fasting plasma glucose value, we used the 2003 American Diabetes Association criteria to define normal fasting glucose (NFG, FPG ≤ 5.6 mmol/L), impaired fasting glucose (IFG, FPG of 5.6 to 6.9 mmol/L), and T2D (FPG ≥ 7.0 mmol/L), as previously described[43,44]. Hyperglycemia was defined as FPG > 5.6 mmol/L. Total adiponectin (μg/mL) was determined by ELISA (Human Adiponectin ELISA Kit, Millipore, St. Charles, MO, USA).

DNA extraction, SNP selection, and genotyping

Genomic DNA was isolated from peripheral blood using a standard extraction protocol on an Autogen FLEX STAR (Holliston, Massachusetts USA). We selected 10 SNPs in *ADIPOQ* (rs2241766, rs266729, rs822393, rs17366568, rs182052, rs4632532, rs7649121), *ADIPOR1* (rs10920533), and *ADIPOR2* (rs11061971, rs16928751) associated with cardio-metabolic traits in literature and harboring minor allele frequencies ≥ 10% in the Mexican population according to the HapMap database. Genotyping of the SNPs was performed using the TaqMan Open Array Real-Time PCR System (Life Technologies, Carlsbad, USA), following the manufacturer's

instructions. Three SNPs (rs4632532, rs7649121, rs16928751) did not reach valid Open Array assay scores. The Open Array experiment involved 64 polymorphisms in total. From the initial sample of 1 559 participants, 102 were excluded from the current analysis because i) no blood sample was collected for DNA extraction; ii) DNA extraction was unsuccessful; iii) the individual genotyping success rate of the Open Array experiment based on the 64 polymorphisms was < 90.6% (≥ 6 genotypes missing). The current analysis included 1 457 children with both genotypic and clinical data available. Only one SNP out of seven did not pass the quality control criteria (rs17366568). The six remaining SNPs harbored a genotyping call rate between 97 and 99%, and no deviation from Hardy-Weinberg equilibrium was observed (p between 0.35 and 0.97; Supplementary Table S1). For quality control purposes, we also compared allele frequencies in our sample with adult Mexican-American reference populations in the 1000 Genomes Project (1000G; Supplementary Table S1). Allele frequencies in our study were not significantly different from the reported frequencies in the 1000G for all SNPs (Supplementary Table 3).

Statistical analyses

The statistical analyses were conducted using the SPSS software (version 20.0) or R (version 3.1.2). We followed the strategy reported previously by Ronald J Feise and considered independent Bonferroni corrections for each question asked[45]. For associations of serum adiponectin with cardio-metabolic traits, two-tailed p-values < 4.2×10^{-3} after Bonferroni correction (0.05/12) were considered statistically significant. For association of SNPs in *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* with serum adiponectin concentration, p-values < 8.3×10^{-3} (0.05/6) were considered statistically significant. For association of the same SNPs with quantitative traits, p-values < 6.9×10^{-4} (0.05/72) were considered statistically significant.

QUANTO software was used for statistical power calculations, assuming normal distribution of quantitative traits, 80% power, and using p-values adjusted for multiple comparisons. Non-biological outlier data were discarded using a Cook's distance test followed by an expert verification. Based on Shapiro-Wilk test (Supplementary Table S8), all the untransformed traits of interest deviated significantly from normality. Hence, rank based inverse normal transformations were applied wherever substantial deviations from normality were observed (Supplementary Figure S1). Differences in *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* SNP allele frequencies were determined by a Chi-square test. Multiple linear and logistic regressions were used to assess associations, while adjusting for covariates of age, sex, and recruitment center. Additional adjustments for BMI or serum adiponectin level were performed for associations with cardio-metabolic traits to investigate the mediation effect of these intermediate traits. An interaction term for Pearson's correlation coefficients and associated p-values were found between all continuous cardio-metabolic traits (Supplementary Table 9). An additive model was used in all the genetic analyses. The minor allele was considered as the effect allele.

RESULTS

Descriptive characteristics of the population

Anthropometric and biochemical characteristics of the 1 457 Mexican children (boys: 52.9%; girls: 47.1%) are summarized in Table 9. The children were 9.24 ± 2.07 years-old and displayed a BMI of 19.65 ± 4.20 kg/m^2 and a SDS-BMI of 0.71 ± 1.09. Within the sample, 20.8% of children were overweight and 23.0% were obese. Insulin resistance was identified in 11.1% of children, 3.1% had hyperglycemia including one child with T2D. Hypertension and dyslipidemia were present in 1.5% and 34.9% of the sample, respectively. The mean serum adiponectin concentration was 5.26 ± 1.23 µg/mL.

Association of serum adiponectin concentration with cardio-metabolic traits

We investigated the association of serum adiponectin concentration with cardio-metabolic traits adjusted for sex, age, and recruitment center (Table 10). Serum adiponectin concentration was negatively associated with BMI ($\beta = -0.27 \pm 0.02$, $p = 4.13 \times 10^{-30}$), SDS-BMI ($\beta = -0.33 \pm 0.03$, $p = 6.50 \times 10^{-32}$), WHR ($\beta = -0.18 \pm 0.02$, $p = 8.20 \times 10^{-12}$), SDS-WHR ($\beta = -0.02 \pm 3.80 \times 10^{-3}$, $p = 2.11 \times 10^{-10}$), LDL cholesterol ($\beta = -0.09 \pm 0.02$, $p= 2.70 \times 10^{-4}$), total cholesterol ($\beta = -0.10 \pm 0.02$, $p = 5.00 \times 10^{-5}$), triglycerides ($\beta = -0.14 \pm 0.03$, $p = 2.98 \times 10^{-8}$), fasting glucose ($\beta = -0.12 \pm 0.02$, $p = 2.00 \times 10^{-6}$), fasting insulin ($\beta = -0.08 \pm 0.03$, $p = 3.00 \times 10^{-3}$), and HOMA-IR ($\beta = -0.10 \pm 0.03$, $p = 3.50 \times 10^{-4}$). Nominal associations ($p < 0.05$) between serum adiponectin concentration and SBP, DBP, SDS-SBP, SDS-DBP and HOMA-B were observed, but did not reach statistical significance after Bonferroni correction ($p > 4.2 \times 10^{-3}$; Table 10). No association between serum adiponectin concentration and HDL cholesterol was observed ($p = 0.49$).

When metabolic traits were classified as binary traits (Table 10), serum adiponectin concentration was negatively associated with dyslipidemia (OR = 0.75, 95% CI = 0.67 - 0.84, $p = 1.00 \times 10^{-6}$), normal weight vs. overweight (OR = 0.39, 95% CI = 0.33 - 0.46, $p = 2.40 \times 10^{-26}$), normal weight vs. obese (OR = 0.40, 95% CI = 0.34 - 0.47, $p = 4.84 \times 10^{-29}$), normal weight vs. overweight and obese participants (OR = 0.41, 95% CI = 0.36 - 0.47, $p = 7.76 \times 10^{-40}$). Associations of serum adiponectin concentration with hypertension, hyperglycemia, and IR were not statistically significant ($p \geq 0.07$).

We then investigated the association of serum adiponectin concentration with continuous and binary cardio-metabolic traits adjusted for sex, age, recruitment center, and BMI (Table 10). Serum adiponectin concentration was positively associated with HOMA-B ($\beta = 0.08 \pm 0.02$, $p =$

1.07 x 10^{-3}) and negatively associated with fasting glucose ($\beta = -0.10 \pm 0.02$, $p = 4.30 \times 10^{-5}$) and HDL cholesterol ($\beta = -0.09 \pm 0.03$, $p = 3.17 \times 10^{-4}$). Nominal associations ($p < 0.05$) between serum adiponectin concentration and fasting insulin, total cholesterol and insulin resistance were observed, but did not reach statistical significance after Bonferroni correction ($p > 4.2 \times 10^{-3}$; Table 2). No association was observed for the other traits ($p \geq 0.08$).

Genotype frequency comparison in Mexican children and adults from 1000G for SNPs in/near ADIPOQ, ADIPOR1, and ADIPOR2

Genotype distributions and allele frequencies of the six selected SNPs are presented in Supplementary Table 3. The MAF for *ADIPOQ*, *ADIPOR1,* and *ADIPOR2* SNPs in Mexican children are as follows: 11% for rs10920533, 18% rs2241766, 36% for rs11061971, 38% for rs266729, 43% for rs822393, and 53% for rs182052. Allelic distributions for all selected SNPs were not significantly different from the reported frequencies in the 1000G reference Mexican adult population ($p \geq 0.07$).

Association of SNPs in/near ADIPOQ, ADIPOR1, and ADIPOR2 with serum adiponectin concentration

We investigated the association of SNPs in *ADIPOQ* (rs182052, rs2241766, rs266729, rs822393), *ADIPOR1* (rs10920533), and *ADIPOR2* (rs11061971) with serum adiponectin concentration under an additive model, adjusted for sex, age, and recruitment center (Table 11). We did not identify any significant associations between these SNP and serum adiponectin concentrations (all $p \geq 0.30$).

Association of SNPs in/near ADIPOQ, ADIPOR1, and ADIPOR2 with cardio-metabolic traits

We subsequently tested the association of the aforementioned SNPs with cardio-metabolic traits, adjusted for sex, age, and recruitment center and with and without adjustment for serum adiponectin concentration (Tables 12 and 13). We observed nominal ($p < 0.05$)

associations for *ADIPOR1* rs10920533 with total cholesterol before and after adjusting for serum adiponectin. *ADIPOR1* rs10920533 was also nominally associated with normal weight vs. obese before adjusting for serum adiponectin. We observed nominal associations for *ADIPOR2* rs11061971 with BMI, SDS-BMI, normal weight vs. overweight and obese, and normal weight vs. overweight before and after adjusting for serum adiponectin, and waist circumference before adjustment. *ADIPOQ* rs182052 was nominally associated with waist circumference and SBP before adjusting for serum adiponectin, with BMI and SDS-BMI both before and after adjustment, with normal weight vs. overweight before adjustment, and with normal weight vs. overweight and obese both before and after adjustment. *ADIPOQ* rs266729 was nominally associated with normal weight vs. overweight after adjustment for serum adiponectin. *ADIPOQ* rs822393 was nominally associated with normal weight vs. overweight and obese after adjustment for adiponectin. However, none of the results remained significant after correcting for multiple testing ($p < 6.9 \times 10^{-4}$).

Statistical power calculations

Statistical power calculations are summarized in Supplementary Tables 4-8. Using a sample of 1 457 participants, our study had at least 80% power to detect effect sizes/beta values of 0.2 or greater for associations between serum adiponectin and SNPs with MAF of 0.2 or greater for p-value = 8.3×10^{-3}.

For associations between serum adiponectin and continuous cardio-metabolic traits, we conducted an example statistical power calculation for the association of serum adiponectin and SBP, for which we had at least 80% power to detect beta values of 0.9 or greater for p-value = 4.2×10^{-3}. For associations between serum adiponectin and binary cardio-metabolic traits, we conducted an example statistical power calculation for the association of serum adiponectin and

insulin resistance. With 127 cases in a sample of 1 457 participants, we had at least 80% power to detect effect sizes/odds ratios of 1.40 or greater for p-value = 4.2×10^{-3}.

