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Association of genetic polymorphisms in *FTO* with Overweight/Obesity and Type 2 Diabetes in the Lebanese Population

By
Veronica Maroun

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Student Name: Veronica Maroun I.D. #: 201402256

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The undersigned certify that they have examined the final electronic copy of this thesis and approved it in Partial Fulfillment of the requirements for the degree of:

Masters of Science in the major of Nutrition

Thesis Advisor's Name: Nadine Zeeni

Signature: [redacted] Date: 3 / 8 / 2021
Day Month Year

Committee Member's Name: Maya Bassil

Signature: [redacted] Date: 3 / 8 / 2021
Day Month Year

Committee Member's Name: Michella Ghassibe Sabbagh

Signature: [redacted] Date: 3 / 8 / 2021
Day Month Year

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Dedication Page

To my loving family and beloved ones

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Association of genetic polymorphisms in *FTO* with Overweight/Obesity and Type 2 Diabetes in the Lebanese Population

Veronica Maroun

ABSTRACT

Background and aims: Overweight and obesity are a worldwide threat to health and quality of life as they increase the risk of many chronic diseases including type 2 diabetes mellitus (T2DM). Several studies have reported associations between the fat-mass and obesity-associated gene (*FTO*) variants, rs1421085 and rs17818499, and overweight/obesity as well as T2DM. However, this association has not yet been studied among the Lebanese population. The present study therefore aimed to investigate the association between the aforementioned *FTO* gene polymorphisms, overweight/obesity and the risk of T2DM in Lebanese adults.

Methods: Secondary cross-sectional retrospective data analysis was conducted, involving 723 Lebanese participants aged ≥ 18 years old. Genotypic data for the *FTO* rs1421085 and rs17817449 variants as clinical and demographic information were available. Binary logistic regressions were used to examine the relationship between *FTO* variants and body mass index (BMI) as well as T2DM.

Results: Rs17817499 showed a significant association with BMI (p=0.012), while rs1421085 did not (p=0.375). Results from the logistic regression analyses showed that the rs17817499-GG, rs17817499-GT and rs1421085-CT genotypes were significantly associated with overweight/obesity after adjusting for age, gender and T2DM (p=0.01, 0.0001, and 0.003 respectively), with the highest risk observed among *FTO* rs17817499-GG carriers. No significant association was observed between rs17817499 and rs1421085 and T2DM.

Conclusion: The rs17817499 variant of the *FTO* gene was highly associated with overweight and obesity in the Lebanese population. On the other hand, the studied *FTO* variants were not significantly associated with T2DM. The present results,

reported for the first time in Lebanon, provide insights about risk factors and chronic disease prevention for Middle Eastern populations.

Keywords: *FTO*, Rs1421085, Rs1781499, BMI, T2DM, Lebanon

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LIST OF ABBREVIATIONS

SNP: single nucleotide polymorphism

***FTO*:** fat-mass and obesity-associated gene

T2DM: Type 2 diabetes mellitus

BMI: Body mass index

GWAS: Genome-wide association study

PA: Physical activity

OHA: Oral hypoglycemic agents

CI: Confidence interval

OR: Odds ratio

SD: Standard deviation

Chapter One

Literature Review

1.1 Overweight and Obesity

1.1.1 Definition

Overweight and obesity have become a significant threat to health and quality of life as they increase the risk of many chronic diseases including type II diabetes (T2DM), cardiovascular diseases, hypertension, and cancer. Overweight and obesity are defined as excess fat accumulation that present risk to health. In 2013, the American Medical Association (AMA) has recognized obesity as a disease due to its serious implications on health (Cheng et al., 2018). The Body Mass Index (BMI) is the surrogate measure that classifies a person with a BMI (weight/ height^2) ≥ 25 kg/m2 as overweight, and a person with a BMI ≥ 30 kg/m2 as obese (Price, Li, & Zhao, 2008).

1.1.2 Global Prevalence and Burden

Over the past decades, the prevalence of overweight and obesity has increased rapidly. According to the World Health Organization (WHO) in 2016, more than 1.9 billion adults (aged 18 years and older) were overweight and more than 650 million were obese. Obesity constitutes a great global public health issue, and it imposes a large economic burden. Indeed, in 2014 the global economic effect of obesity was worth around $2.0 trillion, which is equivalent to 2.8% of the global gross domestic product (GDP) (Tremmel et al., 2017). Low-income and middle-income countries (LMIC) carry most of the obesity and chronic diseases (Malik, Willett, & Hu, 2013).

1.1.3 Obesity in the Middle East Region

The Eastern Mediterranean region is no exception to the progression of overweight and obesity. In fact, obesity prevalence in the Middle East region exceeds at times that reported in Western countries such as the United States and Europe (Nasreddine et al., 2012). According to the WHO, the Middle Eastern countries with the highest levels of overweight and obesity are Egypt, Bahrain, Jordan, Kuwait, Saudi Arabia, and United Arab Emirates. Worldwide, men are more likely to be obese. However, in the Eastern Mediterranean region, higher prevalence of obesity was shown in adult women and overweight in adult men (Mallat et al., 2016).

