

THESIS

ASSOCIATIONS BETWEEN COMMON VARIANTS IN *FTO* AND NEAR *MC4R*
GENES ON BMI, WAIST CIRCUMFERENCE, AND TYPE 2 DIABETES
PREVALENCE AMONG HISPANIC AND NON-HISPANIC WHITE INDIVIDUALS:
THE SAN LUIS VALLEY DIABETES STUDY

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ABSTRACT OF THESIS

ASSOCIATIONS BETWEEN COMMON VARIANTS IN *FTO* AND NEAR *MC4R* GENES ON BMI, WAIST CIRCUMFERENCE, AND TYPE 2 DIABETES PREVALENCE AMONG HISPANIC AND NON-HISPANIC WHITE INDIVIDUALS: THE SAN LUIS VALLEY DIABETES STUDY

Introduction: The prevalence of obesity and type 2 diabetes (T2D) has risen sharply in the United States over the previous 40 years. Heritability estimates for obesity are generally high, and suggest that people may have genotypes that predispose them to obesity when confronted with an obesogenic environment. Genetic variants in *FTO* and near *MC4R* genes have consistently been shown to be associated with risk of obesity.

Methods: Utilizing data from the third examination of the San Luis Valley Diabetes Study (1997-1998, n = 837), we determined the minor allele frequency (MAF) and genotype distribution of single nucleotide polymorphisms (SNP)s rs8050136 and rs17782313 in a cohort of Hispanic and non-Hispanic white individuals. The associations between SNPs rs8050136 and rs17782313 and body mass index (BMI), waist circumference (WC), total energy intake, and T2D prevalence rates were determined.

Results: MAFs and genotype distributions varied between Hispanics and non-Hispanic whites for both SNPs. Hispanics were less likely to be carriers of high-risk A allele at rs8050136 (MAF: 25% vs. 38.3%) and the high-risk T allele at rs17782313 (17% vs. 23.3%) than non-Hispanic whites. After controlling for age, there was a significant association between the rs8050136 SNP in *FTO* and BMI in all Hispanics ($p = 0.0018$) and Hispanic men ($p = 0.0007$), but the association was not significant in Hispanic women ($p = 0.14$). Among all Hispanics, homozygous carriers of the *FTO* high-risk A allele had an average BMI of 31.1 kg/m² (95% CI: 29.0-33.1) compared to an average BMI of 27.1 kg/m² (95% CI: 26.4-27.8) in homozygous carriers of the C allele. In Hispanic men, homozygous carriers of the high-risk A allele had an average BMI of 31.4 kg/m² (95% CI: 28.5-34.3) compared to 25.9 kg/m² (95% CI: 25.0-26.9) in homozygous carriers of the C allele. After controlling for age, there was also a significant association between the rs8050136 SNP and WC in Hispanic men ($p = 0.0048$), but not Hispanic women ($p = 0.24$). Hispanic male homozygous carriers of the A allele had an average WC of 104.2 cm (95% CI: 97.7-110.7) compared to an average WC of 94.1 cm (92.0-96.2) in homozygous carriers of the C allele. A high dietary fat intake was shown to modify the association between the *FTO* variant and BMI. No other significant associations were found between genetic variants in *FTO* or near *MC4R* on BMI, WC, total energy intake, or T2D prevalence.

Discussion: Hispanics are less likely to be carriers of high-risk alleles in *FTO* (rs8050136) and near *MC4R* (rs17782313) than non-Hispanic whites. Our analysis demonstrates that the *FTO* variant is associated with BMI and WC in Hispanics and also that this relationship between the *FTO* variant and BMI is modified by dietary fat intake.

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CHAPTER I

INTRODUCTION

It has been well documented that the prevalence of overweight and obesity has reached epidemic levels in the United States with 68.3% and 33.9% of all adult Americans being classified as either overweight (BMI ≥ 25 kg/m²) or obese (BMI ≥ 30 kg/m²), respectively (Flegal, Carroll, Ogden, Curtin 2010). Obesity has been consistently associated with a number of medical complications including overall mortality, type 2 diabetes mellitus (T2D), cardiovascular disease, cancer, hypertension, dyslipidemia, obstructive sleep apnea, and osteoarthritis (Flegal *et al.* 2010). The development of overweight and obesity is multi-factorial, resulting from an obesogenic environment of limited need for physical activity and a plethora of energy-dense foods interacting with a genome that is predisposed to weight gain. Results from twin studies establish that heritability estimates for BMI range from 0.5 to 0.8 (Schousboe, Visscher, Erbas, Kyvik, Hopper, Henriksen, *et al.* 2004), though it is clear that in the vast majority of cases the genetic contribution to obesity is polygenic in nature.

Genetic variants in and near the *MC4R* gene have been studied as potential contributing factors in obesity because of the known effects of the melanocortin system as a mediator of the effects of leptin on energy intake and expenditure (Garfield, Lam, Marston, Przydzial, & Heisler 2009). For example, the rs17782313 SNP has been found

to be associated with BMI (Loos, Lingren, Li, Wheeler, Zhao, Prokopenko, *et al.* 2008; Qi, Kraft, Hunter, & Hu 2008; Stutzmann, Cauchi, Durand, Calvacanti-Proença, Pigeyre, Hartikainen, *et al.* 2009; Valladares, Domínguez-Vásquez, Obregón, Weisstaub, Burrows, Maiz, & Santos 2010), T2D risk (Qi *et al.* 2008), and eating behavior (Stutzmann *et al.* 2009; Valladares *et al.* 2010).

The advent of genome wide association studies (GWAS) has accelerated the process of identifying single nucleotide polymorphisms (SNPs) that are related to obesity. For example, the rs8050136 SNP in the *fat mass and obesity associated (FTO)* gene have been identified in a number of studies as being associated with BMI in European (Frayling, Timpson, Weedon, Zeggini, Freathy, Lindgren, *et al.* 2007), Hispanic, African-American (Wing, Ziegler, Langfeld, Ng, Haffner, Norris *et al.* 2009), and Asian individuals (Liu, Liu, Song, Zhou, Zhang, Zhao, *et al.* 2010). The FTO gene product (Fto) acts as a DNA demethylase and Fto mRNA has been shown to be most prevalent in the nuclei of the hypothalamus that are involved in energy homeostasis (Gerken, Gizard, Tung, Webby, Saudek, Hewitson *et al.* 2007). There is evidence that Fto levels alter the expression of signal transducer and activator of transcription-3 (Stat3) (Tung, Ayuso, Shan, Bosch, O’Rahilly, Coll, & Yeo 2010), which has been shown to disrupt leptin signaling and depress melanocortin tone resulting in hyperphagia and obesity (Bates, Stearns, Dundon, Schubert, Tso, Wang, *et al.* 2003).

It is possible that genetic variations in *FTO* could alter MC4R functioning by affecting Stat3-dependent leptin action and genetic variations in both genes could have additive effects on obesity. To the best of our knowledge, the combined effects of SNPs in the *FTO* gene and near the *MC4R* gene have only been investigated in European and

Chinese Han populations. In Europeans, the addition of each risk allele was associated with an increasing odds of being obese (OR: 1.21; 1.03 – 1.41; P = 0.02) relative to those carrying no risk alleles (Cauhi, Stutzmann, Cavalcanti-Proença, Durand, Pouta, Hartikainen, *et al.* 2009). In the Chinese Han population, it was found that the BMI of participants carrying no *FTO* or *MC4R* risk alleles was 25.9 ± 4.9 (mean \pm SD) while the BMI of those carrying three or four risk alleles was 33.2 ± 6.3 (Huang, Sun, & Sun 2011).

Statement of Purpose

The purpose of the current study is to determine the cross-sectional associations between common variants in the *FTO* (rs8050136) gene and near the *MC4R* (rs17782313) gene on obesity and T2D prevalence in Hispanic and non-Hispanic white individuals living in the San Luis Valley of Colorado.

Hypotheses

- 1) The adenine for cytosine polymorphism in *FTO* (rs8050136) will be associated with a higher BMI and WC in Hispanic and non-Hispanic white individuals and this association will be modified by dietary fat intake.
- 2) The cytosine for thymine polymorphism near *MC4R* (rs17782313) will be associated with a higher BMI and WC in Hispanic and non-Hispanic white individuals and this association will be modified by dietary fat intake.
- 3) The adenine for cytosine polymorphism in *FTO* (rs8050136) will be associated with a higher total energy intake in Hispanic and non-Hispanic white individuals.

- 4) The cytosine for thymine polymorphism near *MC4R* (rs17782313) will be associated with a higher total energy intake in Hispanic and non-Hispanic white individuals
- 5) The adenine for cytosine polymorphism in *FTO* (rs8050136) will be associated with a higher prevalence of T2D in Hispanic and non-Hispanic white individuals.
- 6) The cytosine for thymine polymorphism near *MC4R* (rs17782313) will be associated with a higher prevalence of T2D in Hispanic and non-Hispanic white individuals.
- 7) Polymorphisms in *FTO* and near *MC4R* will have additive effects on BMI, WC, and T2D in Hispanic and non-Hispanic white individuals.

CHAPTER II

LITERATURE REVIEW

Obesity in America

The obesity epidemic in America has been well documented, but is worthy of further mentioning here. The Centers for Disease Control and Prevention (CDC) utilizes data from the Behavioral Risk Factor Surveillance System (BRFSS) to determine obesity prevalence and trends in America. A total of 405,102 people participated in the 2009 BRFSS survey. According to the 2009 BRFSS survey data, 26.7% of all American adults over age 18 are obese. The prevalence of obesity varies by certain characteristics. The highest prevalences of obesity were reported in adults aged 50-59 (31.1%) and 60-69 (30.9%), non-Hispanic blacks (36.8%), Hispanics (30.7%), and residents of the Midwest (28.2%) and South (28.4%) (Centers for Disease Control and Prevention 2010). The CDC's report on obesity may underestimate the true prevalence of obesity in America due to limitations in the BRFSS data. The BRFSS uses self-reported data collected by phone calls to landline phones. The use of self-reported data raises the possibility that participants may misreport their height and/or weight leading to skewed body mass index calculations. Calling exclusively to landline phone numbers excludes wireless-only households, which are more likely to be younger, black or Hispanic, have lower incomes, and have no health insurance. The exclusion of some people in these groups likely alters

obesity prevalence rates. Also, the median response rate for the 2009 BRFSS survey was only 52.9%. It is possible that obesity characteristics could differ between respondents and non-respondents (Centers for Disease Control and Prevention 2010).