For associations between the six SNPs and continuous cardio-metabolic traits, we also examined the association between SNPs and SBP, for which we had at least 80% power to detect beta values of 2.5 or greater when MAF is 0.2 or greater for p-value = 6.9×10^{-4}. For associations between the six SNPs and binary cardio-metabolic traits, we also examined the association between SNPs and insulin resistance, for which we had at least 80% power to detect effect sizes/odds ratios of 1.3 or greater when MAF is 0.1 or greater for p-value = 6.9×10^{-4}.

DISCUSSION

In the present study, we assessed the relationship between serum adiponectin concentration and cardio-metabolic traits and the association of 6 SNPs in *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* with adiponectin serum levels and cardio-metabolic traits in Mexican children. We also compared the SNP genotypic distributions between Mexican children and adults from the 1000G. We found strong negative associations for adiponectin levels with BMI, WHR, LDL cholesterol, total cholesterol, triglycerides, fasting glucose, fasting insulin, and HOMA-IR, as well as dyslipidemia, overweight and obesity status. Further adjustment for BMI removed most of these associations, to the exception of fasting glucose. The same adjustment resulted in significant association between serum adiponectin concentration, HDL cholesterol and HOMA-B. The 6 SNPs had similar genotypic distribution in Mexican children and adults. We did not find any association between these SNPs and serum adiponectin concentration. While nominal associations were found between *ADIPOR1* rs10920533, *ADIPOR2* rs11061971, and *ADIPOQ* rs182052 and cardio-metabolic traits, none remained significant after Bonferroni correction for

multiple testing. Based on our statistical power calculations, our study was only modestly powered (Supplementary Tables 4-8), and lack of associations may be confirmed in larger samples.

The Mexican population is at high risk for developing obesity, IR, dyslipidemia and T2D due to genetic predisposition in combination with recent demographic, socioeconomic and nutrition transitions[46-53]. Reduced physical activity due to urbanization, together with shifts in dietary patterns away from traditional high-fiber foods in favor of processed foods have resulted in the rise of non-communicable chronic diseases among all age groups[54]. In 2011, the prevalence of overweight and obesity in Mexican children reached 34.4%, representing one of the highest rates in the world[55]. In our sample, the prevalence of overweight / obesity exceeded the national average (43.8%), possibly due to our strategy to recruit children within an urban setting. The prevalence of hypertension in our sample (1.5%) was lower than previously reported (4.7% to 14%)[54,56,57]; however, previous studies classify hypertension using percentiles rather than a threshold, making comparisons difficult. The prevalence of IR in our sample (11%) was also low. A cross-sectional study of Mexican children aged 7-18 estimated the prevalence of IR at 20.3%, while the National Health and Nutrition Examination Survey found 52.1% of obese Mexican-Americans aged 12-19 to have IR[58]. The gradual increase of insulin and glucose concentrations observed during adolescence may partially explain this discrepancy[59,60]. We report an exceptionally high prevalence of dyslipidemia in our sample (34.9%). While this high prevalence may be reflective of a diet rich in refined carbohydrates and animal fats but limited in fiber, we cannot exclude the possibility that it may stem from the employed definition of dyslipidemia within our study[61]. Dyslipidemia is routinely defined by abnormal concentrations of one or two lipids, however we used three lipids, thereby artificially increasing the prevalence of

dyslipidemia in our sample. The mean serum adiponectin concentration in our sample was lower than in previous reports in Mexican children[27,29]. Differences in the prevalence of obesity, blood samples (i.e. serum vs. plasma) and laboratory tests (i.e. radioimmunoassay vs enzyme immunoassay) can significantly affect measured adiponectin concentrations, making comparisons challenging.

Adiponectin is an insulin-sensitizing hormone secreted from the adipose tissue and is negatively associated with obesity and T2D in epidemiological studies[62]. Adiponectin plays an important role in modulating glucose and lipid metabolism by activating AMP-dependent kinase signaling[63]. The relationship between low serum adiponectin and obesity, IR, T2D, dyslipidemia, hypertension, and cardio-vascular disease has been extensively studied in adults[62]. Adiponectin levels have been found to be lower in obese European and East Asian children[64,65]. Here, we extended the negative association between serum adiponectin level and childhood overweight/obesity status to the Mexican population. The associations between serum adiponectin and continuous cardio-metabolic traits have been previously investigated in Mexican children in modestly sized studies. Consistent with our results, Cruz *et al* determined negative associations with plasma adiponectin and BMI, insulin concentrations and HOMA-IR in an independent sample[29]. More recently, plasma adiponectin was inversely associated with insulin concentrations, TG, and HOMA-IR in obese Mexican children with the metabolic syndrome[27]. Our results evidenced an inverse association with adiponectin and WHR, LDL-C, total cholesterol, and fasting glucose, which has previously been shown in Latino and Hispanic youth, but not in a Mexican population[66,67]. We also observed an inverse association with adiponectin and dyslipidemia which is consistent with previous reports in a multiethnic adult population and European children[68,69]. Further adjustment for BMI substantially modified the pattern of

association between serum adiponectin and cardio-metabolic traits, confirming that adiponectin has an important role in the regulation of body weight[22,70]. Taken together, our results suggest that adiponectin levels may contribute to the link between obesity, IR, glucose homeostasis, and dyslipidemia at a young age.

Several common and rare variants at the *ADIPOQ* locus appear to cause substantial changes in circulating adiponectin concentrations[18,71]. The most frequently studied *ADIPOQ* variants associated with altered adiponectin concentrations include rs17300539, rs266729, rs2241766, and rs1501299[18]. The rs17300539 variant is strongly associated with increased circulating adiponectin due to enhanced *ADIPOQ* promoter activity[72]. Associations with rs266729 and serum adiponectin are inconsistent, however the general trend suggests a decrease in adiponectin concentration which is further evidenced by lower *ADIPOQ* promoter activity[72]. *ADIPOQ* rs2241766 is strongly associated with lower adiponectin levels, possibly due to differences in RNA splicing or stability and rs1501299 is mainly associated with lower adiponectin levels[72].

Associations with *ADIPOQ* variants and adiponectin levels have been investigated in various populations, however limited information exists in the Mexican population[72]. *ADIPOQ* rs17300539 was associated with higher adiponectin concentrations in a study of 1 153 Hispanic Americans from San Antonio[73]. In a cross-sectional study of 242 Mexican-Mestizo adults, a positive association with *ADIPOQ* rs1501299 and circulating adiponectin was observed[31]. In the present study, we did not identify any significant associations with the selected *ADIPOQ* SNPs and serum adiponectin concentration, possibly due to limited power, age- or ethnic-dependent effects. To our knowledge, this is the first study to examine the association of genetic variants in

ADIPOQ with serum adiponectin levels in a pediatric Mexican population. Further investigation with a more exhaustive SNP selection and larger sample sizes is warranted.

We subsequently tested the associations of *ADIPOQ* SNPs and cardio-metabolic traits and found nominally significant inverse associations between rs182052 and BMI and obesity status. The association of *ADIPOQ* rs182052 with BMI is consistent with findings by Sutton *et al* who found the A allele of rs182052 associated with lower BMI in 811 Hispanic adults from San Antonio[74]. However, Richardson *et al* found a positive association with the G allele of *ADIPOQ* rs182052 and BMI in 439 Mexican American adults from San Antonio and a trend for increased obesity risk has been observed in a small sample of Mexican children[33,75]. Among Brazilians, the A allele of *ADIPOQ* rs182052 was associated with a greater BMI and risk of obesity[76]. However, studies in European adult populations were unable to identify associations with the A allele of *ADIPOQ* rs182052 and BMI[77,78]. These results suggest possible age-dependent associations of *ADIPOQ* SNPs in children with BMI which may be considered in future replication studies in Mexican children.

We did not observe an association between genetic variants in *ADIPOR1* and *ADIPOR2* and serum adiponectin which is consistent with previous studies. GWAS in diverse ethnic groups did not identify *ADIPOR1* or *ADIPOR2* loci as important contributors to serum adiponectin level variation[22,79,80]. Cohen *et al* investigated the association of *ADIPOR1* and *ADIPOR2* with serum adiponectin levels in Caucasian and African-American women but failed to show an association[81]. Subsequently, Matther *et al* did not find associations with *ADIPOR1* and *ADIPOR2* and serum adiponectin levels in the Diabetes Prevention Program[26]. More recently, a meta-analysis of 2 355 European-Australians failed to find an association with serum adiponectin and genetic variants in adiponectin receptors[82]. We studied these associations in a Mexican

population for the first time and our results are in line with previous publications. We also identified nominally significant associations between *ADIPOR1* rs10920533 and total cholesterol and *ADIPOR2* rs11061971 and obesity risk. Very few studies have examined genetic variation in *ADIPOR1* and *ADIPOR2* in relation to these cardio-metabolic traits, making comparisons challenging. Previous work in adult European populations suggests associations with *ADIPOR1* rs10920533 and *ADIPOR2* rs11061971 and IR, but we were unable to confirm these associations in the present pediatric Mexican population[83]. Further investigation is needed to determine the validity of these associations.

Despite the strong association between adiponectin levels and cardio-metabolic traits, we failed to identify associations with the selected SNPs and cardio-metabolic traits after Bonferroni correction. A possible explanation is that the association between adiponectin and metabolic traits is not causal and can be explained by confounding. Observational epidemiology is prone to confounding, reverse causation, and other sources of bias, thus our results should be interpreted with caution. Adiponectin concentration is inversely associated with obesity and T2D, however it is not yet known whether altered adiponectin concentrations are causal or merely a disease marker. Combining genetic epidemiology with classic epidemiology is one way to strengthen causality. For example, the common *ADIPOQ* variant, rs266729 alters ADIPOQ gene expression and has consistently been associated with lower serum adiponectin concentrations and increased risk of IR and T2D[19,62,84] . Future work in the Mexican population including GWAS for adiponectin levels and Mendelian randomization studies are needed to determine the causal links between this hormone and the development of cardio-metabolic diseases.

Our study has several strengths. It is the first to investigate the association of genetic variation in *ADIPOQ*, *ADIPOR1* and *ADIPOR2*, adiponectin concentrations, and cardio-

metabolic traits in a pediatric Mexican population. Measures of serum adiponectin concentration were available, allowing us to investigate the effects of genetic variants on adiponectin levels in addition to diverse cardio-metabolic traits. Furthermore, our study combines classic and genetic epidemiology to strengthen our conclusions. Children represent a purer phenotype as they have less exposure duration to an obesogenic environment, relative to adults[70]. Studying these associations in children may therefore provide more insight into the early biological determinants of obesity and cardio-metabolic complications. Limitations include the selection of *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* SNPs which was not exhaustive and did not include more recent GWAS discoveries[22,85]. Our study is also modestly powered to identify genetic effects, especially after adjusting for multiple testing correction[86]. Study participants were randomly selected from Mexico City and is therefore representative of the urban population of central Mexico, not of the Mexican population as a whole. Furthermore, the Mexican population is admixed with Native American, European, and West African ancestries with proportions varying within different regions of the country. Because we did not have ancestry-informative markers, we could not adjust for potential population stratification. Also, due to the cross-sectional nature of this study, causality cannot be inferred from the associations between serum adiponectin level and cardio-metabolic traits. Some cardio-metabolic traits are also correlated with each other (Supplementary Table 9), making it difficult to discern whether associations between serum adiponectin and cardio-metabolic traits are direct or indirect, and may be mediated by certain outcomes. However, past Mendelian randomization studies have shown that various cardio-metabolic traits, such as HOMA-IR, have a causal relationship with circulating adiponectin levels[9]. Furthermore, past studies have also identified cardio-metabolic traits, including BMI, WHR, fasting insulin, triglycerides, and HDL-cholesterol, that are affected by genetic determinants of adiponectin

levels[87]. These studies support the idea that these cardio-metabolic traits are largely and often found to be associated with adiponectin levels, thus the possibility of confounding is very difficult to accurately discern and control for.