Regarding Lebanon, WHO data shows that the prevalence of overweight and obesity in adults in 2016 was 67.9% and 32% respectively (WHO, 2016).

1.1.4 Associated Risk Factors

There are several modifiable as well as non-modifiable risk factors associated with overweight and obesity. Modifiable risk factors include an unhealthy lifestyle which is characterized by the absence of physical activity or sedentariness, unhealthy eating habits (e.g., poor-quality diet high in calories from saturated fat, trans fat, and sugars…), along with lack of sleep and increased levels of stress. Other modifiable risk factors include environment, low socioeconomic-status and education level (Hruby et al., 2016). As for non-modifiable factors, race, ethnicity, sex, and family history play a major role in increasing the risk of overweight and obesity. Recent studies have investigated the role of genetics and the interactions between lifestyle factors and genetics in the etiology of obesity (Hruby et al., 2016; Mallat et al., 2016). Another relatively newly discovered risk

factor is the gut microbiota that plays a role in the host metabolism thus affecting body weight (Tseng & Wu, 2019). Hence, there are various modifiable and non-modifiable risk factors for obesity, but genetics is one of the risk factors that is taking a lot of consideration and its interaction with lifestyle factors and obesity is highly investigated.

1.2 Type 2 Diabetes

1.2.1 Definition

Diabetes is a serious chronic metabolic disease characterized by elevated blood glucose levels (hyperglycemia) and is mediated by a complex interaction of behavioral, genetic, and socioeconomic factors. Several of these risk factors are beyond one's control. Diabetes has three main types which are type I diabetes (T1DM), T2DM, and gestational diabetes mellitus (GDM) (Saeedi et al., 2019). According to the WHO, diabetes is diagnosed mainly at glycated hemoglobin (HBA1C) of 48 mmol/mol (6.5%) and/or at fasting blood sugar (FBS) of greater or equal to 126 mg/dl while prediabetes is diagnosed at HbA1C between 5.7% and 6.4% (Wang et al., 2016). While T2DM is the most common form of diabetes, it accounts for approximately 90% of all diabetes cases worldwide (Yang et al., 2017). People with T2DM are susceptible to higher risk of developing serious life-threatening health problems, which leads to increased health care costs, reduced quality of life and higher mortality (Cho et al., 2018).

1.2.2 Global Prevalence and Burden

The prevalence of diabetes is increasing globally. In 2019, approximately 463 million adults aged between 20-79 years old were living with diabetes and this number is expected to rise by 2045 reaching 700 million adults with diabetes (Saeedi et al., 2019). This rising trend can be ascribed to several factors such as the rapid urbanization, aging,

obesogenic environment, and increase in sedentary lifestyle (Saeedi et al., 2019). According to International Diabetes Federation (IDF) Atlas 9th edition 2019, China, India and the United States of America are the countries with the highest prevalence of adults with diabetes and are expected to remain so until 2030 (IDF, 2019). Low- and middle- income (LMIC) carry the biggest burden of this disease. Almost 79% of the total adults with diabetes live in LMIC where 87% of all diabetes-related mortality are found in LMIC. The high prevalence of diabetes and diabetes-related mortality in LMIC, is be due to having the lowest rates of diabetes diagnosis (68.8% undiagnosed diabetes), limited healthcare access, and lack of awareness among the population.

In general, diabetes not only imposes health burdens, but also high economic price where in 2019, diabetes caused almost USD 760 billion dollars health expenditure which is equivalent to 10% of the total healthcare spending on adults (IDF, 2019).

1.2.3 Type 2 Diabetes in the Middle East Region

The Middle East and North Africa (MENA) region is not immune to this rise in diabetes prevalence. According to IDF, MENA region has the highest age-adjusted prevalence of diabetes in adults in 2019, 2030 and 2045 (12.2%, 13.3% and 13.9% respectively) (IDF, 2019). Environmental, genetic, and cultural factors contribute to the high risk of T2DM among Arab. However, the environmental risk factors that contribute to T2DM development did not justify the varied risk among Arab population which indicates that genetic risk factor has a great influence on the overall risk (Almawi et al., 2013). In Lebanon, the prevalence of diabetes was found to be 7.8% for the adult population from cross-sectional data from 2006 (Ahmadieh, Sawaya, & Azar, 2019). An epidemiologic analysis of T2DM risk in subgroups representative of the Lebanese population showed

alarming prevalence of T2DM (40.8% of surveyed population); moreover, it showed a strong positive correlation between T2DM and higher BMI, positive family history, and sedentary lifestyle (Ghassibe-Sabbagh et al., 2014). Lebanon is part of a Levantine area which has a greater European similarity compared to other areas in the MENA region. This fact should be considered when searching for and comparing potential genetic factors and mutations that might be risk factors for T2DM in the Lebanese population (Badro et al., 2013; Ghassibe-Sabbagh et al., 2014).