The National Health and Nutrition Examination Survey (NHANES) is another nationwide survey used by the CDC for tracking obesity prevalence and trends in America. The 2007-2008 NHANES sample included 8082 men and women aged 20 years and older. NHANES data has a major advantage over BRFSS data because height and weight are measured rather than self-reported. As such, the prevalence of obesity determined from NHANES data differs from BRFSS data. According the 2007-2008 NHANES data, 68.3% of Americans aged 20 and older are overweight ($BMI \geq 25 \text{ kg/m}^2$) and 33.9% are obese ($BMI \geq 30 \text{ kg/m}^2$). Similar to the BRFSS report, obesity prevalence was highest among non-Hispanic blacks (44.1%) and Hispanics (37.9%) (Flegal, Carroll, Ogden, Curtin 2010). Obesity has been consistently associated with a number of medical complications including, but not limited to, overall mortality, type 2 diabetes mellitus (T2D), cardiovascular disease, cancer, hypertension, dyslipidemia, obstructive sleep apnea, and osteoarthritis (Flegal, *et al.* 2010). Diseases associated with obesity have been estimated to account for 27% of the increase in medical costs in the United States from 1987 to 2001, and the medical costs associated with obesity totaled \$147 billion in 2006 (Centers for Disease Control and Prevention 2010). These personal and financial burdens make obesity one of the greatest public health concerns in America. The federal government has intensified its efforts to prevent and treat obesity in recent years through new initiatives such as the ‘Let’s Move!’ campaign, the Communities Putting Prevention

to Work program, and the Patient Protection and Affordable Care Act (Centers for Disease Control and Prevention 2010).

The Development of Obesity

The prevalence of obesity in America had remained relatively stable until the mid-1970s when obesity rates began to rise sharply, although the most recent NHANES data suggests that the rise in obesity prevalence rates is slowing down. From 1999-2000 to 2007-2008, obesity rates in men rose 4.7% compared with increases of between 6 and 9% in previous decades. During the same time, obesity rates in women rose a non-significant 2.1% (Flegal *et al.* 2010). The sharp and sudden rise in obesity prevalence over the past four decades implies that the obesity epidemic has been caused by changes in our environment that are affecting energy intake and expenditure as it is unlikely that the gene pool has changed significantly over this time span. However, results from twin studies have established that the heritability estimates for BMI range from 0.5 to 0.8 (Schousboe, Visscher, Erbas, Kyvik, Hopper, Henriksen, *et al.* 2004). The results from these studies are congruent with the “Thrifty Genotype” hypothesis first proposed by James Neel in 1962. This hypothesis states that people with a genetic propensity towards obesity and T2D are “... exceptionally efficient in the intake and/or utilization of food” (Neel 1962, p.354). An efficient genotype of this nature would have been beneficial in preventing starvation during early human evolution, but it has proven to be detrimental in our current obesogenic environment of limited need for physical activity and a plethora of easily obtainable, energy-dense foods.

While it is obvious that behavioral and environmental modifications are necessary to prevent and treat obesity, a greater understanding of the genetic contribution to the development of obesity has clinical relevance as well. For instance, a more individualized plan for preventing and treating obesity could be developed if a patient's genetic predisposition to obesity could be determined through genetic testing, and recent advances in the fields of nutrigenomics and nutrigenetics are providing insight into the ways in which the nutrients we eat affect gene expression and how a specific genotype can alter nutrient requirements, respectively (Debusk, Fogarty, Ordovas, & Kornman 2005). For example, a polymorphism in the *APOA2* gene has been shown to be associated with an increased BMI only in people who are consuming ≥ 22 grams of saturated fat per day (Corella, Peloso, Arnett, Demissie, Cupples, Tucker, *et al.* 2009). Approximately 15% of the population is homozygous for the high-risk C allele in the *APOA2* gene, and this portion of the population would likely benefit from a diet that specifically addressed saturated fat intake. Genetic variants and their association with obesity are detected using a variety of different experimental approaches. Evidence from single-gene mutations resulting in obesity, Mendelian disorders exhibiting obesity, transgenic and knockout murine models, and quantitative trait loci (QTL) from animal cross-breeding experiments provides candidate genes for association studies. More recently, linkages from genome wide association studies (GWAS) have become more popular. As reported in the 2005 human obesity gene map, >600 loci and 135 candidate genes associated with an obese phenotype have been identified using these various methods (Rankien, Zuberi, Chagnon, Weisnagel, Argyropoulos, Walts *et al.* 2006). In the case of candidate gene association studies, a priori hypotheses are made regarding the

relationship with variants in the gene and obesity based on previous knowledge of its involvement in the regulation of energy intake and/or expenditure. Alternatively, GWAS can analyze up to 2,000,000 genetic variations for associations with a given phenotype such as obesity without any a priori knowledge of the functional consequences of those variations. QTLs identified in GWAS can be further narrowed down by fine mapping to discover new potential candidate genes, which can then be studied through candidate gene association studies (Hinney, Vogel, & Hebebrand 2010).

The Melanocortin-4 Receptor and Obesity

The physiological control of energy homeostasis is determined by the integration of both acute and long-term signals of energy balance. The central melanocortin system is one crucial system in the integration and interpretation of these signals. The remainder of this short review on the role of the melanocortin system in energy homeostasis comes from the recent review by Garfield *et al.*, and the reader is referred to this review for a more in depth discussion of the topic (Garfield, Lam, Marston, Przydzial, & Heisler 2009). The melanocortin system is defined by cells expressing any one of the five melanocortin receptors (MC1-MC5R), the pro-opiomelanocortin (POMC)-derived melanocortin receptor agonists, and the melanocortin receptor antagonist agouti-related protein (AgRP). POMC and AgRP expressing neurons are found within the arcuate nucleus (ARC) of the hypothalamus and project to other areas of the hypothalamus known to be involved in the regulation of energy homeostasis. Additionally, POMC expressing neurons are also found within the solitary tract of the caudal brainstem.

Of the five receptors, the MC4R receptor is the most extensively studied and appears to be the most important for the regulation of energy homeostasis. An increased melanocortin tone resulting from the binding of POMC ligands (especially α - and β -melanocyte-stimulating hormone [MSH]) suppresses energy take and enhances energy expenditure. Alternatively, AgRP acts as a competitive antagonist to α - and β -MSH. Increased binding of AgRP to the MC4R promotes energy intake and suppresses energy expenditure. The various acute and long-term signals of energy balance differentially effect POMC and AgRP expressing neurons to determine the net melanocortin tone and resultant effect on energy homeostasis. These energy balance signals originate from both the periphery and the central nervous system.

Peripheral Signals

Leptin is a long-term regulator of energy homeostasis and is secreted from adipocytes in proportion to adipose tissue mass. Both POMC and AgRP expressing neurons express leptin receptors. Congruent with its anorectic effects, leptin stimulates the release of POMC ligands and inhibits the release of AgRP, thereby resulting in an increased melanocortin tone. Similar to leptin, increasing circulating insulin levels correlate with an increasing adipose tissue mass and is considered to be another long-term indicator of energy homeostasis. Intracerebroventricular (ICV) insulin administration inhibits the release of AgRP and decreases AgRP mRNA expression. The effects on insulin on POMC expressing neurons are less clear. ICV insulin administration has been shown to increase POMC expression, but paradoxically hyperpolarizes POMC expressing neurons and inhibits the release of POMC ligands. Ghrelin is an orexigenic hormone secreted from the gut prior to meal initiation. Ghrelin treatment of ApRP expressing neurons has

been shown to increase AgRP mRNA and cause depolarization of these neurons. Electrophysiological data reveal that ghrelin hyperpolarizes POMC neurons. Other peripheral signals such as cholecystokinin, peptide YY, glucose, and fatty acids are also regulators of energy homeostasis and may be involved in the melanocortin system.

Central Signals

Increased serotonin signaling increases melanocortin tone by promoting the depolarization of POMC expressing neurons and hyperpolarizing AgRP expressing neurons. Pituitary adenylate cyclase-activating peptide (PACAP) also promotes an increased melanocortin tone by inducing POMC and MC4R mRNA expression. Orexins and melanin-concentrating hormone act in opposition to serotonin and PACAP by increasing the release of AgRP and decreasing POMC ligand release.