In conclusion, our study suggests strong associations between serum adiponectin level and cardio-metabolic traits in a young Mexican population. Further well-powered studies are needed to elucidate the causal relationship between genetic variation in *ADIPOQ*, *ADIPOR1* and *ADIPOR2*, serum adiponectin level, and development of cardio-metabolic complications.

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Author Contributions

JH, CS, JPR, MC and DM designed the experiment. JPR, RKA and MC contributed to the recruitment of participants and the clinical and biochemical measurements in the study. JPR, FS and JGZ performed the DNA extraction and genotyping experiments. JH, CS, ABG and DM prepared the dataset for analysis. JH, CS, HR, AA and DM conducted statistical analyses. JH, CS and DM wrote the manuscript and prepared all tables and figures. HR, JPR, RKA, FS, JGZ, ABG, AA and MC critically reviewed the manuscript. DM had primary responsibility for final content. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Table 10: General characteristics of the studied population of Mexican children

Trait	Total N=1 457
Boys/Girls, N (%)	771/686 (52.9/47.1)
Age (years)	9.24 ± 2.07
Adiponectin (μg/ml)	5.26 ± 1.23
BMI (kg/m^2)	19.65 ± 4.20
SDS-BMI	0.71 ± 1.09
Waist to hip ratio	0.85 ± 0.06
SDS-Waist to hip ratio	2.95 ± 0.33
Systolic blood pressure (mmHg)	98.57 ± 10.86
SDS-Systolic blood pressure	-0.32 ± 1.01
Diastolic blood pressure (mmHg)	66.24 ± 8.80
SDS-Diastolic blood pressure	0.59 ± 0.78
LDL Cholesterol (mg/dL)	102.39 ± 26.42
HDL Cholesterol (mg/dL)	50.60 ± 12.82
Total cholesterol (mg/dL)	157.25 ± 33.56
Triglycerides (mg/dL)	93.62 ± 49.70
Fasting glucose (mmol/L)	4.57 ± 0.53
Fasting insulin (mIU/L)	8.68 ± 7.10
HOMA-IR	1.86 ± 1.52
HOMA-B	36.36 ± 30.36
Hypertension, N (%)	22 (1.5)
Hyperglycemia, N (%)	45 (3.1)
Insulin resistance, N (%)	127 (11.1)
Dyslipidemia, N (%)	509 (34.9)
Underweight, N (%)	30 (2.1)
Normal weight, N (%)	788 (54.1)
Overweight, N (%)	303 (20.8)
Obese, N (%)	335 (23.0)

Abbreviations: BMI, body mass index; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SDS, standard deviation scores. Data are means ± standard deviation for continuous traits, and numbers and percentages for categorical traits.

Table 11: Association of serum adiponectin concentrations with cardio-metabolic traits

	No additional adjustments	Additional adjustment for BMI
Continuous Traits	**β ± SE (p-value)**	
BMI (kg/m^2)[a]	**-0.27 ± 0.02 (4.13×10^{-30})**	NA
SDS-BMI	**-0.33 ± 0.03 (6.50×10^{-32})**	NA
WHR[a]	**-0.18 ± 0.02 (8.20×10^{-12})**	-0.02 ± 0.02 (0.43)
SDS-WHR	**$-0.02 \pm 3.80 \times 10^{-3}$ (2.11×10^{-10})**	$-4.45 \times 10^{-3} \pm 3.46 \times 10^{-3}$ (0.20)
SBP (mmHg)[a]	-0.07 ± 0.02 (4.00×10^{-3})	0.02 ± 0.02 (0.47)
SDS-SBP	-0.06 ± 0.03 (0.03)	0.02 ± 0.03 (0.55)
DBP (mmHg)[a]	-0.07 ± 0.02 (8.00×10^{-3})	$4.20 \times 10^{-3} \pm 0.03$ (0.87)
SDS-DBP	-0.05 ± 0.02 (0.02)	$-8.24 \times 10^{-3} \pm 0.02$ (0.71)
LDL Cholesterol (mg/dL)[a]	**-0.09 ± 0.02 (2.70×10^{-4})**	-0.04 ± 0.03 (0.14)
HDL Cholesterol (mg/dL)[a]	0.02 ± 0.02 (0.49)	**-0.09 ± 0.03 (3.17×10^{-4})**
Total cholesterol (mg/dL)[a]	**-0.10 ± 0.02 (5.00×10^{-5})**	-0.07 ± 0.03 (4.52×10^{-3})
Triglycerides (mg/dL)[a]	**-0.14 ± 0.03 (2.98×10^{-8})**	-0.02 ± 0.02 (0.47)
Fasting glucose (mmol/L)[a]	**-0.12 ± 0.02 (2.00×10^{-6})**	**-0.10 ± 0.02 (4.30×10^{-5})**
Fasting insulin (mIU/L)[a]	**-0.08 ± 0.03 (3.00×10^{-3})**	0.06 ± 0.02 (0.01)
HOMA-IR[a]	**-0.10 ± 0.03 (3.50×10^{-4})**	0.04 ± 0.03 (0.08)
HOMA-B[a]	-0.07 ± 0.03 (0.02)	**0.08 ± 0.02 (1.07×10^{-3})**
Binary Traits	**OR [95% CI] (p-value)**	
Hypertension	0.75 [0.50-1.13] (0.17)	0.80 [0.53-1.23] (0.31)
Hyperglycemia	0.95 [0.69-1.32] (0.78)	1.03 [0.74-1.45] (0.85)
Insulin resistance	0.83 [0.67-1.02] (0.07)	1.30 [1.01-1.66] (0.04)
Dyslipidemia	**0.75 [0.67-0.84] (1.00×10^{-6})**	0.95 [0.94-1.08] (0.42)
Normal weight vs. overweight	**0.39 [0.33-0.46] (2.40×10^{-26})**	NA
Normal weight vs. obese	**0.40 [0.34-0.47] (4.84×10^{-29})**	NA
Normal weight vs. overweight and obese	**0.41 [0.36-0.47] (7.76×10^{-40})**	NA

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure; SDS,

standard deviation scores; WHR, waist-to-hip ratio. Continuous traits: Data presented are β ± SE (p_{value}). Data was adjusted for age, sex, and recruitment center. Values in bold indicate significant associations after Bonferroni correction ($p < 4.2 \times 10^{-3}$). Binary traits: Data presented are OR [95% CI] (p_{value}). Data was adjusted for age, sex, and recruitment center; additional adjustments are for BMI where NA denotes a non-applicable adjustment. Values in bold indicate significant associations after Bonferroni correction ($p < 4.2 \times 10^{-3}$). [a]Inverse normal transformed variables.

Table 12: Association of SNPs in/near *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* with serum adiponectin concentration[a]

SNP		β ± SE	p-value
ADIPOQ	rs182052	0.02 ± 0.04	0.60
	rs2241766	-0.01 ± 0.05	0.81
	rs266729	0.01 ± 0.04	0.80
	rs822393	0.03 ± 0.04	0.40
ADIPOR1	rs10920533	-0.06 ± 0.60	0.30
ADIPOR2	rs11061971	0.04 ± 0.04	0.33

Data presented are β ± SE (p_{value}). Data presented follow an additive model, adjusting for age, sex, and recruitment center. [a]Inverse normal transformed variables

Table 13: Association of SNPs in/near *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* with continuous metabolic traits