In conclusion, T2DM is major public health concern that is on the rise worldwide causing humane and economic burdens. However, much about its pathology and etiology is still unclear. Researchers acknowledge that there are several risk factors (i.e., environmental, genetic…) for T2DM among different areas in the world, yet more region and ethnic specific studies are needed.

1.3 Genetics behind Obesity

1.3.1 The Predisposition to Excess Weight Gain

The earliest hypothesis of innate biologic or endogenous cause of obesity was proposed by von Noorden back in 1907 (Thaker, 2017). This concept paved the way for more studies on the genetic causes of obesity. Heritability of human characteristics (i.e., the individual difference in characteristic explained by genetic variability) was usually assessed by twin studies. The classical study of the Swedish adopted and separated twins by Stunkard et al., (1990) showed that there was no significant correlation between the BMI of the twin to that of his/her foster parents. However, the twin's BMI was closer to his/her biological parent and his/her twin sibling (O'Rahilly & Farooqi, 2008; Stunkard, Harris, Pedersen, & McClearn, 1990). BMI examination of twins who were raised

together and separated showed that the genetic factor contributes to about 70% in predicting BMI. In other words, these studies highlight the role of the genetic factors over the shared familial environment in predicting BMI (Herrera & Lindgren, 2010). Although environmental factors, such as sedentariness and poor-quality diet, have increased the number of people who are overweight or obese, genetic factors are estimated to account for 40–90% of the population variation in BMI (Fawcett & Barroso, 2010). Nowadays, more advanced and modern genetic technology studies with precise definition of single nucleotide changes have increased the knowledge of the molecular mechanisms of weight regulation and even paved the way for more advanced therapeutic interventions (Fawcett & Barroso, 2010). Overall, numerous studies confirmed that genetic factors are important in the predisposition to excess weight, and recent research is trying to further elucidate the mechanisms behind these genetic factors and their interaction with the environment.

1.3.2 Genome Wide Associated Studies

The National Institute of Health (NIH)/ The National Human Genome Institute has launched the genome-wide association study (GWAS) which is an approach that involves a computerized database tool that scans markers across complete sets of people's DNA to detect genetic variations associated with exposure to common heritable diseases and traits. This tool uses a reference human genome sequence, a map of human genetic variations and a set of new technologies that can quickly and precisely analyze whole-genome samples for genetic variations and assess their contribution to the onset of a disease (Ducan & Brown, 2018; Evangelou & Ioannidis, 2013; Lee, Eskin, & Han, 2017). So far, the GWAS have identified more than 10,000 loci for common human diseases and traits (Duncan & Brown, 2018). The International HapMap Project among other genome-

wide databases of patterns of human genetic variations, guided genetic studies including GWAS that examine the association of genetics to diseases, traits, and shown phenotypes (Manolio & Collins, 2009).

Genetic variations could be an extra base pair inserted in the DNA (insertion), a missing section in the DNA (deletion), or a substitution known as single nucleotide polymorphism (SNP). SNPs represent the majority of the common human genetic variations (Dehghan, 2018).

Therefore, the GWAS has used genotyping technologies to identify these common disease-causing variants and to assay common SNPs and relate them to clinical conditions and measurable traits (Lee, Eskin, & Han, 2017). Various GWAS have revealed numerous genetic susceptibility loci and numerous SNPs related to the risk of obesity (Srivastava et al., 2016). In addition, in 2007, several independent GWAS identified an association between SNPs in intron 1 of *FTO* (i.e., fat mass and obesity associated gene which was first identified by the GWAS to be correlated with increasing odds of obesity and higher BMI) and human obesity in different populations worldwide (Europeans, East and South Asians Africans, Hispanics, and Native Americans) (Frayling et al., 2007; Loos & Yeo, 2014; Merkestein & Sellayah, 2015; Scuteri et al., 2007).

1.3.3 The *FTO* Gene

1.3.4 History of *FTO* Gene

The history of the *FTO* gene dates back to at least 450 million years ago as it was conserved across many vertebrae including fish and chicken (Fredriksson et al., 2008; Peng et al., 2011). Late in the 20th Century, the *FTO* gene was first identified in mice in a study later known as "fused toe (ft) mouse study" where the gene was shown to play a

role in programmed cell death (Peters, Ausmeier, & Rüther, 1999; Scuteri et al., 2007). In the study, they inserted a 1.6 mega-base deletion on chromosome 8, which is supposed to be responsible for the "fused toes" phenotype. Heterozygote mice developed fused toes of their forelimbs along with enlargement of the thymus (thymic hyperplasia). However, the *FTO* gene was only one of the deleted six genes, thus they could not tell the sole impact of *FTO* on the phenotype.