Effects of Genetic Variations in and near MC4R

Given the crucial role for the MC4R in energy homeostasis, it is not surprising that genetic variations in *MC4R* have been associated with BMI and obesity. Partial or complete loss-of-function mutations in *MC4R* are the commonest cause of monogenic obesity. Such mutations have been shown to be responsible for 5.8% of early onset, severe obesity cases in the United Kingdom (Farooqi, Keogh, Yeo, Lank, Cheetham, & O'Rahilly 2003). In 2008, Loos *et al.* identified a cluster of single nucleotide polymorphisms (SNPs) on chromosome 18q21 in a cohort of 16,876 individuals of European descent. Two of these SNPs (rs17782313 and rs17700633) were found to be significantly associated with BMI and overweight/obesity. Of the two, rs17782313 had the greatest effect with each copy of the risk allele (C>T) being associated with a difference in BMI of 0.22 kg/m², an 8% increase in odds of being overweight, and a 12%

increase in the odds of being obese. It is important to note, however, that genetic variants near *MC4R* explain only a very modest amount of the total variance in adult BMI (~0.14%). The rs17792313 SNP is located 188 kb downstream of *MC4R*, which is located on chromosome 18q22. The location of SNP implies that the expression of *MC4R* is altered in these individuals rather than an alteration in the function of the receptor (Loos, Lingren, Li, Wheeler, Zhao, Prokopenko, *et al.* 2008). The minor allele frequency (MAF) for the rs1778213 SNP has been shown to vary by ethnicity. Loos, *et al.* (2008) reported a MAF of 24% (6% homozygous) in those of European descent. The MAFs have been reported in African American (27%; 7.5% homozygous) (Liu, Zhu, Lagou, Gutin, Barbeau, Treiber, *et al.* 2010) and Chinese Han (Obese: 48.7%, 24.1% homozygous; Controls: 40.6%, 17.5% homozygous) populations (Huang, Sun, & Sun 2011). To the best of my knowledge, the MAF for the rs17782313 SNP has not been reported in the Hispanic population. Several subsequent studies have replicated the association of SNP rs17782313 and obesity (Qi, Kraft, Hunter, & Hu 2008; Stutzmann, Cauchi, Durand, Calvacanti-Proença, Pigeyre, Hartikainen, *et al.* 2009; Valladares, Domínguez-Vásquez, Obregón, Weisstaub, Burrows, Maiz, & Santos 2010), as well as significant associations with higher intakes of total energy and fat, diabetes risk (Qi *et al.* 2008), snacking behavior, hunger, eating large meals (Stutzmann *et al.* 2009), satiety responsiveness, and enjoyment of food (Valladares *et al.* 2010).

The *Fat Mass and Obesity Associated Gene and Obesity*

In a 2007 GWAS, Frayling *et al.* discovered multiple SNPs in the *Fat Mass and Obesity Associated (FTO)* gene that predispose individuals to T2D through an effect on BMI. All

of the SNPs associated with BMI were highly correlated with one another (r^2 from 0.52-1.0). The associations with obesity and SNP rs9939609 were reported and further studied by the authors. They found that each additional copy of the risk allele (A>T) was associated with an increase in BMI of ~ 0.4 kg/m², a 31% increase in the odds of being overweight, and an 18% increase in the odds of being obese. Similar to *MC4R*, genetic variants in *FTO* explain a very modest amount of the overall variance in adult BMI ($\sim 1\%$) (Frayling, Timpson, Weedon, Zeggini, Freathy, Lindgren, *et al.* 2007). Although Frayling *et al.* identified multiple SNPs in *FTO*, rs9939609 and rs8050136 have been the most intensely studied. The overall MAF for the rs8050136 SNP has been reported as 38.5% in postmenopausal women, but also that it varies significantly by ethnicity. The MAF was 40.9% in non-Hispanic white (18% homozygous), 41.8% in African American (19.0% homozygous), 28.1% in Hispanic (9.6% homozygous), and 18.8% in Asian (5.4% homozygous) postmenopausal women (Song, You, Hsu, Howard, Langer, Manson *et al.* 2008). Frayling *et al.* first described the association between obesity and genetic variants in *FTO* among Europeans. Subsequent studies of SNPs rs8050136 and/or rs9939609 have demonstrated similar results in African American, Hispanic (Wing, Ziegler, Langfeld, Ng, Haffner, Norris *et al.* 2009), and Asian populations (Liu, Liu, Song, Zhou, Zhang, Zhao, *et al.* 2010). It has been noted that the relationship between SNPs in *FTO* and obesity may be accentuated by high fat intake (Sonestedt, Roos, Gullberg, Ericson, Wirfält, & Orho-Melander 2009) and low physical activity (Sonestedt *et al.* 2009; Ahmad, Chasman, Mora, Paré, Cook, Buring, *et al.* 2010; Rankinen, Rice, Teran-Garcia, Rao, & Bouchard 2010). Additionally, Rankinen *et al.* (2010) found that homozygotes for the A/A risk allele (rs8050136) were more resistant to exercise-induced reductions in

fat mass than homozygotes for the low risk C/C allele. While the effects of genetic variants in *FTO* seem to be modified by dietary and physical activity habits, the mechanism for the increased risk of obesity in individuals with a *FTO* high-risk genotype has not been resolved. Some studies have found that SNPs in *FTO* are not associated with energy intake or leisure-time physical activity levels (Hubáček, Pikhart, Peasey, Kubínová, & Bobák 2010; Liu, Zhu, Lagou, Gutin, Stallmann-Jorgensen, Treiber, *et al.* 2010; Hakanen, Raitakari, Lehtimäki, Peltonen, Pakkala, Silanmäki, *et al.* 2009), while others have found significant associations with total energy (Speakman, Rance & Johnstone 2008) and fat intake (Cecil, Tavendale, Watt, Hetherington & Palmer 2008; Timpson, Emmett, Frayling, Rogers, Hattersley, McCarthy & Davey Smith 2008) as well as resting metabolic rate (Hubáček *et al.* 2010).

Function of the FTO Gene Product

The function of the *FTO* gene product (hereafter referred to as Fto) has been intensely researched ever since Frayling *et al.* discovered the association between *FTO* genetic mutations and obesity. Shortly after Frayling *et al.* published their results, Gerken *et al.* performed a sequence analysis of Fto and determined that it contained a double-stranded beta-helix fold homologous to those of (Fe)II and 2-oxoglutarate oxygenases. These oxygenases are involved in various functions in the body including DNA repair, fatty acid metabolism, and posttranslational modifications, including hydroxylation and demethylation reactions. Further analysis determined that Fto catalyzes the demethylation of DNA and localizes to the nucleus of transfected cells. In situ hybridization of hypothalamic slices revealed that *FTO* mRNA was highly expressed in the arcuate, paraventricular, dorsomedial, and ventromedial nuclei (Gerken, Gizard,

Tung, Webby, Saudek, Hewitson *et al.* 2007). DNA methylation is known to be a major epigenetic modification and occurs on cytosine residues that are followed by a guanine residue (CpG areas). These CpG areas have been found in the promoter region of nearly half of human genes, and methylation at these sites typically silences the expression of that gene (Campión, Milagro, & Martínez 2010). Taken together, it is possible that perturbations to Fto activity and/or abundance could alter the expression of genes involved in energy homeostasis.

Indeed, Church *et al.* developed a mouse model which displays decreased Fto expression, and revealed increased expression of multiple genes involved in fat and carbohydrate metabolism. The adipose tissue of mutant mice also demonstrated an improved inflammatory profile relative to wild-type mice (Church, Lee, Bagg, McTaggart, Deacon, Gerken, *et al.* 2009). Consistent with this data, inactivation of the *FTO* gene has been shown to protect from obesity in a mouse model (Fischer, Koch, Emmerling, Vierkotten, Peters, Brüning, & Rütther 2009), and overexpression of *FTO* in mice increases food intake and results in obesity (Church, Moir, McMurray, Girand, Banks, Teboul, *et al.* 2010). A second line of evidence on the function of Fto contradicts this data. It has been reported that fasting reduces Fto mRNA and high fat diet increases Fto mRNA in the ARC (Tung, Ayuso, Shan, Bosch, O’Rahilly, Coll, & Yeo 2010). This implies that a reduction in Fto would stimulate food intake while an increase would be expected to inhibit food intake. Tung *et al.* (2010) confirmed this implication by demonstrating that a 2.5 fold increase in ARC Fto levels decreased daily food intake by 14%, whereas ARC overexpression of Fto by 40% increased food intake by 16%. Interestingly, the expression of *AgRP* and *POMC* were unaffected by Fto levels, but

overexpression of Fto increased signal transducer and activator of transcription-3 (Stat3) mRNA. The ability of leptin to regulate energy homeostasis is dependent on intact Stat3 signaling (Buettner, Poci, Muse, Etgen, Myers, & Rossetti 2006). Although Tung *et al.* (2010) observed no effect on *POMC* or *AgRP* expression levels; the s/s Stat3 knockout mouse has been shown to exhibit decreased hypothalamic *POMC* expression and increased *AgRP* expression and, therefore, decreased melanocortin tone (Bates, Stearns, Dundon, Schubert, Tso, Wang, *et al.* 2003). It was recently discovered that *FTO* transcription is regulated by the cut-like homeobox 1 (CUX1) transcription factor via a regulatory site within the first intron of the *FTO* gene. This is the same region where the previously mentioned SNPs associated with human obesity are located. The presence of the risk allele at rs8050136 results in a reduced affinity for CUX1 and subsequent reduction in Fto as well as a diminished cellular response to leptin (Stratigopoulos, LeDuc, Cremona, Chung, & Leibel 2011). These equivocal findings regarding Fto's function clearly demonstrate the need for continued effort in this field so its physiological effects on the development of obesity as well as possible interactions with other genetic variants can be elucidated.

Combined Associations Between Variants in *FTO* and near *MC4R* and Obesity

It is possible that genetic variations in *FTO* could alter MC4R functioning by affecting Stat3-dependent leptin action. Loos *et al.* (2008) were the first to notice that variants in *FTO* and near *MC4R* have additive effects on BMI. Compared to individuals with no risk alleles in either gene (19% of the population), the BMI of individuals who are homozygous in both (1%) was found to be 1.17 kg/m² higher. A second study in the

European population found that children carrying three or four risk alleles were three times more likely to be obese than those carrying no risk alleles. The association seen in adults was more modest with high-risk individuals being 1.8 times more likely to be obese than those carrying no risk alleles (Cauhi, Stutzmann, Cavalcanti-Proença, Durand, Pouta, Hartikainen, *et al.* 2009). A similar study in Chinese Han populations found that the BMI of individuals with no risk alleles was 25.9 ± 4.9 , one risk allele was 26.4 ± 5.1 , two risk alleles was 28.1 ± 5.5 , and three or four risk alleles was 33.2 ± 6.3 (Huang, Sun, & Sun 2011). The intent of the present study was to determine the combined and separate associations between genetic variants in *FTO* and near *MC4R* and obesity and T2D risk in Hispanics and non-Hispanic whites living in the San Luis Valley of Colorado.