Continuous traits	Additional adjustment	β ± SE (p-value)					
		rs10920533	**rs11061971**	**rs182052**	**rs2241766**	**rs266729**	**rs822393**
BMI (kg/m^2)	None	0.10 ± 0.06 (0.08)	-0.08 ± 0.04 (0.03)	-0.08 ± 0.03 (0.02)	-0.02 ± 0.0 (0.59)	0.08x10^{-1} ± 0.03 (0.82)	0.03 ± 0.03 (0.37)
	Adiponectin	0.08 ± 0.02 (0.14)	-0.07 ± 0.03 (0.048)	-0.07 ± 0.03 (0.02)	-0.03 ± 0.04 (0.54)	0.01 ± 0.03 (0.77)	0.04 ± 0.03 (0.25)
SDS-BMI	None	0.12 ±0.07 (0.08)	**-0.11 ± 0.04 (0.01)**	**-0.11 ± 0.04 (4.61 x 10^{-3})**	-0.03 ± 0.05 (0.64)	0.02 ± 0.04 (0.60)	004 ± 0.04 (0.28)
	Adiponectin	0.09 ± 0.06 (0.14)	**-0.10 ± 0.04 (0.02)**	**-0.10 ± 0.04 (6.43 x 10^{-3})**	-0.03 ± 0.05 (0.58)	0.02 ± 0.04 (0.53)	0.05 ± 0.04 (0.16)
WHR	None	0.06 ± 0.06 (0.33)	-0.03 ± 0.04 (0.48)	-0.03 ± 0.04 (0.39)	0.04 ± 0.05 (0.44)	-0.08 x 10^{-1} ± 0.04 (0.82)	0.02 ± 0.04 (0.54)
	Adiponectin	0.05 ± 0.06 (0.43)	-0.02 ± 0.04 (0.62)	-0.02 ± 0.04 (0.55)	0.03 ± 0.05 (0.50)	-0.07 x 10^{-1} ± 0.04 (0.84)	0.03 ± 0.04 (0.44)
SDS-WHR	None	4.16x10^{-3}±8.91x10^{-3} (0.64)	-5.67x10^{-3}±5.70x10^{-3} (0.32)	-3.90x10^{-3}±5.45x10^{-3} (0.47)	1.46x10^{-3}±7.14x10^{-3} (0.84)	3.70x10^{-3}±5.61x10^{-3} (0.51)	7.00x10^{-3}±5.40x10^{-3} (0.20)
	Adiponectin	2.27x10^{-3}±8.81x10^{-3} (0.80)	-4.44x10^{-3}±5.64x10^{-3} (0.43)	-2.74x10^{-4}±5.39x10^{-3} (0.61)	9.79x10^{-4}±7.05x10^{-3} (0.89)	4.005x10^{-3}±5.54x10^{-3} (0.46)	8.00x10^{-3}±5.33x10^{-3} (0.13)
SBP (mmHg)	None	0.08 ± 0.06 (0.12)	-0.06 ± 0.04 (0.10)	-0.07 ± 0.03 (4.97 x 10^{-2})	0.03 ± 0.04 (0.54)	-0.03 ± 0.04 (0.38)	-0.01 ± 0.03 (0.71)
	Adiponectin	0.08 ± 0.06 (0.16)	-0.06 ± 0.04 (0.12)	-0.06 ± 0.03 (0.06)	-0.03 ± 0.40 (0.55)	-0.03 ± 0.04 (0.37)	-0.01 ± 0.03 (0.75)
SDS-SBP	None	0.09 ± 0.06 (0.14)	-0.04 ± 004 (0.29)	-0.05 ± 0.04 (0.18)	0.04 ± 0.05 (0.46)	-0.06 ± 0.04 (0.17)	-0.02 ± 0.04 (0.67)
	Adiponectin	0.09 ± 0.06 (0.18)	0.04 ± 0.04 (0.33)	-0.05 ± 0.04 (0.20)	0.04 ± 0.05 (0.47)	-0.06 ± 0.04 (0.17)	-0.01 ± 0.04 (0.71)
DBP (mmHg)	None	0.02 ± 0.06 (0.69)	-0.04 x 10^{-1} ± 0.04 (0.92)	-0.05 ± 0.04 (0.14)	0.03 x 10^{-1} ± 0.05 (0.94)	0.02 ± 0.04 (0.60)	-0.02 ± 0.03 (0.63)
	Adiponectin	0.01 ± 0.06 (0.82)	-0.01 x 10^{-1} ± 0.04 (0.98)	-0.05 ± 0.04 (0.15)	-0.03 x 10^{-1} ± 0.05 (0.94)	0.02 ± 0.04 (0.59)	-0.01 ± 0.03 (0.68)
SDS-SBP	None	-3.03x10^{-3} ± 0.05(0.95)	1.10 x 10^{-3} ± 0.03 (0.97)	-0.05 ± 0.03 (0.13)	0.03 ± 0.04 (0.52)	0.01 ± 0.03 (0.78)	-0.04 ± 0.03 (0.20)
	Adiponectin	-0.01 ± 0.05 (0.84)	4.66 x 10^{-3} ± 0.03 (0.88)	-0.05 ± 0.03 (0.14)	0.02 ± 0.04 (0.56)	0.01 ± 0.03 (0.74)	-0.04 ± 0.03 (0.24)
LDL cholesterol (mg/dL)	None	0.11 ± 0.06 (0.05)	0.05 ± 0.04 (0.18)	0.03 x 10^{-1} ± 0.04 (0.93)	0.03 ± 0.05 (0.53)	-0.01 ± 0.04 (0.71)	0.04 x 10^{-1} ± 0.04 (0.90)
	Adiponectin	0.11 ± 0.06 (0.07)	0.06 ± 0.04 (0.12)	-0.01 ± 0.04 (0.79)	0.02 ± 0.05 (0.60)	-0.01 ± 0.04 (0.72)	0.08x10^{-1} ± 0.04 (0.83)
HDL cholesterol (mg/dL)	None	0.04 ± 0.06 (0.54)	-0.01 ± 0.04 (0.71)	-0.02 x 10^{-1} ± 0.04 (0.96)	0.02 ± 0.05 (0.61)	-0.06 ± 0.04 (0.10)	-0.03 ± 0.03 (0.47)
	Adiponectin	0.04 ± 0.06 (0.48)	-0.09 x 10^{-1} ± 0.04 (0.80)	0.02 x 10^{-1} ± 0.04 (0.95)	0.02 ± 0.05 (0.68)	-0.06 ± 0.04 (0.09)	-0.02 ± 0.03 (0.48)
Total cholesterol (mg/dL)	None	0.15 ± 0.06 (8.00 x 10^{-3})	0.03 ± 0.04 (0.35)	-0.03 x 10^{-1} ± 0.04 (0.92)	0.04 ± 0.04 (0.39)	-0.04 ± 0.04 (0.22)	-0.01 ± 0.03 (0.72)
	Adiponectin	0.14 ± 0.06 (0.01)	0.04 ± 0.04 (0.23)	0.04 x 10^{-1} ± 0.03 (0.91)	0.03 ± 0.04 (0.46)	-0.04 ± 0.04 (0.22)	-0.09 x 10^{-1} ± 0.03 (0.80)
Triglycerides (mg/dL)	None	0.04 ± 0.06 (0.54)	0.02 ± 0.04 (0.65)	-0.05 ± 0.04 (0.15)	-0.03 ± 0.05 (0.50)	-0.01 ± 0.04 (0.72)	-0.04 x 10^{-1} ± 0.04 (0.90)
	Adiponectin	0.03 ± 0.06 (0.63)	0.02 ± 0.04 (0.55)	-0.05 ± 0.04 (0.18)	-0.03 ± 0.05 (0.52)	-0.01 ± 0.04 (0.72)	-0.02 x 10^{-1} ± 0.04 (0.96)
Fasting glucose (mmol/L)	None	0.04 ± 0.06 (0.49)	0.02 x 10^{-1} ± 0.04 (0.96)	-0.03 ± 0.04 (0.44)	-0.02 ± 0.04 (0.68)	-0.06 ± 0.04 (0.10)	0.02 ± 0.03 (0.58)
	Adiponectin	0.04 ± 0.06 (0.50)	0.08 x 10^{-1} ± 0.04 (0.83)	-0.02 ± 0.03 (0.57)	-0.02 ± 0.04 (0.59)	-0.06 ± 0.04 (0.08)	0.02 ± 0.03 (0.55)
Fasting insulin (mIU/L)	None	0.07 ± 0.06 (0.26)	-0.06 ± 0.04 (0.12)	-0.01 ± 0.04 (0.80)	-0.03 ± 0.05 (0.53)	0.01 ± 0.04 (0.73)	0.05 ± 0.04 (0.21)
	Adiponectin	0.06 ± 0.06 (0.30)	-0.06 ± 0.04 (0.13)	-0.08 x 10^{-1} ± 0.04 (0.84)	-0.04 ± 0.05 (0.47)	0.01 ± 0.04 (0.75)	0.05 ± 0.04 (0.19)
HOMA-IR	None	0.08 ± 0.06 (0.22)	-0.07 ± 0.04 (0.09)	-0.01 ± 0.04 (0.73)	-0.04 ± 0.05 (0.44)	0.05 x 10^{-1} ± 0.04 (0.89)	0.05 ± 0.04 (0.19)
	Adiponectin	0.07 ± 0.06 (0.25)	-0.07 ± 0.04 (0.10)	-0.01 ± 0.04 (0.79)	-0.04 ± 0.05 (0.38)	0.05 x 10^{-1} ± 0.04 (0.91)	0.05 ± 0.04 (0.17)
HOMA-B	None	0.06 ± 0.06 (0.32)	-0.06 ± 0.04 (0.12)	-0.05 x 10^{-1} ± 0.04 (0.90)	-0.03 ± 0.05 (0.53)	0.02 ± 0.04 (0.60)	0.04 ± 0.04 (0.26)
	Adiponectin	0.06 ± 0.06 (0.36)	-0.06 ± 0.04 (0.13)	-0.03 x 10^{-1} ± 0.04 (0.93)	-0.04 ± 0.05 (0.47)	0.02 ± 0.04 (0.62)	0.04 ± 0.04(0.24)

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure; SDS, standard deviation scores; WHR, waist-to-hip ratio. Continuous traits: Data presented are β ± SE (p_{value}). All models were adjusted for age, sex, and recruitment center.

Table 14: Association of SNPs in/near *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* with binary metabolic traits

Binary traits	Additional adjustment	OR [95% CI] (p-value)					
		rs10920533	**rs11061971**	**rs182052**	**rs2241766**	**rs266729**	**rs822393**
Hypertension	None	1.37 [0.54 - 3.50] (0.51)	1.56 [0.83 - 2.92] (0.17)	0.76 [0.42 - 1.40] (0.38)	0.95 [0.43 - 2.10] (0.91)	0.77 [0.40 - 1.46] (0.43)	0.53 [0.28 - 1.03] (0.06)
	Adiponectin	1.35 [0.53 - 3.44] (0.53)	1.57 [0.84 - 2.93] (0.16)	0.77 [0.42 - 1.42] (0.40)	0.98 [0.44 - 2.16] (0.95)	0.77 [0.40 - 1.48] (0.44)	0.54 [0.28 - 1.04] (0.07)
Hyperglycemia	None	1.04 [0.51 - 2.10] (0.92)	0.77 [0.49 - 1.23] (0.28)	1.02 [0.66 - 1.57] (0.93)	0.92 [0.52 - 1.62] (0.76)	0.67 [0.42 - 1.07] (0.09)	0.94 [0.62 - 1.44] (0.79)
	Adiponectin	1.04 [0.52 - 2.12] (0.90)	0.77 [0.48 - 1.22] (0.26)	1.02 [0.66 - 1.57] (0.94)	0.92 [0.52 - 1.62] (0.77)	0.66 [0.41 - 1.06] (0.09)	0.94 [0.62 - 1.43] (0.76)
Insulin resistance	None	1.28 [0.84 - 1.94] (0.24)	0.94 [0.70 - 1.26] (0.68)	0.94 [0.71 - 1.23] (0.64)	0.85 [0.59 - 1.22] (0.38)	1.04 [0.79 - 1.36] (0.80)	1.09 [0.84 - 1.43] (0.52)
	Adiponectin	1.26 [0.83 - 1.91] (0.28)	0.94 [0.70 - 1.26] (0.69)	0.94 [0.72 - 1.24] (0.68)	0.84 [0.59 - 1.21] (0.36)	1.04 [0.79 - 1.37] (0.78)	1.10 [0.84 - 1.44] (0.48)
Dyslipidemia	None	1.12 [0.87 - 1.44] (0.38)	0.90 [0.77 - 1.06] (0.22)	0.89 [0.76 - 1.04] (0.15)	1.03 [0.84 - 1.26] (0.77)	1.04 [0.89 - 1.22] (0.61)	1.00 [0.86 - 1.17] (1.00)
	Adiponectin	1.10 [0.85 - 1.41] (0.47)	0.92 [0.78 - 1.08] (0.31)	0.90 [0.77 - 1.06] (0.21)	1.02 [0.83 - 1.25] (0.84)	1.04 [0.89 - 1.23] (0.60)	1.01 [0.86 - 1.18] (0.92)
Normal weight vs. Overweight	None	0.91 [0.65 - 1.26] (0.57)	0.75 [0.61 - 0.92] (6.00×10^{-3})	0.77 [0.63 - 0.92] (6.00×10^{-3})	1.03 [0.80 -1.31] (0.84)	1.21 [0.99 - 1.48] (0.06)	1.09 [0.90 - 1.32] (0.35)
	Adiponectin	0.92 [0.65 - 1.30] (0.64)	0.74 [0.59 - 0.92] (7.00×10^{-3})	0.78 [0.63 - 0.95] (0.01)	0.97 [0.74 - 1.26] (0.80)	1.25 [1.01 - 1.55] (0.04)	1.13 [0.92 - 1.38] (0.24)
Normal weight vs. Obese	None	1.42 [1.08 - 1.87] (0.01)	0.85 [0.71 - 1.02] (0.09)	0.90 [0.75 - 1.07] (0.24)	0.92 [0.73 - 1.15] (0.46)	1.08 [0.90 - 1.28] (0.43)	1.13 [0.95 - 1.34] (0.15)
	Adiponectin	1.27 [0.95 - 1.71] (0.10)	0.87 [0.71 - 1.06] (0.15)	0.90 [0.74 - 1.09] (0.27)	0.91 [0.71 - 1.17] (0.47)	1.10 [0.90 - 1.33] (0.35)	1.20 [1.00 - 1.44] (0.05)
Normal weight vs. Overweight and Obese	None	1.17 [0.92 - 1.49] (0.19)	0.80 [0.69 - 0.94] (6.00×10^{-3})	0.84 [0.72 - 0.97] (0.02)	0.96 [0.79 - 1.16] (0.68)	1.14 [0.98 - 1.32] (0.10)	1.12 [0.97 - 1.30] (0.13)
	Adiponectin	1.12 [0.86 - 1.45] (0.39)	0.80 [0.68 -0.95] (9.00×10^{-3})	0.83 [0.71 - 0.98] (0.02)	0.94 [0.76 - 1.16] (0.57)	1.16 [0.98 - 1.37] (0.08)	1.17 [1.00 - 1.38] (4.60×10^{-2})

Data presented are OR [95% CI] (p_{value}). All models were adjusted for age, sex, and recruitment center.