The *FTO* gene, originally named ''*Fatso*'' (*Fto*) because of its large size, is located on 16q12.2 and is composed of nine exons (F. van der Hoeven et al., 1994; Fawcett & Barroso, 2010; Tung, Yeo, O'Rahilly, & Coll, 2014). More studies were conducted on the *FTO* genes and with the advance in research, *FTO* changed its name and labeled acronyms. In the well-known study conducted by Fraying et al. in 2007, *FTO* was clearly correlated to metabolism and obesity, and it was labeled as the "fat mass and obesity associated gene" (Tung, Yeo, O'Rahilly, & Coll, 2014).

1.4 The *FTO* Single Nucleotide Polymorphisms

1.4.1 Effect of *FTO* SNPs on Obesity and Adipogenic Capacity

FTO SNPs have an indispensable role in obesity. SNPs are viewed as the most common form of genetic variation in humans (Thusberg, et al., 2011). As its name implies, SNPs are substitution in one nucleotide (i.e., basic structural unit of DNA). An individual's genome carries 4-5 millions SNPs. While many SNPs have no effect on individuals, some predict people's risk of developing certain diseases (Wray, Goddard, & Visscher, 2007). There are correlations between the SNPs known as linkage disequilibrium (LD), whereby an allele of one SNP can be inherited or associated with an allele of another SNP (Bell, 2002; Bush & Moore, 2012). Among these SNPs, around 445 SNPs and hundreds of

genes are related to obesity (Cheng et al., 2018). Nevertheless, the *FTO* gene is the earliest to be discovered and strongest obesity-related gene (Loos & Bouchard, 2008). While the BMI-increasing allele in the *FTO* gene is prevalent in 42% of the European population, it is only 12% prevalent in the African population (Deng et al., 2018; Surendran, Jayashri, Drysdale, 2019). Studies carried on different populations worldwide (Asians, Africans, Hispanics, and Native Americans) confirmed the association between *FTO* variants in intron 1 (i.e., rs9939609, rs17817449, rs3751812, rs1421085, rs9930506, and rs7202116) and obesity in both children and adults (Deng, Su, Stanford, & Chen, 2018) (figure1). The three primary SNPs are rs9939609, rs1421085, and rs17817449, which are in strong LD (Price, Li, & Zhao, 2008). These SNPs are also known as, rs9939609T>A, rs1421085T>C, rs9939609T>A, rs17817449 T>G to show the exact nucleotide substitution (Antonio et al., 2019).

The variation in the rs1421085 is a T>C nucleotide substitution. In a recent study, it was shown that individuals who carried the allele C had an increased risk for obesity and an increased percentage body fat (Antonio, Knafo, Kapoor, & Tartar, 2018). In a meta-analysis, *FTO* rs17817449 risk allele is shown to have a significant positive association with obesity (OR:1.54,95% CI:1.41-1.68) (Peng et al., 2011). Moreover, in a study conducted by Price et al., three SNPs rs1421085, rs17817449, and rs9939609 were assessed in 583 women with current BMI greater than 35 kg/m^2 and lifetime BMI more than 40 kg/m^2, and 544 controls who were normal weight and had never been overweight during their lifetimes. After extracting the DNA from lymphoblastoid cell lines and genotyping, Price *et al.,* estimated the power to compare cases and controls. The results

from the association analyses showed that the three SNPs were highly significantly associated with increased obesity risk (Price, Li, & Zhao, 2008).

We can conclude that some SNPs influence people's health by affecting the risk of some diseases such as diabetes. Moreover, *FTO* obesity related SNPs have a role in adipogenesis.

1.4.2 Effect of *FTO* rs1421085 and rs17817449 on Obesity and Type 2 Diabetes

Obesity and T2DM are global endemic that result from the interaction of environmental influences with genetic variants. Several early GWAS suggest the association of *FTO* SNPs with obesity and T2DM (Frayling et al., 2007); however, throughout the years, studies have shown inconsistent results depending on the race and geographic location. The effect of *FTO* rs1421085 and rs17817449 on obesity as well as T2DM has been studied across different regions of the world. To investigate whether there is an association between *FTO* SNPs, obesity, and T2DM risk and if this relation is region-associated, a meta-analysis of 62 case-control studies was conducted. It included above 60,000 T2DM cases and 90,000 non-cases. This meta-analysis studied the association between different *FTO* SNPs (including rs1421085 and rs17817499) and risk of T2DM in various areas (i.e., across Asia, Europe and Northern America). *FTO* SNPs rs9939609 and rs8050136 presented a susceptibility to T2DM even after adjusting for BMI. However, subgroup analysis showed different results across different regions (rs9939609 and rs8050136 showed comparable findings in East Asia but had no significant relation in North America). Meanwhile, no association was observed with rs1421085 or rs17817499 *FTO* variants irrespective of the adjustment for BMI. This suggests that more studies are needed to investigate the *FTO* SNPs association with obesity and T2DM in

different regions (Yang et al., 2017). This study was also in line with previous meta-analysis of Asian populations (Vasan et al., 2014; Yang et al., 2017).