CHAPTER III

MATERIALS AND METHODS

Subjects

Subjects were participants of the San Luis Valley Diabetes Study (SLVDS). Detailed methods have been published previously (Hamman, Marshall, Baxter, Kahn, Mayer, Orleans, *et al.* 1989). The SLVDS was designed to examine the prevalence, risk factors and natural history of type 2 diabetes among Hispanic and non-Hispanic white men and women from the San Luis Valley in southern Colorado using a geographically based case-control design. Participants without a history of diabetes were initially examined between 1984 and 1988 [$n = 1351$ including 71 with undiagnosed types 2 diabetes and 173 with impaired glucose tolerance (IGT)]. From 1988 to 1992, a second examination was conducted, and from 1997 to 1998 a third examination was conducted on individuals without a diagnosis of diabetes at the initial visit ($n = 837$). The data for the present cross-sectional study comes from those participants who took part in the third examination. Informed consent was obtained from all subjects, and the University of Colorado at Denver and Health Sciences Center Institutional Review Board approved all of the protocols. The Colorado State University Institutional Review Board approved the analysis of this data.

Clinical Characteristics

During the clinical visit between 1997 and 1998, weight was measured in kilograms with a balance beam scale calibrated weekly with standard weights. Height was measured using a stadiometer to the nearest 0.1 cm. Participants were measured in light clothing or a hospital scrub suit for both height and weight measurements. WC was measured by trained data collectors at the 10th rib with a steel tape to the nearest 0.1 cm. BMI was calculated as the current weight (kg) divided by height squared (m^2). To establish diabetic status, an oral glucose tolerance test (Orangedex, Koladex, Custom Lab Inc. Baltimore, MD) was performed on all subjects. Diabetes mellitus was diagnosed according to ADA criteria (American Diabetes Association 2003), which requires a fasting venous plasma glucose greater than or equal to 126 mg/dl or a two-hour glucose level greater than or equal to 200 mg/dl after a 75 g oral glucose load. Subjects on insulin or oral hypoglycemic medication were classified as diabetic, regardless of their glucose tolerance test

Dietary patterns were determined by a 24-hour dietary recall. The 24-hour recall estimates intake of 93 nutrients for which food composition has been determined, including total calories, the macronutrients, subtypes of carbohydrate and fat (including individual fatty acids), alcohol, dietary fiber, (including soluble and insoluble), vitamins, and minerals. Interviews were conducted by bilingual interviewers trained and certified by Nutrition Coordinating Center (NCC) at the University of Minnesota with annual recertification. The NCC database includes a large number of Hispanic foods added for studies including the SLVDS who have used the system, as well as for NHANES III.

Participants reported other clinical characteristics on a validated health history questionnaire including medications and smoking status.

SNP Genotyping

The rs8050136 and rs17782313 SNPs were genotyped by Illumina , Inc (San Diego, Ca) as part of a larger analysis of the SLVDS data of 384 loci in 1678 DNA samples (1398 genomic DNA samples and 280 whole genomic amplification (WGA) samples) in 2009-10. The genotyping success rate for the whole genomic samples and WGA samples were 98.4% and 93.9%, respectively.

Statistical Analysis

Descriptive statistics and frequencies were calculated by gender for each ethnic group. General linear models were used to estimate least-square means with 95% confidence intervals for the association of BMI, WC, and total energy intake with each of the SNPs by ethnic group. An additive genetic model was used and adjusted for age at the time of the clinical visit for BMI and WC and for both age and gender for total energy intake. The same analysis was used to determine the combined association of both SNPs on BMI and WC. Logistic regression was used to determine the association between diabetes status and each SNP again using an additive genetic model and adjusting for age at the time of the clinical visit. The same analysis was used to determine the combined association of both SNPs on T2D status. All analyses were run using SAS 9.3 for Windows.

CHAPTER IV

RESULTS

Participant Characteristics

Participant characteristics are presented in Table 1 and genotype distributions for genetic variants in *FTO* and *MC4R* are presented in Table 2. The alleles at rs17782313 and rs8050136 were found to be in Hardy-Weinberg Equilibrium. The minor allele frequency (MAF) for *FTO* rs8050136 was 25.1% (males: 24.1%; females: 25.9%) and 38.2% (males: 38.9%; females: 37.7%) for Hispanics and non-Hispanic whites, respectively. The MAF for *MC4R* rs17792313 was 16.9% (males: 17.6%; females: 16.4%) and 23.4% (males: 22.2%; females: 24.4%) in Hispanics and non-Hispanic whites, respectively. Hispanics were less likely than non-Hispanic whites to be homozygous carriers for the risk allele in both *FTO* and *MC4R* genes (*FTO* in males: 5.9% vs. 14.7%; *FTO* in females: 7.7% vs. 13.9%; *MC4R* in males: 4.4% vs. 7.6%; *MC4R* in females: 1.8% vs. 6.4%).

Associations Between *FTO* and *MC4R* Variants and BMI and WC in Hispanics

The associations between genetic variants in *FTO* and *MC4R* and BMI and WC in Hispanics are presented in Figures 1a – 1d, and all associations were adjusted for age at the time of the clinical visit. Among all Hispanics, there was a significant association

Table 1: Participant Characteristics	Hispanics		Non-Hispanic Whites	
	Male	Female	Male	Female
	N = 153 Mean±SD	N = 200 Mean±SD	N = 228 Mean±SD	N = 256 Mean±SD
Age	62.9±12.4	61.6±12.3	63.4±11.0	63.9±11.6
BMI (kg/m ²)	26.9±4.5	28.0±5.2	27.5±3.9	26.9±5.4
WC (cm)	96.1±9.7	89.1±11.2	98.8±9.5	86.3±10.7
Energy Intake (kcal/day)	2110±882	1517±692	2376±1133	1726±639
Fat Intake (g/day)	82.6±45.1	58.9±35.9	93.8±63.9	67.5±32.3
% Diabetic	19.6	19.5	11.5	10.3
% Current Smoker	34.0	27.7	22.0	31.8

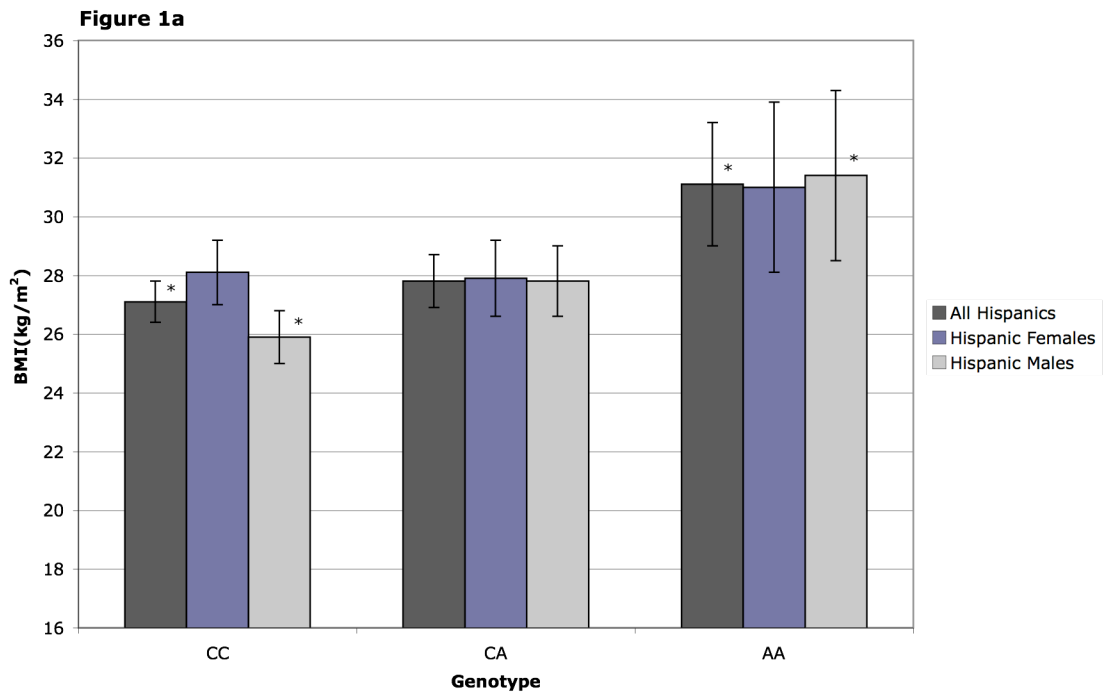
Table 2: Genotype Distribution	Hispanics		Non-Hispanic Whites	
	Males	Females	Males	Females
FTO (rs8050136)	N = 135	N = 170	N = 198	N = 231
	N (%)	N (%)	N (%)	N (%)
CC	78 (57.8)	95 (55.9)	73 (36.9)	89 (38.5)
CA	49 (36.3)	62 (36.5)	96 (48.5)	110 (47.6)
*AA	8 (5.9)	13 (7.7)	29 (14.7)	32 (13.9)
MAF	24.1%	25.9%	38.9%	37.7%
MC4R (rs17782313)	N = 136	N = 171	N = 198	N = 236
	N (%)	N (%)	N (%)	N (%)
TT	94 (69.1)	118 (69.0)	125 (69.1)	136 (57.6)
TC	36 (26.5)	50 (29.2)	58 (29.3)	85 (36.0)
*CC	6 (4.4)	3 (1.8)	15 (7.6)	15 (6.4)
MAF	17.6%	16.4%	22.2%	24.4%