Supplementary Table 3: Description of the six adiponectin SNPs studied

SNP (*Gene*)	Major / Minor Allele	Mexican children allele count		1000 Genomes allele count		1000G MAF	Study MAF	Call Rate	HWE P-value	Genotype count Chi^2 (P-value)
rs10920533 (*ADIOPR1*)	G / A	G	A	G	A	0.11	0.11	0.966	0.35	0.02 (0.89)
		2519	297	114	14					
rs11061971 (*ADIPOR2*)	T / A	T	A	T	A	0.42	0.36	0.982	0.97	2.01 (0.16)
		1831	1031	74	54					
rs182052 (*ADIPOQ*)	G / A	G	A	G	A	0.47	0.53	0.984	0.76	2.11 (0.15)
		1336	1532	68	60					
rs2241766 (*ADIPOQ*)	T / G	T	G	T	G	0.14	0.18	0.972	0.67	1.61 (0.21)
		2310	524	110	18					
rs266729 (*ADIPOQ*)	C / G	C	G	C	G	0.30	0.38	0.989	0.61	3.22 (0.07)
		1776	1104	89	39					
rs822393 (*ADIPOQ*)	C / T	C	T	C	T	0.41	0.43	0.986	0.38	1.43 (0.23)
		1529	1345	75	53					

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism. Pearson's Chi^2 test was used to compare the allele frequencies of our study with the reference frequencies from 1000G.

Supplementary Table 4: Sample sizes needed to detect significant association between serum adiponectin and the six SNPs with a power of 80% and a two-sided p-value of 8.3 x 10^{-3} (adjusted) by beta coefficient and allele frequency for risk allele

Minor allele frequency							
β	0.01	0.05	0.1	0.2	0.3	0.4	0.5
0.10	92594	19294	10180	5724	4359	3814	3661
0.20	23144	4819	2540	1426	1085	949	911
0.30	10283	2138	1126	631	479	418	401
0.40	5781	1200	631	352	267	233	223
0.50	3698	766	401	223	168	147	141
0.60	2566	530	277	153	115	100	96
0.70	1884	388	202	111	83	72	69
0.80	1441	295	153	83	62	53	51
0.90	1137	232	120	64	48	41	39
1.00	920	187	96	51	37	32	30
1.50	405	80	39	19	12	10	9
2.00	225	42	19	6	< 1	< 1	< 1
2.50	142	24	9	< 1	< 1	< 1	< 1
3.00	97	15	< 1	< 1	< 1	< 1	< 1
3.50	69	8	< 1	< 1	< 1	< 1	< 1

Abbreviations: SBP, systolic blood pressure; SNP: single nucleotide polymorphism. Calculations are based on serum adiponectin mean 5.26 and standard deviation 1.23.

Supplementary Table 5: Sample sizes needed in a cohort design to detect significant association between serum adiponectin and SBP across a range of beta coefficients with statistical power of 80% and two-sided p-values of 0.05 (unadjusted) and 4.2 x 10^{-3} (adjusted)

β	P=0.05	P=4.2 x 10^{-3}
0.10	61183	107112
0.20	15293	26773
0.30	6795	11895
0.40	3820	6688
0.50	2444	4278
0.60	1696	2969
0.70	1245	2179
0.80	952	1667
0.90	751	1316
1.00	608	1064
1.10	502	878
1.20	421	737
1.30	358	627
1.40	308	540
1.50	268	469

Abbreviation: SBP, systolic blood pressure. Calculations are based on serum adiponectin standard deviation 1.23, SBP mean 98.57 and standard deviation 10.86.

Supplementary Table 6: Number of cases per 10 controls to detect significant association between serum adiponectin and IR across a range of odds ratios with statistical power of 80% and two-sided p-values of 0.05 (unadjusted) and 4.2 x 10^{-3} (adjusted)

OR	P=0.05	P=4.2 x 10^{-3}
1.10	629	1102
1.20	173	303
1.30	84	147
1.40	52	91
1.50	36	63
1.60	27	48
1.70	22	38
1.80	18	31
1.90	15	27
2.00	13	23
2.10	12	21
2.20	11	19
2.30	10	17
2.40	9	16
2.50	8	15

Abbreviation: IR, insulin resistance. Calculations are based on serum adiponectin standard deviation 1.23, and 11% baseline risk for IR.

Supplementary Table 7: Sample sizes needed in a cohort design to detect significant association between the six SNPs and SBP with statistical power of 80% and two-sided p-values of 0.05 (unadjusted) and 6.9 x 10^{-4} (adjusted)

β	P=0.05					P=6.9 x 10^{-4}				
	Minor allele frequency					Minor allele frequency				
	0.05	0.10	0.20	0.40	0.50	0.05	0.10	0.20	0.40	0.50
0.10	974411	514270	289275	192849	185135	>1000000	>1000000	660515	440340	422726
0.20	243600	128565	72316	48209	46281	556222	293557	165122	110078	105675
0.30	108264	57138	32138	21424	20567	247205	130465	73383	48919	46962
0.40	60897	32138	18076	12049	11567	139049	73383	41274	27513	26412
0.50	38973	20567	11567	7710	7402	88988	46962	26412	17605	16900
0.60	27063	14281	8032	5353	5139	61794	32610	18339	12223	11734
0.70	19882	10491	5900	3932	3774	45398	23956	13471	8978	8618
0.80	15221	8032	4516	3009	2889	34755	18339	10312	6871	6596
0.90	12026	6345	3567	2377	2282	27459	14488	8146	5427	5210
1.00	9740	5139	2889	1925	1847	22240	11734	6596	4395	4218
1.50	4327	2282	1282	853	819	9880	5210	2927	1948	1870
2.00	2432	1282	719	478	459	5553	2927	1642	1092	1048
2.50	1555	819	459	305	292	3551	1870	1048	696	667
3.00	1079	567	317	210	202	2463	1296	725	480	461
3.50	792	416	232	153	147	1807	950	530	350	336

Abbreviation: SBP, systolic blood pressure; SNP, single nucleotide polymorphism. Calculations are based on SBP mean 98.57 and standard deviation 10.86.

Supplementary Table 8: Number of cases per 10 controls to detect significant association between the six SNPs and IR with statistical power of 80% and two-sided p-values of 0.05 (unadjusted) and 6.9 x 10^{-4} (adjusted)

OR	P=0.05					P=6.9 x 10^{-4}				
	Minor allele frequency					Minor allele frequency				
	0.05	0.10	0.20	0.40	0.50	0.05	0.10	0.20	0.40	0.50
1.10	9568	5076	2885	1963	1903	21865	11601	6593	4485	4345
1.20	2513	1340	769	533	522	5743	3063	1758	1219	1191
1.30	1171	628	364	257	253	2677	1435	832	586	578
1.40	690	372	217	156	155	1576	849	497	356	353
1.50	461	250	148	107	107	1055	571	337	245	245
1.60	335	182	108	80	80	765	416	248	183	183
1.70	256	140	84	63	63	586	320	193	144	145
1.80	204	112	64	51	52	467	257	156	117	119
1.90	168	93	57	43	44	384	212	130	99	100
2.00	142	79	48	37	38	324	180	111	85	87
2.10	122	68	42	33	33	278	155	96	75	76
2.20	106	59	37	29	30	242	136	85	66	68
2.30	94	53	33	26	27	214	121	76	60	61
2.40	84	47	30	24	24	191	108	69	55	56
2.50	76	43	27	22	22	173	98	63	50	51
3.00	50	29	19	16	16	115	67	44	36	37
3.50	37	22	15	12	13	85	51	34	29	29

Abbreviation: IR, insulin resistance; SNP, single nucleotide polymorphism. Calculations are based on 11% baseline risk for IR.

Supplementary Table 9: Correlation table for continuous cardio-metabolic traits showing Pearson's correlation coefficient and associated p-values.

	BMI	SDS-BMI	WHR	SDS-WHR	SBP	SDS-SBP	DBP	SDS-DBP
BMI	1	0.86 (0.00)	0.48 (0.00)	-0.10 ($1.46x10^{-4}$)	0.40 (0.00)	0.22 (0.00)	0.29 (0.00)	0.14 (0.00)
SDS-BMI	0.86 (0.00)	1	0.54 (0.00)	0.17 (0.00)	0.28 (0.00)	0.22 (0.00)	0.20 (0.00)	0.15 (0.00)
WHR	0.48 (0.00)	0.54 (0.00)	1	0.43 (0.00)	0.08 ($2.00x10^{-3}$)	0.13 (0.00)	0.04 (0.09)	0.09 ($2.00x10^{-3}$)
SDS-WHR	-0.10 ($1.46x10^{-4}$)	0.17 (0.00)	0.43 (0.00)	1	-0.26 (0.00)	0.03 (0.28)	-0.15 (0.00)	0.07 (0.01)
SBP	0.40 (0.00)	0.28 (0.00)	0.08 ($2.00x10^{-3}$)	-0.26 (0.00)	1	0.92 (0.00)	0.63 (0.00)	0.53 (0.00)
SDS-SBP	0.22 (0.00)	0.22 (0.00)	0.13 ($3.00x10^{-6}$)	0.03 (0.28)	0.92 (0.00)	1	0.57 (0.00)	0.62 (0.00)
DBP	0.29 (0.00)	0.20 (0.00)	0.04 (0.09)	-0.15 (0.00)	0.63 (0.00)	0.57 (0.00)	1	0.96 (0.00)
SDS-DBP	0.14 (0.00)	0.15 (0.00)	0.09 ($2.00x10^{-3}$)	0.07 (0.01)	0.53 (0.00)	0.62 (0.00)	0.96 (0.00)	1
LDL	0.18 (0.00)	0.20 (0.00)	0.16 (0.00)	0.06 (0.03)	0.11 ($2.40x10^{-5}$)	0.12 (0.00)	0.07 (0.01)	0.07 (0.01)
HDL	-0.36 (0.00)	-0.30 (0.00)	-0.20 (0.00)	-0.07 (0.01)	-0.08 ($2.00x10^{-3}$)	-0.01 (0.62)	-0.05 (0.07)	$1.00x10^{-3}$ (0.97)
TC	0.08 ($4.00x10^{-3}$)	0.10 ($1.42x10^{-4}$)	0.09 ($1.00x10^{-3}$)	0.02 (0.40)	0.11 ($4.80x10^{-5}$)	0.14 (0.00)	0.08 ($3.00x10^{-3}$)	0.10 ($1.00x10^{-3}$)
TG	0.46 (0.00)	0.40 (0.00)	0.27 (0.00)	-0.02 (0.50)	0.22 (0.00)	0.16 (0.00)	0.13 (0.00)	0.07 ($9.00x10^{-3}$)
FG	0.13 ($1.00x10^{-6}$)	0.09 ($1.00x10^{-3}$)	0.04 (0.11)	-0.16 (0.00)	0.18 (0.00)	0.11 ($4.00x10^{-4}$)	0.09 ($1.00x10^{-3}$)	0.05 (0.09)
FI	0.62 (0.00)	0.46 (0.00)	0.29 (0.00)	-0.07 (0.02)	0.28 (0.00)	0.16 (0.00)	0.19 (0.00)	0.09 ($6.00x10^{-3}$)
HOMA-IR	0.61 (0.00)	0.44 (0.00)	0.28 (0.00)	-0.08 ($6.00x10^{-3}$)	0.29 (0.00)	0.17 (0.00)	0.19 (0.00)	0.09 ($3.00x10^{-3}$)
HOMA-B	0.56 (0.00)	0.40 (0.00)	0.25 (0.00)	-0.05 (0.13)	0.23 (0.00)	0.13 ($2.70x10^{-5}$)	0.15 (0.00)	0.06 (0.04)

Abbreviations: BMI, body mass index; FG, fasting glucose; FI, fasting insulin; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SDS, standard deviation scores; TC, total cholesterol; TG, triglycerides. Data are Pearson's correlation coefficient (p-value). P-values of 0.00 indicate that the value is $< 1.00 \times 10^{-36}$.