In Europe, a case control study was conducted in France to analyze the contribution of 24 obesity-related SNPs (including *FTO* rs1421085) to T2DM risk, pancreatic beta-cells function, and diabetic indices in 2077 T2DM cases and 3085 controls aged 45 years and older. Rs1421085 *FTO* significantly increased T2DM risk (insulin resistance) which is characterized by increased homeostasis model assessment of insulin resistance (HOMA-IR), and/or decreased insulin sensitivity index (ISI), even after adjusting for sex and age. However, the association was not maintained after adjusting for BMI. This validates the fact that *FTO* increases the predisposition to T2DM primarily via its effect on adiposity (Robiou-du-Pont, et al., 2013). This French study is in line with previous studies conducted on European populations (Frayling et al., 2007; Robiou-du-Pont, et al., 2013). Similarly, a study genotyped *FTO* rs17817449 variant in participants of Czech origin, of which 814 patients were T1DM, 848 individuals T2DM and 2,339 healthy controls. The homozygous genotype (GG) in rs17817449 was confirmed to have a significant association with T2DM but not T1DM. The variant was also associated with increased risk of certain complications like diabetic neuropathy (Hubacek et al., 2018).

Different ethnicities and regions in the world showed different associations. A study conducted by Bressler et al., compared association of four different *FTO* SNPS (including rs17817449 and rs1421085) and risk of obesity and T2DM between two ethnic groups: 1,004 cases and 10,038 controls in the white ethic group, and 670 cases and 2,780 controls in the African-American population. In the white ethnic group, all *FTO* SNPs significantly increased the risk of obesity and T2DM. However, in African Americans,

the carriers of C allele in the *FTO* rs1421085 increased obesity risk, but surprisingly it showed a protective role against T2DM (OR=0.79, p=0.03). Results showed an association with T2DM even after adjusting for BMI; however, the adjustment attenuated the results in the Whites. These results contradict with the previous European theory. This statistical difference emphasizes that the interaction between *FTO* variants, obesity, and diabetes is context, race, and region dependent (Bressler et al., 2010).

In 612 Pakistani individuals, the *FTO* variant rs1421085 (CT genotype) was observed to increase the risk of overweight/obesity; however, rs1421085 was not associated with obesity related metabolic parameters including fasting blood glucose (FBG), fasting insulin, and HOMA-IR (Rana S., & Bhatti A., 2020).

A GWAS in Canada verified the relationship of the *FTO* SNPs rs17817449 and rs1421085 with adiposity measures (body weight, waist circumference, plasma leptin…) in 908 subjects who participated in the Quebec Family Study. These two obesity risk alleles were also found to increase the risk of diabetes via affecting fasting insulin and insulin sensitivity measures such as HOMA-IR. After adjusting for BMI, the latter association was eliminated, thus showing that the effect of these SNPs on insulin sensitivity is mediated via adiposity (Do, Bailey et al., 2008).

The MENA region also showed variable results. In Iraq, a case control study genotyped *FTO* gene variants rs9939609 and rs17817449 among 400 T2DM obese cases and 400 healthy obese participants to examine the association of these variants with diabetes in obese Iraqi population. Results showed that rs9939609 and rs17817449 variants increased the development of insulin resistance thus increased the risk of T2DM in obese patients. The heterozygous genotype (TG) in rs17817449 significantly increased the risk of T2DM

more than two folds even after adjustment for age, sex, and BMI (Younus, Algenabi, Abdul-Zhara, Hussein, 2017). In Tunisian subjects, *FTO* rs1421085T>C variant showed a significant association with metabolic syndrome (MetS) and impaired fasting glucose (IFG) (Elouej et al., 2016). In Egyptian female population, *FTO*rs1421085 and rs9939609 polymorphisms were genotyped in 105 obese patients and 100 healthy controls aged between 14-60 years. Both SNPs were associated with increased risk of obesity in T2DM individuals. *FTO* rs1421085 variant showed significantly higher frequency of the CC genotype in total obese cases compared with control adjusted by diabetes and age. However, there is no correlation between the genotypes and obesity-related parameters (Abdel Rahman et al., 2018). In Lebanon, a case control replication study genotyped 19 GWAS T2DM risk variants including *FTO* rs8050136 and rs17817449 among 995 T2DM cases and 1076 non-cases individuals. *FTO* rs8050136 and rs17817449 were initially correlated with higher risk of T2DM, but this association was lost upon multiple adjustments (i.e., age, gender, BMI). Almawi et al., suggest that the *FTO* rs8050136 and rs17817449 variants may impact T2DM risk, since these variants had effect sizes that were comparable to those found in Europeans (Almawi et al., 2013).

In conclusion, genetic variants in the *FTO* gene have consistently been reported to be associated with BMI and diabetes in Europe (France, Czech), Canada, and Lebanon but findings have been inconsistent with other ethnicities including some countries in the MENA region, Asia, North America, and African-American region. This emphasizes that the association between *FTO* SNPs rs1421085 rs17817449 and the risk of obesity as well as T2DM is ethnic and region specific. Moreover, the effect of the variants on T2DM was mostly mediated through its role on adiposity.