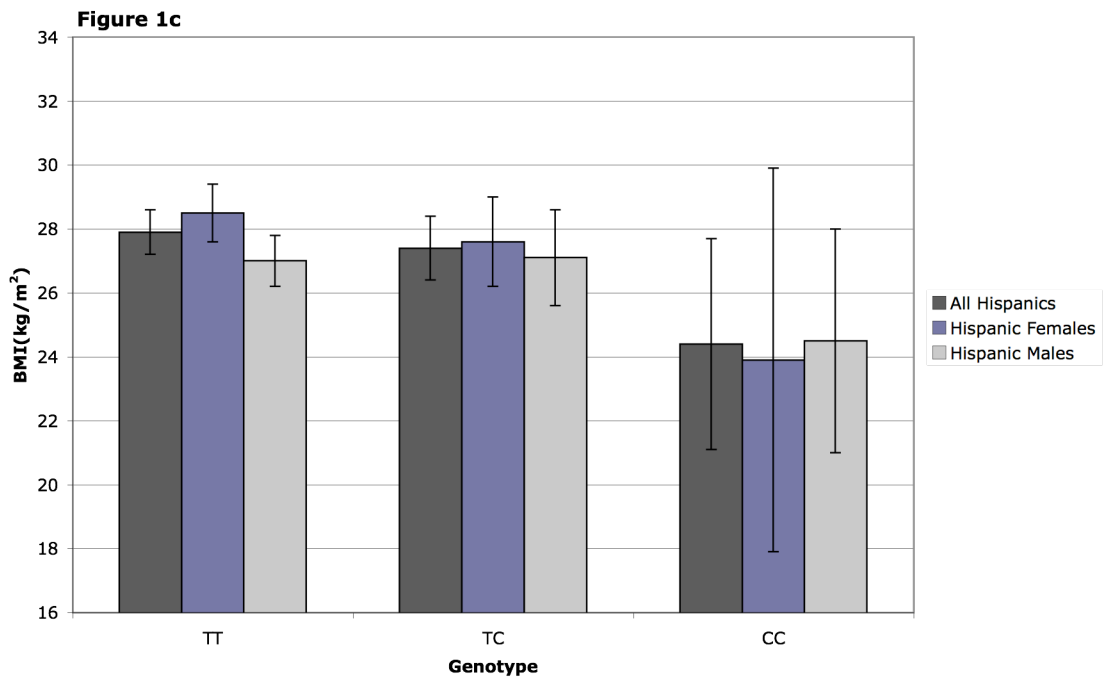
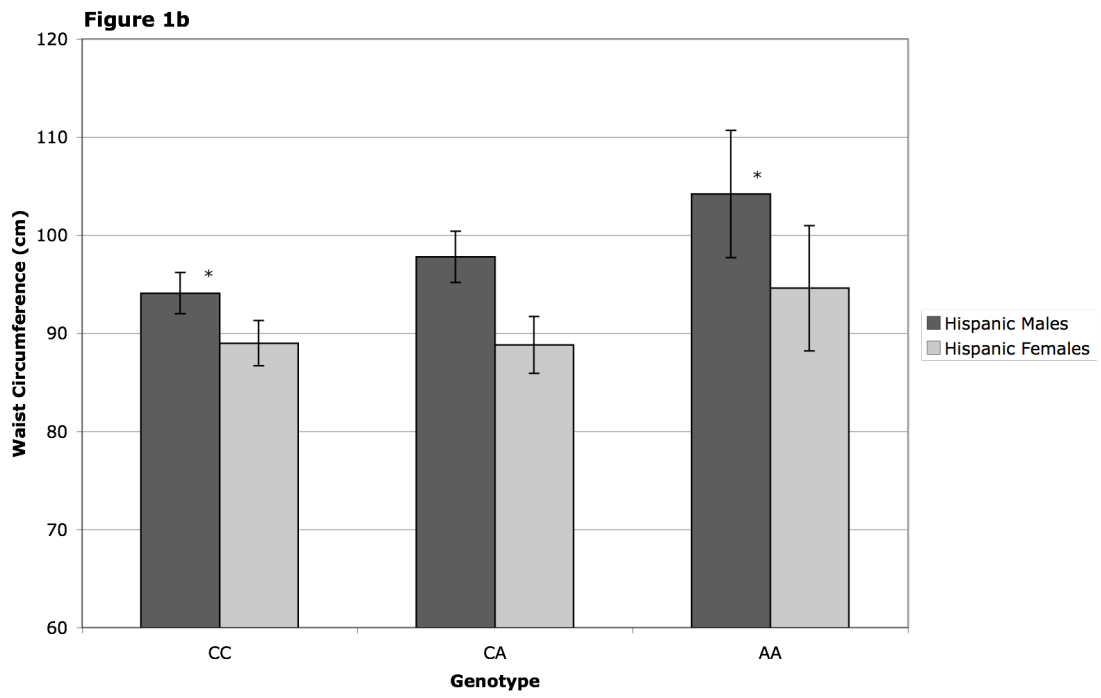
* Denotes homozygous risk allele

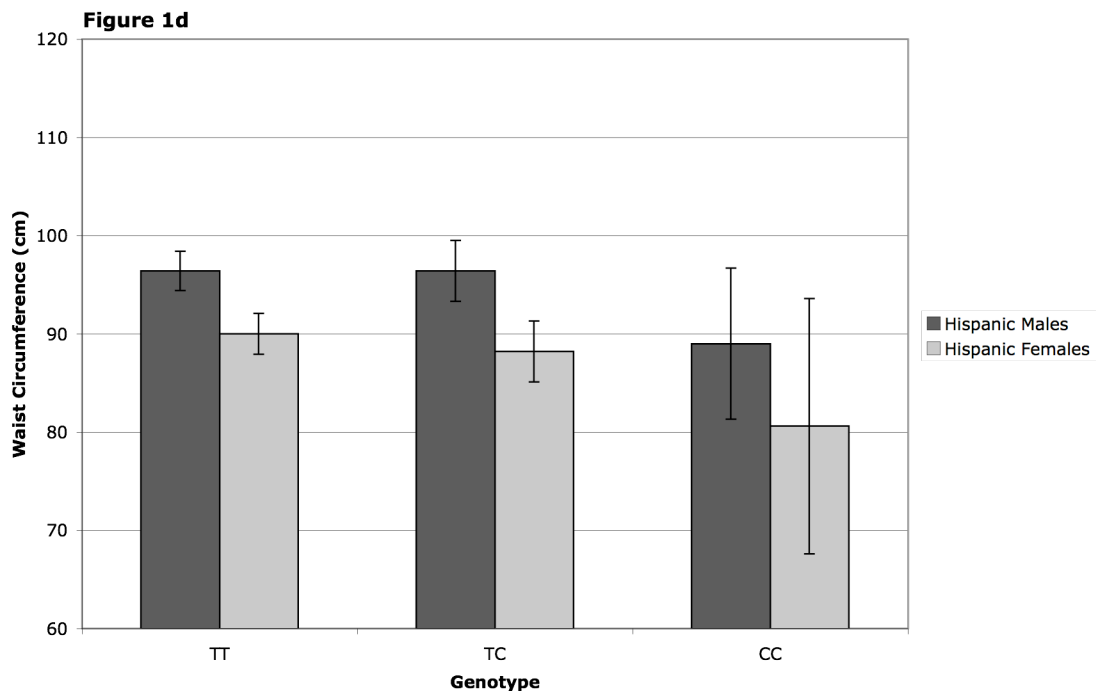
between the rs8050136 *FTO* allele and BMI ($p = 0.0018$). Homozygous carriers of the high-risk A allele had an average BMI of 31.1 kg/m^2 (95% CI: 29.0-33.1) compared to an average BMI of 27.1 kg/m^2 (95% CI: 26.4-27.8) in homozygous carriers of the C allele (Figure 1a). This association was significant in Hispanic males ($p = 0.0007$), but not females ($p = 0.14$). Hispanic males who were homozygous carriers of the high-risk A allele had average BMI of 31.4 kg/m^2 (95% CI: 28.5-34.3) compared to an average BMI of 25.9 kg/m^2 (95% CI: 25.0-26.9) in homozygous carriers of the C allele. A similar trend was seen for the association between genetic variants in *FTO* and WC (Figure 1b). There was a significant association between the rs8050136 SNP in *FTO* and WC in Hispanic men ($p = 0.0048$), but not Hispanic women ($p = 0.24$). Hispanic males who were homozygous for the high-risk A allele had a WC of 104.2 cm (95% CI: 97.7-110.7) compared to 94.1 cm (95% CI: 92.0-96.2) in those homozygous for the C allele. Among Hispanic females, homozygous carriers of the high-risk A allele had an average WC of 94.6 cm (95% CI: 88.2-101.1) and homozygous carriers of the C allele had average WC of 89.0 cm (95% CI: 86.7-91.3).

The associations between the rs17782313 SNP near *MC4R* and BMI and WC in Hispanics are presented in Figures 1c and 1d, respectively. The association between the rs17782313 SNP and BMI was not statistically significant among all Hispanics ($p = 0.092$), Hispanic males ($p = 0.38$), or Hispanic females ($p = 0.21$). Among all Hispanics, participants who were homozygous for the *MC4R* high-risk C allele tended to have a lower BMI than those who were homozygous for the T allele. Homozygous carriers of the high-risk C allele had an average BMI of 24.4 kg/m^2 (95% CI: 21.2-27.6) compared to an average BMI of 27.9 kg/m^2 (95% CI: 27.2-28.5) in homozygous carriers of the T

allele. Evaluating these results by gender did not affect the overall trend. A similar, non-significant trend was seen for WC ($p = 0.18$ in males; $p = 0.27$ in females). Hispanic males who were homozygous for the high-risk C allele had a WC of 88.9 cm (95% CI: 81.3-96.6) compared to 96.4 cm (95% CI: 94.4-98.3) in those homozygous for the T allele. Among Hispanic women, those homozygous for the C allele had a WC of 80.6 cm (95% CI: 67.6-93.6) compared to 90.0 cm (95% CI: 87.9-92.0) in those homozygous for the T allele.