Supplementary Table 10 Continued: Correlation table for continuous cardio-metabolic traits showing Pearson's correlation coefficient and associated p-values.

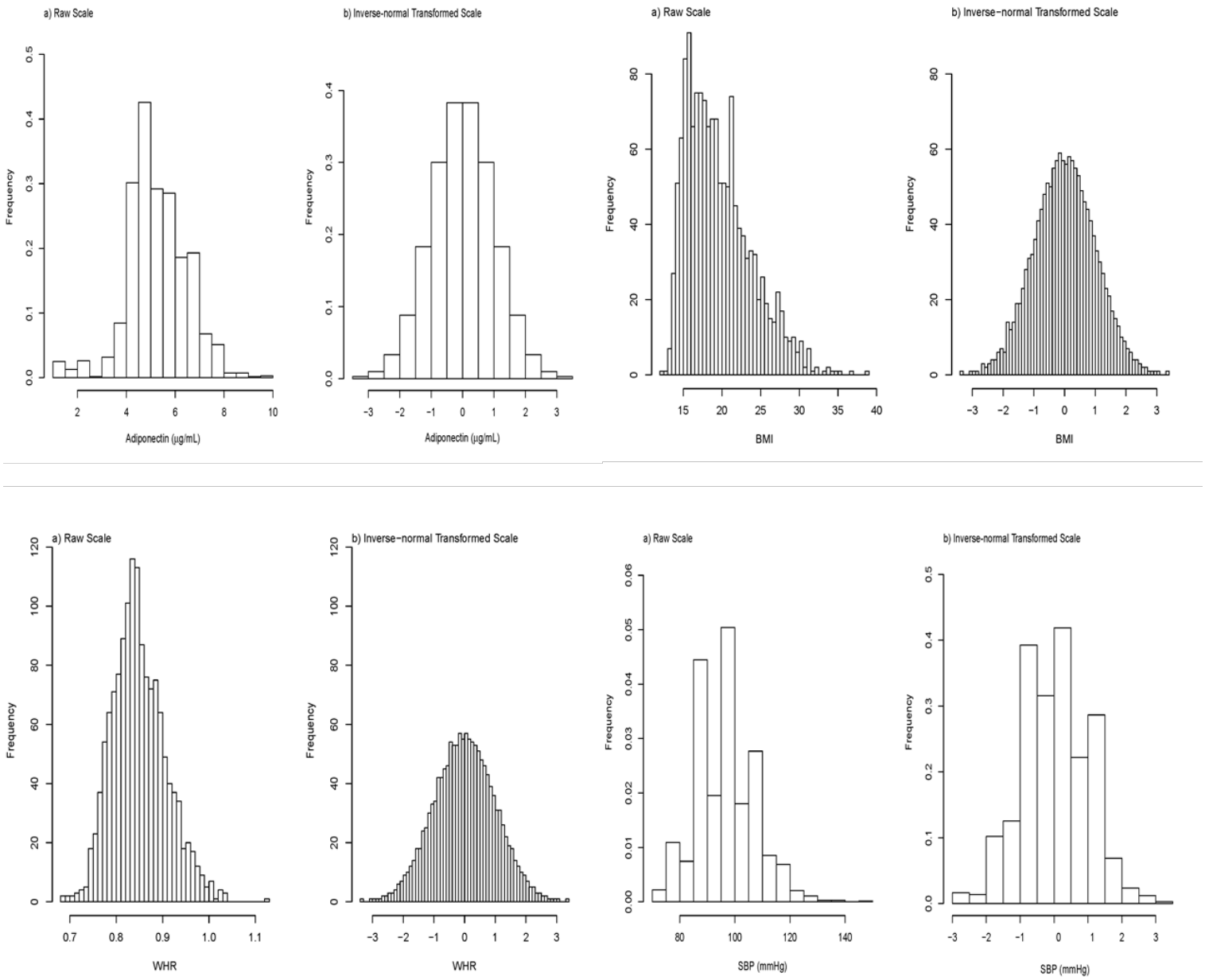
	LDL	HDL	TC	TG	FG	FI	HOMA-IR	HOMA-B
BMI	0.18 (1.75×10^{-11})	-0.36 (1.00×10^{-13})	0.08 (4.00×10^{-3})	0.46 (1.00×10^{-13})	0.13 (1.00×10^{-6})	0.62 (1.00×10^{-13})	0.61 (1.00×10^{-13})	0..53 (1.00×10^{-13})
SDS-BMI	0.20 (1.25×10^{-13})	-0.30 (1.00×10^{-13})	0.10 (1.42×10^{-4})	0.40 (1.00×10^{-13})	0.09 (1.00×10^{-3})	0.46 (1.00×10^{-13})	0.44 (1.00×10^{-13})	0.40 (1.00×10^{-13})
WHR	0.16 (4.06×10^{-10})	-0.20 ($1.17\text{x}0^{-13}$)	0.09 (1.00×10^{-3})	0.27 (1.00×10^{-13})	0.04 (0.11)	0.29 (1.00×10^{-13})	0.28 (1.00×10^{-13})	0.25 (1.00×10^{-13})
SDS-WHR	0.06 (0.03)	-0.07 (0.01)	0.02 (0.40)	-0.02 (0.50)	-0.16 (1.01×10^{-9})	-0.07 (0.02)	-0.08 (6.00×10^{-3})	-0.05 (0.13)
SBP	0.11 (2.40×10^{-5})	-0.08 (2.00×10^{-3})	0.11 (4.80×10^{-5})	0.22 (1.00×10^{-13})	0.18 (1.08×10^{-11})	0.28 (1.00×10^{-13})	0.29 (1.00×10^{-13})	0.23 (1.00×10^{-13})
SDS-SBP	0.12 (1.00×10^{-5})	-0.01 (0.62)	0.14 (7.05×10^{-7})	0.16 (1.54×10^{-8})	0.11 (1.00×10^{-4})	0.16 (1.00×10^{-6})	0.17 (9.20×10^{-8})	0.13 (2.68×10^{-5})
DBP	0.07 (0.01)	-0.05 (0.07)	0.08 (3.00×10^{-3})	0.13 (7.63×10^{-7})	0.09 (1.00×10^{-3})	0.19 (1.85×10^{-10})	0.19 (4.51×10^{-11})	0.15 (2.44×10^{-7})
SDS-DBP	0.07 (0.01)	1.00×10^{-3} (0.97)	0.10 (1.00×10^{-3})	0.07 (9.00×10^{-3})	0.05 (0.09)	0.09 (6.00×10^{-3})	0.09 (3.00×10^{-3})	0.06 (0.04)
LDL	1	0.10 (8.10×10^{-5})	0.89 (0.00)	0.42 (1.00×10^{-13})	0.28 (1.00×10^{-13})	0.08 (5.00×10^{-3})	0.12 (6.20×10^{-5})	0.03 (0.27)
HDL	0.10 (8.10×10^{-5})	1	0.34 (1.00×10^{-13})	-0.37 (1.00×10^{-13})	0.18 (3.61×10^{-12})	-0.32 (1.00×10^{-13})	-0.28 (1.00×10^{-13})	-0.33 (1.00×10^{-13})
TC	0.89 (0.00)	0.34 (1.00×10^{-13})	1	0.38 (0.00)	0.34 (1.00×10^{-13})	-0.02 (0.55)	0.03 (0.28)	-0.08 (6.00×10^{-3})
TG	0.42 (1.00×10^{-13})	-0.37 (1.00×10^{-13})	0.38 (1.00×10^{-13})	1	0.20 (1.03×10^{-13})	0.41 (1.00×10^{-13})	0.42 (1.00×10^{-13})	0.33 (1.00×10^{-13})
FG	0.28 (1.00×10^{-13})	0.18 (3.61×10^{-12})	0.34 (1.00×10^{-13})	0.20 (1.03×10^{-13})	1	0.16 (6.49×10^{-8})	0.29 (1.00×10^{-13})	4.00×10^{-4} (0.99)
FI	0.08 (5.00×10^{-3})	-0.32 (1.00×10^{-13})	-0.02 (0.55)	0.41 (1.00×10^{-13})	0.16 (6.49×10^{-8})	1	0.98 (0.00)	0.98 (0.00)
HOMA-IR	0.12 (6.20×10^{-5})	-0.28 (1.00×10^{-13})	0.03 (0.28)	0.42 (1.00×10^{-13})	0.29 (1.00×10^{-13})	0.98 (0.00)	1	0.94 (0.00)
HOMA-B	0.03 (0.27)	-0.33 (1.00×10^{-13})	-0.08 (6.00×10^{-3})	0.33 (1.00×10^{-13})	4.00×10^{-4} (0.99)	0.98 (0.00)	0.94 (0.00)	1

Abbreviations: BMI, body mass index; FG, fasting glucose; FI, fasting insulin; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SDS, standard deviation scores; TC, total cholesterol; TG, triglycerides. Data are Pearson's correlation coefficient (p-value). P-values of 0.00 indicate that the value is $< 1.00 \times 10^{-36}$.