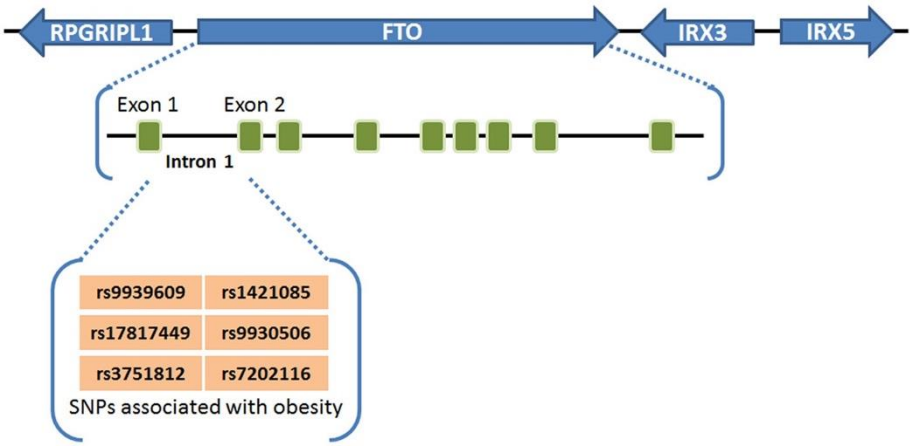

Figure 1 Association of *FTO* SNPs with Overweight/Obesity.

Association of *FTO* SNPs in intron 1 (i.e., "rs9939609, rs17817449, rs3751812, rs1421085, rs9930506, and rs7202116") with overweight/obesity (Deng, Su, Stanford, & Chen, 2018; Zhao, Yang, Sun, Zhao, & Yang, 2014).

Chapter Two

Relevance to the Field, Aim, and Study Hypothesis

2.1 Relevance to the Field

To date, there is no study conducted in Lebanon that evaluates the association of two of the three main *FTO* SNPs rs1421085 and rs17817449 with both overweight/obesity and risk of T2DM and whether the latter association is attenuated when adjusting for BMI, age, and sex. Moreover, it has been shown in the literature that the association of *FTO* SNPs and obesity and T2DM are race and region depending. Therefore, it is novel to study this association in the Lebanese population.

2.2 Research Question

The main question to be answered in this research is whether *FTO* SNPs rs1421085 and rs17817449 influence overweight/obesity and type II diabetes in Lebanese adults.

2.3 Objective

The objective of the present study is to investigate the association of the *FTO* rs1421085 and rs17817449 gene polymorphisms with obesity and obesity-related parameters in Lebanese adults. The aims are:

1. To describe the prevalence of *FTO* gene risk genotypes in a sample of Lebanese adults.
2. To investigate the association between *FTO* variants and overweight/obesity.
3. To examine the impact of these SNPs on the risk of T2DM, and whether this relationship is mediated by BMI.

Chapter Three

Methodology

3.1 Recruitment

This cross-sectional retrospective study is a secondary data analysis where the participants were initially recruited in three phases. The first phase of recruitment was conducted in collaboration with the Lebanese American University Medical Center. It occurred in the suburbs of the Lebanese capital Beirut and led to the recruitment of 506 subjects. The second phase occurred in North Lebanon and included 492 participants. Finally**,** 2,292 subjects were enrolled in the Functional Genomic Diagnostic Tools for Coronary Artery Disease project initiative (FGENTCARD) from two hospitals in Lebanon (i.e., Rafic Hariri University Hospital and Centre Hospitalier du Nord) (Ghassibe-Sabbagh et al., 2014).

3.2 Ethics

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the LAU institutional review board (IRB) and local ethics committees on human research (Reference number SMPZ08072010-4). All subjects signed an informed consent for participation prior to their inclusion in the study (Ghassibe-Sabbagh et al., 2014).

3.3 Research Procedure and Tools

At the end of the first two recruitment phases, participants completed a comprehensive questionnaire, gave blood for DNA testing as well as for HbA1C, FBS, and lipid profile. The remaining participants from the FGENTCARD project filled a

comprehensive questionnaire and provided blood sample for DNA and metabolites analysis. Finally, medical history, lab tests, and prescribed medications were coded from the medical charts (Ghassibe-Sabbagh et al., 2014).

3.4 Genotyping

Ghassibe-Sabbagh et al., have previously presented details of the procedure.
The extraction of DNA was done using a standard phenol chloroform extraction method. Samples were genotyped for almost 700,000 SNPs using Illumina HumanOmniExpress-12V1-1 multi-use while 550,000 SNPs were genotyped using Illumina Human610-660W Quad BeadChips. Plink was utilized for quality control and for the management of the data. Genotype information was transferred to NCBI genome build 37 via Lift Over. Chromosomes' phasing was done through SHAPEIT via applying the 1000 Genomes Phase I haplotypes found on the IMPUTE2 website according to sequence information for 1,092 TGP samples. Imputing genotypes blocks of ~5 Mb was done through IMPUTE2 and utilizing all individuals in the 1000 Genomes Phase I data. Accuracy of imputation was measured using IMPUTE2's information metric ''info'' (Ghassibe-Sabbagh et al., 2014).