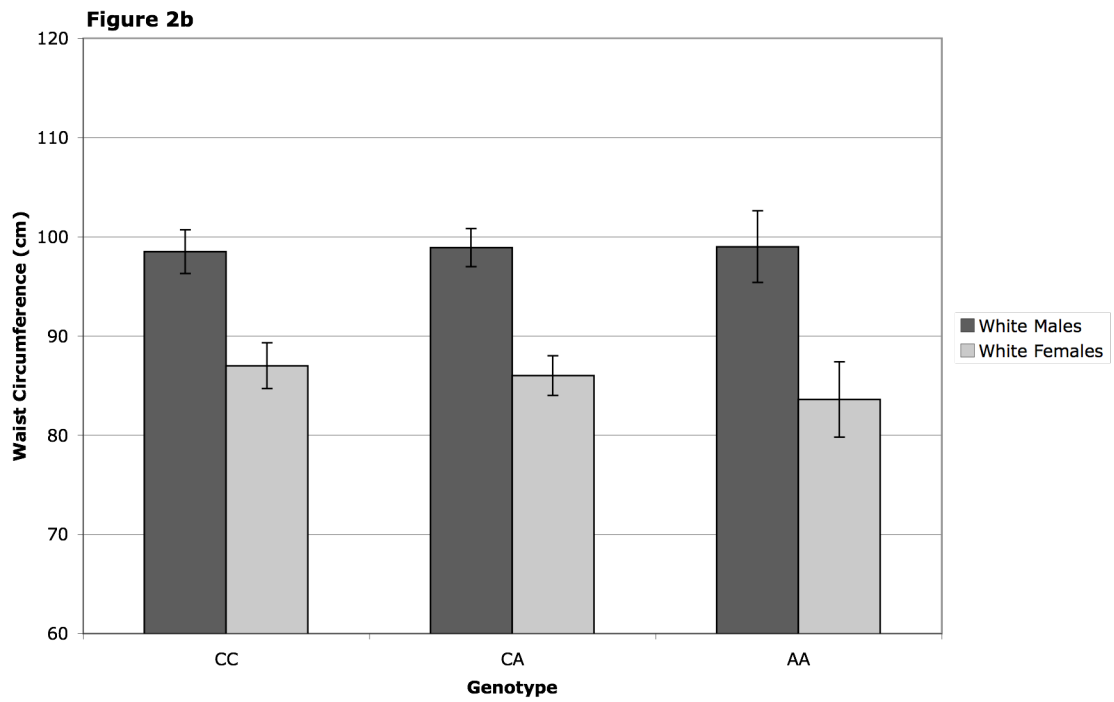
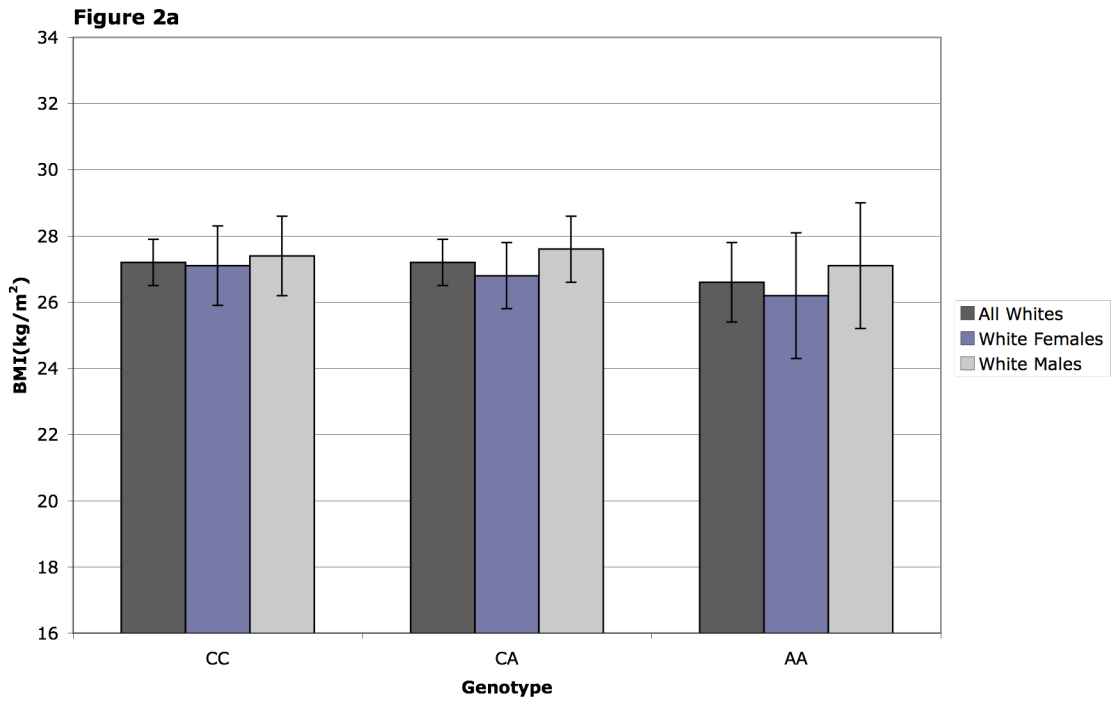


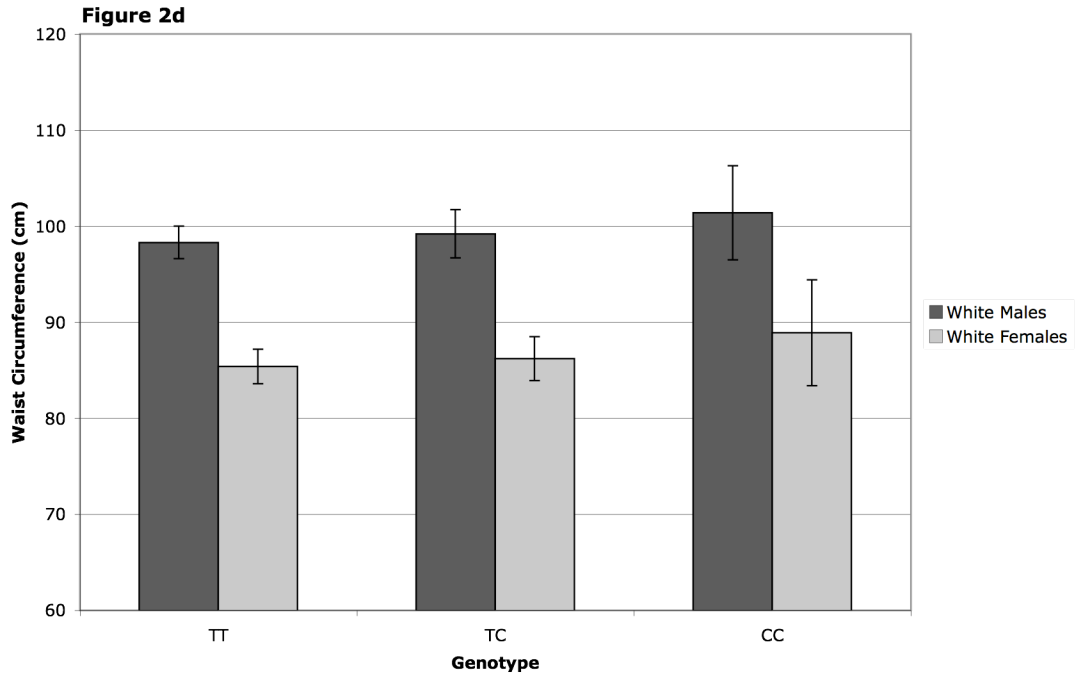
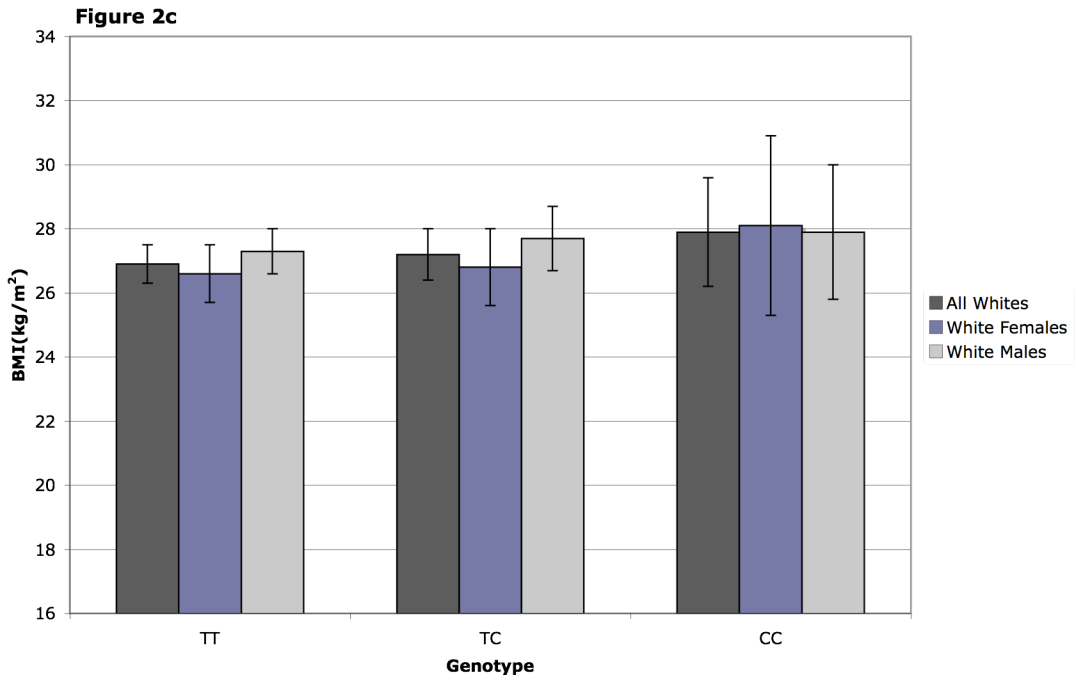
Figures 1a – 1d: Associations between 1a) *FTO* and BMI in Hispanics. 1b) *FTO* and Waist Circumference in Hispanics. 1c) *MC4R* and BMI in Hispanics. 1d) *MC4R* and Waist Circumference in Hispanics. For Figures 1a and 1b, A is the risk allele. For Figures 1c and 1d, C is the risk allele. Error bars represent 95% confidence intervals.