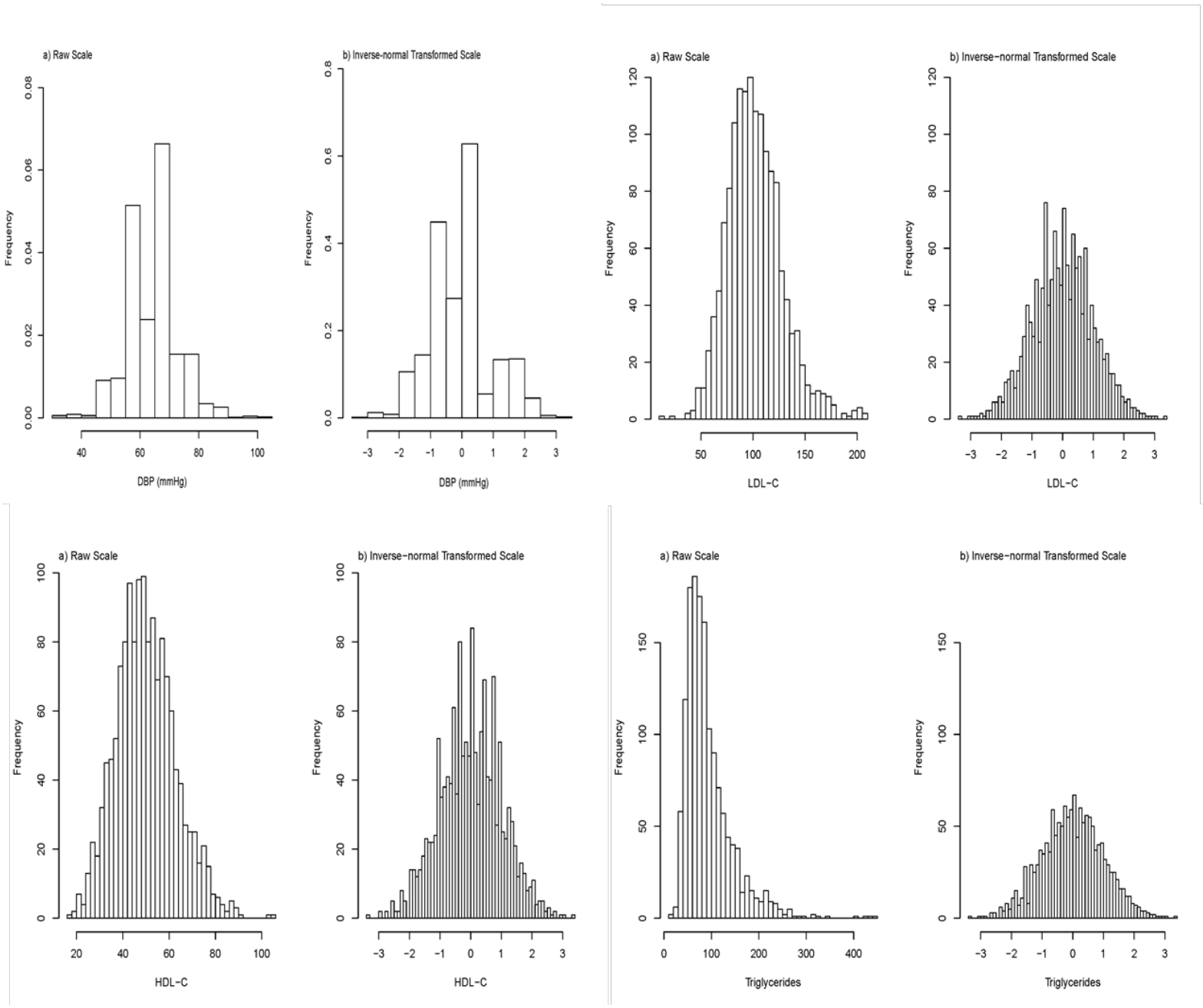
Supplementary Table 10: Shapiro-Wilk test for normality of continuous traits before and after inverse normal transformations

Trait	P-value before transformation	P-value after transformation
Adiponectin (μg/ml)	1.50×10^{-14}	6.21×10^{-1}
BMI (kg/m^2)	8.71×10^{-20}	1.00
WHR	2.92×10^{-7}	1.00
SBP (mmHg)	6.69×10^{-13}	8.93×10^{-10}
DBP (mmHg)	7.20×10^{-17}	8.43×10^{-14}
LDL Cholesterol (mg/dL)	5.66×10^{-12}	1.00
HDL Cholesterol (mg/dL)	6.67×10^{-8}	7.55×10^{-1}
Total cholesterol (mg/dL)	3.76×10^{-8}	1.00
Triglycerides (mg/dL)	1.93×10^{-32}	9.71×10^{-1}
Fasting glucose (mmol/L)	1.00×10^{-3}	3.02×10^{-1}
Fasting insulin (mIU/L)	5.20×10^{-36}	1.00
HOMA-IR	6.67×10^{-37}	1.00
HOMA-B	1.14×10^{-35}	1.00

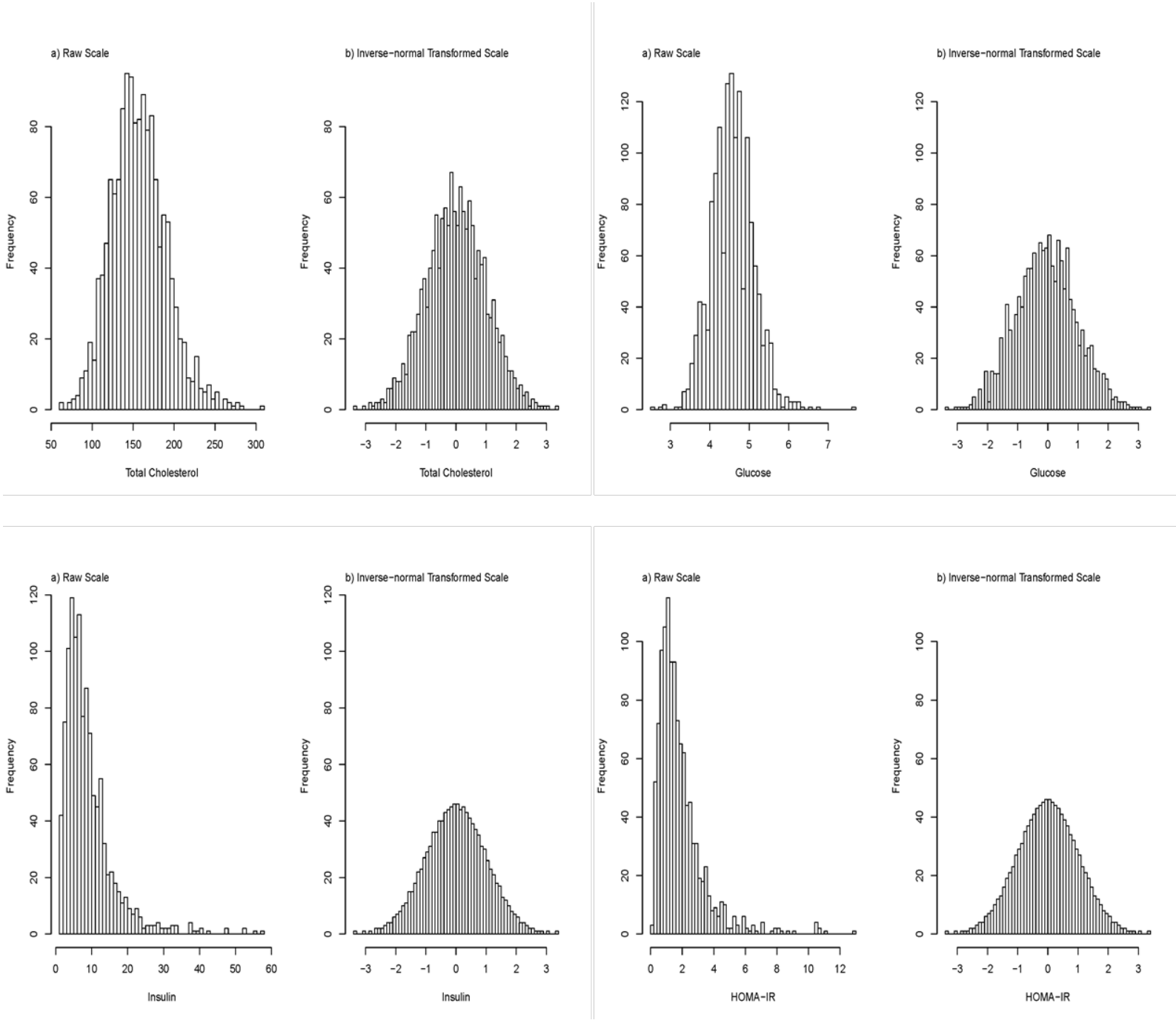
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-B, homeostatic model assessment of beta cell function; LDL, low density lipoprotein cholesterol; SBP, systolic blood pressure; WHR, waist-to-hip ratio.



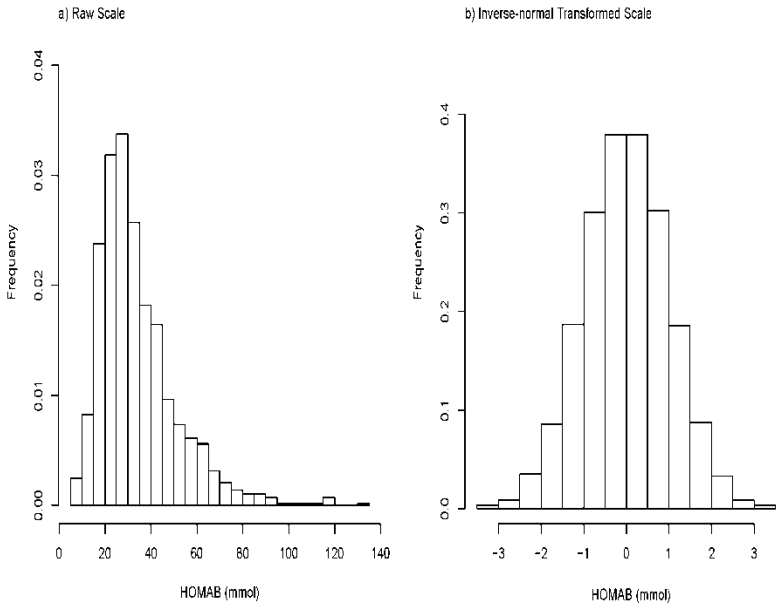
Supplementary Figure 3: Histograms illustrating raw distribution (panel A) and corrected distributions following inverse normal transformations (panel B) of variables of interest.



Supplementary Figure 3 Continued: Histograms illustrating raw distribution (panel A) and corrected distributions following inverse normal transformations (panel B) of variables of interest.



Supplementary Figure 3 Continued: Histograms illustrating raw distribution (panel A) and corrected distributions following inverse normal transformations (panel B) of variables of interest.



Supplementary Figure 3 Continued: Histograms illustrating raw distribution (panel A) and corrected distributions following inverse normal transformations (panel B) of variables of interest.

CHAPTER 6: DISCUSSION

This thesis addresses the contribution of genetic variants on the development of obesity and its metabolic complications in a multi-ethnic context and provides several novel contributions to the field of genetic epidemiology. Chapter 1 provides a comprehensive discussion of the ethnic differences in the genetic architecture of obesity and provides heritability estimates of BMI across various ethnic groups. The effects of the *PPARγ* Pro12Ala polymorphism on T2D related traits were investigated in a young, at-risk population in Chapter 2. For the first time, significant gene-environment interaction between *PPARγ* Pro12Ala, circulating lipids and markers of insulin resistance are reported, showing that genetic predisposition can alter metabolic traits early in life in presence of an obesogenic environment. Subsequent chapters move from the genetics of obesity to understanding the inflammatory mechanisms of obesity. Strong associations between circulating adiponectin concentration and metabolic traits were observed in Mexican children. However, no significant associations between inflammation-related genes and metabolic traits were identified, consistent with previous work in pediatric European populations. How these results contribute to better understanding the underlying causes of obesity and associated complications in the context of genetic epidemiology are discussed below. Future directions are also described.