3.5 Selection of Participants in this Study

In this cross-sectional retrospective study, 765 participants had available data for the *FTO* rs142108 and rs17817449 variants. After excluding non-Lebanese nationalities, a total of 723 Lebanese adults were included (i.e., n=709 Lebanese, n=3 Lebanese Armenian, n=3 Lebanese Greek, n=5 Lebanese Syrian, n=1 Lebanese Mexican, n=1 Lebanese Palestinian) =. The sample size is justified based on previous work done in the literature (Rana S., & Bhatti A., 2020; Bakhashab et al., 2020; Elouej et al., 2016;

Younus, Algenabi, Abdul-Zhara, Hussein, 2017). The subjects were older than 18 years old with a mean age 65.39 ± 11.272 SD. Body mass index (BMI) was analyzed with response to standard units. Female subjects represented 424 (58.6%) of the participants. BMI is classified as (weight/ height^2) < 18.5 kg/m2 as underweight, BMI between 18.5-24.5 kg/m2 as normal weight, 25-29.9 kg/m2 as overweight, and a person with a BMI ≥ 30 kg/m2 as obese (Price, Li, & Zhao, 2008). To evaluate the impact of the *FTO* variants on overweight/obesity, participants were classified in two categories (1) normal weight n=182 (29.2%) and (2) overweight obese n=442 (70.8%) excluding the underweight. We excluded missing data. T2DM was categorized into 3 categories (diabetic 254 (35.2%), prediabetic 104 (14.4%), and healthy 364 (50.4%)) according to questionnaire & HbA1C test & medication. The cut-off points for diagnosing T2DM was HbA1C of 48 mmol/mol or 6.5% while prediabetes was HbA1C between 5.7% and 6.4% (Wang et al., 2016). These cut-off points are in line with the ADA and WHO guidelines. As for the data obtained from the FGENTCARD project, diabetes was asserted by a medical doctor's diagnosis along with HbA1C test and/or two-hour post load plasma glucose levels after an oral glucose tolerance test from medical records.

Chapter Four

Statistics

4.1 Variables, Outcome, Exposures, and Confounders

While assessing the association of *FTO* variants on overweight/obesity, *FTO*-rs1421085 and rs17817449 SNPs are the independent variables while BMI is the dependent variable. This association was adjusted for confounders: age, gender, and T2DM.

While assessing the effect of *FTO* variants on T2DM, *FTO*-rs1421085 and rs17817449 SNPs are the independent variables while T2DM is the dependent variable. This association was adjusted for age, gender, and BMI confounders.

Several parameters were also included in the analysis such as the intake of medications: oral hypoglycemic agents (OHA) (i.e., metformin), which might affect body weight. Other exposures were physical activity level which is categorized into four groups (I do not exercise, less than once per week, once or twice per week, 3 or more times per week), family history of T2DM, and other comorbidities (i.e., hypertension).

4.2 Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 24.0 for Windows. Descriptive statistics for categorical data (ex: gender, exercise, BMI, T2DM) was described in terms of frequency and percentage N (%) while for continuous data (ex: age) mean ± standard deviation (SD), median, and interquartile. Univariate data was analyzed using Independent Sample T test for parametric continuous variable, and Chi-square (Monte Carlo) for categorical variables. The association of the *FTO* variants and BMI was assessed by binary logistic regression represented as odds ratio (OR) and

95% confidence intervals (CI) after adjusting for age, gender, and T2DM. Logistic regression was also used to measure odds ratio (OR) for T2DM (yes-no) adjusted for age, gender, and BMI. Statistical significance was considered as p-value ≤ 0.05

Chapter Five

Results

5.1 Population Characteristics

We assessed the association of baseline, metabolic characteristics, and *FTO* rs1421085 and *FTO* rs17817499 genotypes according to BMI. The characteristics of the population studied are given in Table 1. Analysis was based on subjects being divided into two binary categories representing normal weight individuals (BMI <25 kg/m2) and overweight-obese individuals (BMI ≥ 30 kg/m2). Our population sample included 624 normal, overweight, and obese participants of whom 367 were females and 277 were males. We did not observe any difference between males and females when comparing normal versus overweight-obese BMI (*p*- = 0.929). There was no significant association between age of individuals and BMI (*p-value* = 0.183). The proportion of overweight/obese or normal weight participants who do not exercise did not differ between those who exercise less than once a week, once or twice per week, or three or more times per week (*p-value* = 0.106). The *FTO* rs1421085 and *FTO* rs17817499 genotypes frequencies were presented in Table 1. We found that carriers of the *FTO* rs1421085 variant included n=442 (70.8%) overweight-obese subjects; however, we obtained no significant association with BMI (*p-value* = 0.375) while *FTO* rs17817499 variant was significantly associated with BMI (*p-value* = 0.012). Of the 461 hypertensive participants, 331(71.8%) were overweight/obese and 111 (68.5%) of the 162 non-hypertensive participants were overweight/obese. The relationship between BMI and hypertension was not significant (*p-value* = 0.429). Similarly, we observed no significant difference between T2DM, and

overweight-obese subjects compared to normal BMI subjects (*p-value* = 0.119). There was significant difference between the intake of OHA medications and the two BMI (*p-value* = 0.034) where intake of OHA medications was more common overweight and obese subjects (70.9%) compared to normal BMI (29.1%) (Table 1).