* Denotes significant difference between genotypes within gender, $p < 0.05$.

Associations Between *FTO* and *MC4R* Variants and BMI and WC in Non-Hispanic Whites

The associations between genetic variants in *FTO* and *MC4R* and BMI and WC in non-Hispanic whites are presented in Figures 2a – 2d, and all associations were adjusted for age at the time of the clinical visit. No associations between genetic variants in *FTO* and BMI or WC were found among non-Hispanic whites (BMI: $p = 0.71$; WC in men: $p = 0.95$; WC in women: $p = 0.32$). Similarly, no associations between genetic variants in *MC4R* and BMI or WC were found (BMI: $p = 0.54$; WC in men: $p = 0.45$, WC in women: $p = 0.48$).





Figures 2a – 2d: Associations between 2a) *FTO* and BMI in non-Hispanic whites. 2b) *FTO* and Waist Circumference in non-Hispanic whites. 2c) *MC4R* and BMI in non-Hispanic whites. 2d) *MC4R* and Waist Circumference in non-Hispanic whites. For Figures 2a and 2b, A is the risk allele. For Figures 2c and 2d, C is the risk allele. Error bars represent 95% confidence intervals.

Effect Modification by Dietary Fat Intake for BMI and WC

It has been shown previously that increased dietary fat intake accentuates the association between *FTO* variants and BMI (Sonestedt *et al.* 2009). Gene x dietary fat interactions was tested for by comparing the BMI of individuals with a high dietary fat intake by genotype.. A high fat intake was defined as a daily intake greater than or equal to the median intake, and a low fat intake was defined as a daily intake of less than the median intake. The median dietary fat intake was 60.6 g/day and 72.0 g/day for Hispanics and non-Hispanic whites, respectively. Similar to Sonestedt *et al.* (2009), we found that there was only a significant difference in BMI between Hispanic homozygous carriers of the high-risk A allele and the low-risk C allele when a high-fat diet was consumed. When adjusted for clinical age and gender, Hispanics who were homozygous for the high-risk A allele who reported eating a high-fat diet had an average BMI of 33.4 kg/m² (95% CI: 29.8-37.0) compared to an average BMI of 27.0 kg/m² (95% CI: 25.9-28.1) in homozygous carriers of the C allele who reported consuming a high-fat diet. Alternatively, the difference in BMI between genotypes was not significantly different among individuals who reported consuming a low-fat diet. Homozygous carriers of the high-risk A allele who reported consuming a low-fat diet had an average BMI of 29.8 kg/m² (95% CI: 27.3-32.3), and homozygous carriers of the C allele who reported consuming a low-fat diet had an average BMI of 27.3 kg/m² (95% CI: 26.3-28.3). There was no evidence of an *FTO* gene x dietary fat interaction in non-Hispanic white individuals. Also, there was no evidence of an *MC4R* gene x dietary fat interaction in Hispanic or non-Hispanic white individuals.

Associations Between *FTO* and *MC4R* Variants and Total Energy Intake in Hispanics

The associations between *FTO* and *MC4R* variants and total energy intake are presented in Table 3 and all associations were adjusted for age at the time of the clinical visit and gender. Among all Hispanics, reported daily energy intake did not differ significantly between homozygous carriers of the *FTO* high-risk A allele (1898 kcals, 95% CI: 1542-2254) and homozygous carriers of the C allele (1781 kcals, 95% CI: 1658-1905). Similar to the results for BMI, homozygous carriers of the *MC4R* high-risk C allele tended to report a lower daily energy intake (1350 kcals, 95% CI: 790-1910) than homozygous carriers of the T allele (1825 kcals, 95% CI: 1713-1936), though this difference was not statistically significant.

Associations Between *FTO* and *MC4R* Variants on Total Energy Intake in Non-Hispanic Whites

Among all non-Hispanic whites, reported daily energy intake did not differ significantly between homozygous carriers of the *FTO* high-risk A allele (2033 kcals, 95% CI: 1808-2258) and homozygous carriers of the C allele (2149 kcals, 95% CI: 2008-2290). A similar, non-significant trend was seen for the rs17782313 SNP near *MC4R*. Homozygous carriers of the *MC4R* high-risk C allele reported a daily energy intake of 2377 kcals (95% CI: 2059-2694), compared to 2003 kcals (95% CI: 1894-2113) reported by homozygous carriers of the T allele.

Table 3. <i>FTO</i> and <i>MC4R</i> and Total Energy Intake	Hispanics		Non-Hispanic Whites	
	kcal/day (95% CI)		kcal/day (95% CI)	
FTO (rs8050136)	CC 1782 (1658-1905)	*AA 1898 (1542-2254)	CC 2149 (2008-2290)	*AA 2032 (1808-2258)
MC4R (rs17782313)	TT 1825 (1713-1936)	*CC 1350 (790-1910)	TT 2003 (1894-2113)	*CC 2377 (2059-2694)

* Denotes homozygous for risk allele.

Associations Between *FTO* and *MC4R* Variants on T2D Prevalence

Odds ratios for diabetes prevalence were calculated for heterozygote and homozygous carriers of the risk allele (A in *FTO* rs8050136 and C in *MC4R* rs17782313) relative to homozygous carriers of the low-risk/wild-type allele (C in *FTO* rs8050136 and T in *MC4R* rs17782313) and are presented in Table 4. There was no significant association found between genetic variants in *FTO* and T2D prevalence in Hispanics (OR: 0.93; 95% CI: 0.52-1.68) or non-Hispanic whites (OR: 1.11; 95% CI: 0.57-2.15). Similarly, there was no significant association found between genetic variants near *MC4R* on T2D prevalence in Hispanics (OR: 1.11; 95% CI: 0.59-2.06) or non-Hispanic whites (OR: 1.43; 95% CI: 0.76-2.71).

Table 4. <i>FTO</i> and <i>MC4R</i> and T2D Status	Hispanics	Non-Hispanic Whites
	OR (95% CI)	OR (95% CI)
<i>FTO</i> (rs8050136)	0.93 (0.52-1.68)	1.11 (0.57-2.15)
<i>MC4R</i> (rs17782313)	1.11 (0.59-2.06)	1.43 (0.76-2.71)

Combined Effects of *FTO* and *MC4R* on BMI, WC, and T2D Prevalence

The low prevalence of carriers of the risk alleles in *FTO* and near *MC4R* and the relatively small sample size of the present study made it impossible to accurately determine the combined effects of the genetic variants on BMI, WC, or T2D prevalence.

CHAPTER V

DISCUSSION

Genotype Distribution

Similar to other studies (Loos *et al.* 2008; Liu *et al.* 2010; Song *et al.* 2008), we found that genotype distributions in *FTO* (rs8050136) and near *MC4R* (rs17782313) vary between ethnicities. Our analysis demonstrates that Hispanics are less likely to be carriers of risk alleles at SNPs rs8050136 and rs17782313 than their non-Hispanic white counterparts. To the best of our knowledge, the MAF and genotype distribution of SNP rs8050136 has not been previously reported in Hispanic males and has only been reported in Hispanic females in one study by Song *et al.* (2008). Our reported MAF of 25.9% in Hispanic females is slightly lower the 28.1% reported by Song *et al.* (2008). Similarly, we found that only 7.7% of Hispanic females were homozygous for the high-risk A allele, while Song *et al.* (2008) reported that 9.6% were homozygous. Song *et al.* (2008) utilized data from the Women's Health Initiative-Observational Study, which only included postmenopausal women. The female Hispanic participants in our study were fairly similar to those in Song *et al.* (2008) in regards to age (61.6 y and 60.2 y, respectively), BMI (28.0 kg/m² and 29.0 kg/m², respectively), and WC (89.1 cm and 87.3 cm, respectively). To the best of our knowledge, the MAF and genotype distribution of SNP rs17782313 near *MC4R* had not been previously reported in Hispanics. Our analysis shows a MAF of only 16.9% in Hispanics, compared to 23.4% in non-Hispanic

whites. Similarly, only 4.4% of Hispanic males and 1.8% of Hispanic females were found to be homozygous for the high-risk C allele, compared to 7.6% and 6.4% in non-Hispanic white males and females, respectively. The lower prevalence of risk alleles in Hispanics relative to non-Hispanic whites needs to be considered in the design of future experiments to be certain that adequate power is achieved.

Associations Between *FTO* and *MC4R* Variants on BMI and WC

The rs8050136 SNP in *FTO* was found to be significantly associated with BMI in all Hispanics ($p = 0.0018$) and Hispanic males ($p = 0.0007$) as well as WC in Hispanic males ($p = 0.0048$). This association was not statistically significant Hispanic females for BMI ($p = 0.14$) or WC ($p = 0.24$). These results suggest that Hispanic women may be affected differently than Hispanic males by genetic variants in *FTO*. Rankinen *et al.* reported a significant gene-by-sex interaction for percent body fat ($p = 0.0053$) and fat mass ($p = 0.013$) in a study of 276 African American and 503 white subjects. They found that male homozygous carriers of the high-risk A allele had a larger total fat mass and higher percent body fat than homozygous carriers of the C allele, but that fat mass and percent body fat did not differ by genotype in females (Rankinen, Rice, Teran-Garcia, Rao, & Bouchard 2010). However, Frayling *et al.* (2007) reported that the gene-by-sex interaction was not significant ($p = 0.13$) when they pooled the data from 13 cohorts with 38,759 European participants, and Ahmad *et al.* reported that among their cohort of 21,674 apparently healthy white women that homozygous carriers of the *FTO* high-risk A allele had a higher BMI than homozygous carriers of the C-allele (25.6 kg/m^2 vs. 24.6 kg/m^2 ; $p < 0.0001$) (Ahmad, Chasman, Mora, Paré, Cook, Buring, *et al.* 2010). Larger

sample sizes may be necessary to see the effects of variants in *FTO* on BMI in women, and researchers should consider a gene-by-sex interaction when looking at these variants.