Obesity rates have escalated globally, with varying prevalence across ethnic groups. Given that only a subset of individuals will develop obesity in a shared obesogenic environment, underlying genetic differences in the susceptibility to obesity have been suggested. For the first time, ethnic and population differences in the genetic architecture of obesity of obesity are discussed in a comprehensive review. A meta-analysis of heritability estimates of BMI from 19 twin and 20 family studies from various ethnic groups was also performed. Heritability estimates

for BMI obtained from family studies were not significantly different in African, admixed and Asian populations, relative to Europeans. Due to the limited number of twin studies from non-European populations, we were unable to assess ethnic differences in the heritability of BMI.

This chapter highlights the strengths and challenges of multi-ethnic studies in understanding the genetics of obesity. Identifying obesity predisposing genes in European populations has been undeniably successful with over 90% of obesity-susceptibility loci having been identified in European populations[1]. Pathway analysis of genes associated with BMI provide strong support for a role of the central nervous system, adipose tissue, the musculoskeletal system and digestive tract, highlighting the complex etiology of obesity that encompasses biological pathways in multiple organ systems[2]. In recent years, more GWAS for obesity traits have been conducted in non-European populations and have been critical in confirming European obesity loci and identifying novel, ethnic-specific loci[1]. Moving beyond GWAS, whole-exome and whole-genome sequencing can be employed to assess rare variants and copy number variants to reveal novel loci implicated in obesity. Multi-ethnic studies allow researchers to identify which genetic signals are shared across diverse ethnic groups, identifying ethnic specific disease predisposing variants and private mutations, identifying gene – gene and gene – environment interactions, and understanding how a population's history and societal practices have shaped the present genetic susceptibility to obesity. However, issues of reproducibility and transferability make the process challenging[3]. Ethnic-minorities are often under-represented in multi-ethnic studies and sample sizes of non-European cohorts are often much smaller, limiting statistical power. The effect sizes and minor allele frequencies of variants identified in European GWAS are generally larger than in non-European populations[3]. It is often unclear if the lack of significance in the replication cohort is the result of limited power/ sample

size or truly an absence of genetic association[4]. The formation of large, international genomic consortia for replication in various ethnic groups should be encouraged[5]. Funding initiatives expanding gene identification efforts in non-European or isolated populations should also be encouraged, especially in populations at high or low risk for obesity. Together the unique ethnic patterns of genetic predisposition to obesity stress the limitations of a 'one size fits all' approach for obesity treatment and emphasizes the importance of considering ethnicity in prevention strategies[6].

On the theme of multi-ethnic studies, subsequent analyses were performed in a pediatric Mexican cohort which is disproportionately affected by obesity and metabolic complications[7]. Associations with *PPARγ* Pro12Ala and BMI and T2D are well established in European populations, however few studies have examined these associations in the Mexican population[8,9]. The present work supports an association of the *PPARγ* Pro12Ala polymorphism with insulin resistance in Mexican children and suggests that this relationship is modified by circulating lipids. Previous studies have shown interactions between total, saturated or polyunsaturated fat intake on obesity and T2D related traits, however this is the first study to report significant interactions between *PPARγ* genotype and circulating lipids on IR. The use of circulating lipids as a surrogate for a high-fat diet strengthens the results and overcomes the limitations of food frequency questionnaires which are subject to bias and underestimate dietary intake[10]. However, it is important to acknowledge that circulating LDL is not a good marker of dietary intake as increased saturated fat intake is documented to increase LDL concentrations[11]. Circulating LDL levels are also strongly influenced by both monogenic and polygenic factors. Indeed, LDL lowering alleles in *PCSK9*, *HMGCR*, *NPC1L1* are associated with increased risk of T2D, which can underestimate the effects of *PPARγ* interactions with LDL[12].

Nonetheless, these findings suggest that diet and genetic background can significantly impact the development of metabolic complications. Gene - environment interactions with *FTO* and physical activity have been well established where physical activity reduced the risk of obesity by 27% in a predominately European population[13]. However, gene - environment interactions in the context of obesity and metabolic complications remain largely unexplored and should be investigated further. Once a more comprehensive understanding of gene – gene, gene – diet and gene – environment interactions is established, more effective interventions can be implemented to reduce the adverse health effects associated with obesity.

Chapter 2 also suggests that polygenic variants have a more profound effect on obesity-related complications. Rare cases of monogenic non-syndromic obesity have previously shown the importance of the central nervous system in the development of obesity, resulting in fully penetrant, early-onset obesity[14]. For example, when clinical and phenotypic characteristics of subjects with *MC4R* mutations were examined, all subjects were hyperphagic, euglycemic, and had serum lipid concentrations within normal ranges although they had significantly elevated insulin concentrations[15]. Those with *MC4R* mutations were also found to be protected from hypertension[16]. Conversely, a lack of adipose tissues, as seen in familial partial lipodystrophy syndrome can cause severe metabolic complications such as T2D, dyslipidemia and heart disease[2]. For most of the population, obesity is polygenic in nature with genetic variants involved in various biological pathways including adipocyte differentiation, insulin signaling, lipid metabolism, muscle and liver biology, gut microbiota having been identified. These diverse genetic variants demonstrate the obesity is a complex disease involving multiple, interconnected metabolic pathways which can have a profound effect on obesity-related complications.

Obesity is characterized by a state of chronic low-grade inflammation due to dysregulated adipokine secretion and macrophage infiltration, which is one of the suggested pathophysiological mechanism linking obesity to other metabolic complications. Low concentrations of the anti-inflammatory adipokine, adiponectin have been associated with obesity, insulin resistance, T2D, dyslipidemia, hypertension and cardiovascular disease[17]. The present work extends this negative association between serum adiponectin level and childhood overweight/obesity status to the Mexican population. Inverse associations with serum adiponectin and WHR, LDL-C, total cholesterol, and fasting glucose were also observed, suggesting that low adiponectin concentrations may influence the development of metabolic complications and that this may begin at an early age.

It is important to clarify that the pathogenesis of insulin resistance in the context of dyslipidemia is multifactorial, and the definition provided in Chapter 3 is an oversimplification. Dyslipidemia is commonly defined as high TG, low HDL and increased concentration of small dense LDL and is highly correlated with hyperinsulinemia[18]. This dyslipidemia is caused by an increase in free fatty acid flux into the liver which is secondary to insulin resistance and is worsened by increased concentrations inflammatory adipokines[19]. This is evidenced by genetic studies of those with insulin receptor mutations who do not develop dyslipidemia despite extreme insulin resistance[18].

Furthermore, those with insulin receptor mutations and severe insulin resistance were found to have elevated plasma adiponectin levels. Plasma adiponectin levels were not only higher than those reported in other states of severe IR, but were also significantly higher than in the normal population[20]. This challenges the assumption that low adiponectin concentrations result in insulin resistance and implies that the insulin receptor has a critical role in adiponectin

production and / or clearance, or that disruptions to the insulin receptor influence adiponectin levels[21,22]. While the precise mechanisms remain to be elucidated, rare adiponectin- lowering genetic variants have not been convincingly associated with insulin resistance, suggesting a complex relationship between adiponectin and insulin sensitivity[21].

Associations between inflammation-associated genes and metabolic traits were also investigated in the Mexican population and did not reach statistical significance, consistent with previous reports in European children. These are the first studies to explore the associations of a representative list of genetic variants related to inflammation with metabolic traits in a pediatric Mexican population. Further investigation with a more exhaustive SNP selection reflecting recent GWAS discoveries for inflammatory markers, larger sample sizes and the availability of serum inflammatory-markers, is warranted[23].

These projects also establish divergent results between classic observational studies and genetic epidemiology in the context of obesity-associated inflammation and metabolic complications. Observational studies propose that obesity-associated inflammation is the suggested pathophysiological mechanism linking obesity to other metabolic complications. However, as observational epidemiology is subject to bias, confounding, and reverse causation, causality is difficult to assess[24]. This is particularly problematic when the results are disseminated and cannot be confirmed by costly, large-scale randomized trails[25]. Genetic studies are not confounded by environmental or lifestyle factors, thus combining genetic epidemiology with classic observational epidemiology can circumvent the limitations of traditional observational studies[26]. A further approach is Mendelian randomization which can be used to show the causal direction of associaitons[26]. Deemed “nature’s randomized trials” due to the random and independent assortment of genetic variation at conception, Mendelian randomization

can provide a better understanding of the etiology of a disease[26]. Both monogenic and polygenic variants can be used in Mendelian randomization studies[27]. Monogenic analyses provide the most reliable assessments of causal relationships as the gene region encodes either the risk factor itself or a biologically relevant risk factor in the causal pathway[27]. Loss – of – function mutations care possible candidate variants for monogenic Mendelian randomization studies[27]. Polygenic Mendelian randomization studies rely on genetic variants from multiple gene regions associated with a risk factor and can be summarized in a gene score. Polygenic gene scores account for a greater proportion of variance, thus increasing statistical power and can avoid weak instrument bias[28]. Although Mendelian randomization studies are hampered by insufficient statistical power, lack of replication, genetic variants in linkage disequilibrium, and the use of surrogate tag-SNPs, they have already successfully identified causal associations with increased BMI and higher risk of metabolic and cardiovascular disease and lower adiponectin concentrations and increased insulin resistance[17,29]. Future studies combining classic and genetic epidemiology will have great utility in determining the direction of associations for obesity-associated inflammation and metabolic complications.

With more than 940 loci associated with BMI and / or obesity, there is considerable interest in using this information to predict one's risk for developing obesity. Prevention and treatment strategies are rarely developed for the individual, but at-risk subgroups may be identified more effectively using genetic testing. This is the basis of precision medicine which many believed would emerge quickly after the Human Genome Project sequenced the human genome and revolutionize modern medicine[30]. For rare cases of monogenic obesity, where individuals are easily identified through genetic testing (direct sequencing of *LEP, LEPR, PCSK1, POMC* and *MC4R*), actionable interventions are available; subcutaneous injections of

leptin in those with *LEP* mutations results in weight loss and reduced food intake[31]. The MC4R agonist, setmelanotide also results in substantial weight loss and management of hyperphagia in those who are deficient in LEPR, POMC and MC4R[32-34]. For most however, obesity is polygenic meaning that each induvial will have a unique combination of risk and protective variants. Since the completion of the Human Genome Project, other high-throughput technologies have emerged and provide unique information about the contribution of the epigenome, transcriptome, proteome, metabolome, and microbiome on obesity susceptibility. While the human genome is largely static, various "-omes" (i.e. the epigenome, transcriptome, proteome etc) are dynamic systems, which will pose additional questions surrounding the use of dynamic predictors of obesity. Although each field provides insight into the etiology of obesity, understanding tissue-specific regulation, cross-talk between tissues, and response to various environmental or physiological triggers, makes understanding the biological mechanisms of complex disease a difficult task[35].

It is now clear that after much excitement for precision medicine, much more work needs to be done before these results can be translated to clinical practice. The generation, management, integration, analysis and interpretation of omics data remains expensive and represents a major bottleneck[6]. Others argue that the utility of precision medicine for obesity is limited due to modest heritability and poor predictive ability that will never accurately predict common obestiy[36]. Despite these challenges, genetic epidemiology has made significant contributions to the understanding of the biology of obesity and this cannot be overlooked. Applying genomic medicine to designing clinical trails, guide the development of more effective treatments and identifying individuals who most likely respond to treatment has the potential to revolutionize modern medicine and should be pursued.

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