Sonestedt *et al.* (2009) reported that the association between *FTO* variants and BMI was accentuated by a high dietary fat intake. Our results in Hispanics are congruent with these results. We found that the difference in BMI between homozygous carriers of the high-risk A allele and homozygous carriers of the C allele was only statistically significant when a high-fat diet was consumed. In those who reported consuming a low-fat diet, homozygous carriers of the high-risk A allele tended to have a higher average BMI than those who were homozygous for the C allele (29.8 kg/m² vs. 27.3 kg/m²), but this association was no longer statistically significant.

In regards to the rs17782313 allele near *MC4R*, no significant associations were found with BMI or WC in Hispanics or non-Hispanic whites. In fact, Hispanics who were homozygous carriers of the high-risk C allele tended to have a lower BMI (24.1 kg/m² vs. 27.9 kg/m²) and smaller WC (males: 89.0 cm vs. 96.4 cm; females: 80.2 cm vs. 89.9) than homozygous carriers of the low-risk T allele. While it is tempting to speculate on the potentially protective effects of this variation in Hispanics, it is important to remember that only 4.4% (6 out of 136) of Hispanic males and 1.8% (3 out of 171) of Hispanic females were found to be homozygous for the high-risk C allele, and also that variations near *MC4R* have been found to explain only 0.14% of the variation in BMI in Europeans (Loos *et al.* 2008). Due to the lack of available data on the rs17782313 allele in Hispanics, this relationship should be explored further with a larger sample size to confirm or disprove this seemingly paradoxical trend.

Associations Between *FTO* and *MC4R* Variants on Total Energy Intake

No significant associations between genetic variants in *FTO* or near *MC4R* were found in the present study. Our results regarding the *FTO* variant are congruent with those by Hubáček *et al.* (2010). The study conducted by Hubáček *et al.* included subjects from the Czech part of the Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE) project. Their study sample was much larger than ours ($N = 6024$ vs. 837), but fairly similar in regards to age (58.1 years vs. 63.0 years) and BMI (28.3 kg/m^2 vs. 27.2 kg/m^2). Alternatively, Speakmann *et al.* (2008) found that polymorphisms of the *FTO* gene were significantly associated with an increase in total daily energy intake. Relative to our study sample, their study sample was smaller ($N = 150$ vs. 837) and younger (43.7 years vs. 63.0 years), but similar in BMI (26.5 kg/m^2 vs. 27.2 kg/m^2). We similarly found no evidence of a significant association between SNP rs17782313 near *MC4R* and total energy intake in Hispanics or non-Hispanic whites. Congruent with these results, Hasselbalch *et al.* reported that variants near *MC4R* had no influence on energy intake among their sample of 756 Danish twins (Hasselbalch, Ängquist, Chirstiansen, Heitmann, Kyvik & Sørensen 2010). Other studies have demonstrated that variants near *MC4R* do influence energy intake. Significant associations between genetic variants near *MC4R* have been demonstrated with higher intakes of total energy and fat, (Qi *et al.* 2008), snacking behavior, hunger, eating large meals (Stutzmann *et al.* 2009), satiety responsiveness, and enjoyment of food (Valladares *et al.* 2010). Much like the results we found regarding the *MC4R* high-risk C allele and BMI, Hispanic homozygous carriers of the C allele tended to eat less than those homozygous for the T allele. As mentioned

previously, the association between genetic variants near *MC4R* and BMI and energy intake should be explored in a larger study sample.

Associations Between *FTO* and *MC4R* Variants on T2D Prevalence

The associations between genetic variations in *FTO* on T2D prevalence have previously been found to be mediated by their effects on BMI. Prior to adjustment for BMI, Frayling *et al.* (2007) found that the rs9939609 SNP in *FTO* was associated with an increased odds of having T2D (OR: 1.27, 95% CI: 1.16-1.37, $p = 5 \times 10^{-8}$). However, further adjustment for BMI completely abolished the effect (OR: 1.03, 95% CI: 0.96-1.10, $p = 0.44$). Given the lack of consistent associations of genetic variants in *FTO* on obesity found in the present study, it is not surprising that we were unable to detect any significant associations with this genetic variant and T2D prevalence rates.

The associations between variants near *MC4R* on T2D prevalence are less clear. Qi *et al.* (2008) reported that each rs17782313 high-risk C allele was associated with a 14% increased risk of T2D after adjustment for BMI and other covariates in a cohort of 5724 women of European ancestry participating in the Nurses' Health Study. Alternatively, no association between genetic variants near *MC4R* and T2D were found in a cohort of 7705 Chinese (Ng, Tam, So, Ho, Chan, Lee *et al.* 2010) or a meta-analysis of over 18,000 Japanese individuals (Takeuchi, Yamamoto, Katsuya, Tabika, Sugiyama, Fujioka, *et al.* 2011). The results of these studies suggest that the effect of genetic variations near *MC4R* may vary by ethnicity. Though none of the associations in the present study were significant, a similar trend was found. There was no trend for increased odds of being diabetic among Hispanic carriers of the high-risk C allele relative

to homozygous carriers of the low-risk T allele (OR: 1.11, 95% CI: 0.59-2.06). However, there was a tendency for non-Hispanic white carriers of the high-risk C allele to have an increased odds of being diabetic relative to homozygous carriers of the low-risk T allele (OR: 1.43, 95% CI: 0.76-2.71).

Combined Effects of Variants in *FTO* and near *MC4R*

Additive effects of variants both in *FTO* and near *MC4R* on BMI have been reported in European (Cauchi *et al.* 2009; Loos *et al.* 2008) and Chinese Han individuals (Huang *et al.* 2010). Based on these results, we hypothesized that there would be a linear effect of carrying risk alleles at both *FTO* rs8050136 and *MC4R* rs17782313 on BMI, WC, and T2D prevalence. The combination of a low prevalence of homozygous carriers of high-risk alleles, especially among Hispanics, and the relatively small sample size of the current study resulted in a very small number of carriers of 3 or 4 risk alleles. In fact, our study sample included no homozygous carriers of both risk alleles in any Hispanic nor non-Hispanic white men.

Strengths and Limitations

The measurement of fasting blood glucose, height, weight, and WC during a clinical visit represents a major strength of the present study and decreases the likelihood of misclassification bias. Illumina, Inc. ran genotyping tests for gender and compared those to the reported gender from the clinical visit to control for potential misclassification bias. The alleles at rs17782313 and rs8050136 were both found to be in Hardy-Weinberg equilibrium, suggesting that the population was randomly mating and that the genotyping

by Illumina, Inc. was successful. The associations between the genetic variants and BMI, WC, and total energy intake were adjusted for potential confounders including age and gender. Reliance on 24-hr dietary recall for total energy intake estimates is a common limitation to observational studies. Karvetti and Knuts (1985) established that product-moment correlation coefficient between observed and recalled nutrient intake was in the range of 0.58 to 0.74. They concluded that this relationship was unsatisfactory at the individual level, but satisfactory on a population-based level. Differences in admixture among different groups of Hispanics could be a source of confounding by population stratification. The admixture of Hispanics living in the San Luis Valley has been estimated as $62.7 \pm 2.1\%$ European, $34.1 \pm 1.9\%$ Native American, and $3.2 \pm 1.5\%$ West African (Bonilla, Parra, Pfaff, Dios, Marshall, Hamman, *et al.* 2004). It has been shown that T2D risk varies with the proportion of Native American ancestry in San Luis Valley Hispanic individuals (Parra, Hoggart, Bonilla, Dios, Norris, Marshall, *et al.* 2004). Also, we report here that non-Hispanic white individuals are more likely to be carriers of the risk allele at both rs8050136 and rs17782313 than Hispanics. It is possible that differences in the proportion of Native American and/or European ancestry could be confounding the associations in this study. However, Bonilla *et al.* (2004) reported that there was no significant difference in admixture proportions between diabetics and controls in the SLVDS cohort. The lack of movement into or out of the area could represent a limitation in the present study whereby results from the SLVDS may not be generalizable to the overall U.S. Hispanic population (Hamman *et al.* 1989). Hanis *et al.* (1991) had previously reported the admixture in Mexican Americans to be 61% European, 31% Native American, and 8% African. The admixture in Hispanic

Americans from Puerto Rico and Cuba was quite different. Puerto Ricans were found to be 45% European, 18% Native American, and 37% African, and parallel estimates for Cubans were 62%, 18%, and 20%. Based on the available data, results from the SLVDS would be highly generalizable to the Mexican American population, but less so in regards to Hispanics of Puerto Rican or Cuban ancestry.

Concluding Remarks

The present study presents, for what we believe to be the first time, the MAF and genotype distributions for the rs17782313 common genetic variant near *MC4R* in Hispanics. The lower prevalence of risk alleles in *FTO* rs8050136 and near *MC4R* rs17782313 in Hispanics relative to non-Hispanic whites should be considered when designing future experiments to be sure that adequate power is achieved. We found that Hispanic male, but not female, homozygous carriers of the high-risk A allele at rs8050136 had a higher BMI and WC than homozygous carriers of the low-risk C allele. Further analysis demonstrated that the association between the *FTO* variant and BMI was modified by dietary fat intake such that there was only a statistically significant difference in BMI between genotypes when a high-fat diet was reported. No significant relationships between genetic variations in *FTO* and BMI or WC in non-Hispanic whites or near *MC4R* and BMI or WC in Hispanics or non-Hispanic whites were found.

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