Likewise, we assessed the association of baseline, metabolic characteristics, and *FTO* rs1421085 and *FTO* rs17817499 genotypes in diabetic versus non-diabetic subjects in Table 2. There was no significant association between age, gender, physical activity, BMI, and T2DM (*p-value* = 0.670, *p-value* = 0.851, *p-value* = 0.985, *p-value* = 0.087, respectively). Also, we obtained no significant association between people with diabetes versus non-diabetic and *FTO* rs1421085 and *FTO* rs17817499 variants (*p-value* = 0.508, *p-value* = 0.580, respectively). However, the association of T2DM with hypertension and family history of T2DM was significant (*p-value* = 0.027, *p-value* = 0.0001, respectively).

Table 1 Association of baseline, metabolic characteristics, and *FTO* rs1421085 and *FTO* rs17817499 genotypes according to normal versus overweight/obese body mass index categories

	Normal	**Overweight Obese**	**Total**	**P-value**
Sex	182(29.2)	442(70.8)	624	0.929
Female n(%)	102(29.4)	245(70.6)	367	
Male n(%)	80(28.9)	197(71.1)	277	
Age N	182	441	623	0.183
Mean(SD)	65.05 (10.224)	63.78 (10.649)	64.14(10.534)	
Exercise	182(29.3)	440(70.7)	622	0.106
I do not exercise n(%)	98(26.3)	274(73.7)	372	
Less than once a week n(%)	9(28.1)	23(71.9)	32	
Once or twice per week n(%)	16(27.6)	42(72.4)	58	
Three or more times per week n(%)	59(36.9)	101(63.1)	160	
rs1421085	182(29.2)	442(70.8)	624	0.374
TT n(%)	49(31.6)	106(68.4)	155	
CT n(%)	98(30.0)	229(70.0)	327	
CC n(%)	35(24.6)	107(75.4)	142	
rs17817499	182(29.2)	442 (70.8)	624	**0.012***
TT n(%)	68(37.6)	113(62.4)	181	
GT n(%)	84(26.0)	239(74.0)	323	
GG n(%)	30(25.0)	92(75.0)	120	
Hypertension according to questionnaire & test & medication	181(29.1)	442(70.9)	623	0.429
Yes n(%)	130(28.2)	331(71.8)	461	
No n(%)	51(31.5)	111(68.5)	162	
Type 2 Diabetes (T2DM)	182(29.2)	442(70.8)	624	0.119
Yes	58(25.1`)	173(74.9)	231	
No	99(33.0)	201(67.0)	300	
Family History of T2DM =	174(28.4)	438(71.6)	612	0.101
Yes	87(25.7)	251(74.3)	338	
No	87(31.8)	187(68.2)	274	
OHA Medications	181(29.1)	441(70.9)	622	**0.034***
Yes n(%)	47(28.2)	153(76.5)	200	
No n(%)	134(31.8)	288(68.2)	422	

Data are presented as mean ± standard deviation of the *N* (%) or mean (SD). *p*-values: chi-square, Independent sample T test as appropriate; ***p*-value** < 0.05 was considered significant

Table 2 Association of baseline, metabolic characteristics and *FTO* rs1421085 *and FTO* rs17817499 genotypes for diabetic versus non-diabetic individuals

	Non-diabetic	**Diabetic**	**Total**	**P-value**
Sex	254(35.2)	104(34.8)	722	0.851
Female n(%)	273(65.2)	250(35.5)	423	
Male n(%)	195(65.2)	104(34.8)	299	
Age N	254	466		0.670
Mean(SD)	65.50 (11.611)	65.13 (10.620)		
Exercise	445(64.2)	248(35.8)	693	0.985
I do not exercise n(%)	273(63.9)	154(36.1)	427	
Less than once a week n(%)	22(64.7)	12(35.3)	34	
Once or twice per week n(%)	39(62.9)	23(37.1)	62	
Three or more times per week n(%)	111(65.3)	59(34.7)	170	
rs1421085	468(64.8)	254(35.2)	722	
TT n(%)	115(61.5)	72(38.5)	187	0.508
CT n(%)	107(64.8)	58(35.2)	165	
CC n(%)	246(66.5)	124(33.5)	370	
rs17817499	468(64.8)	254(35.2)	722	0.580
TT n(%)	136(63.6)	78(36.4)	241	
GT n(%)	94(68.6)	42(31.4)	137	
GG n(%)	238(64.2)	133(35.8)	371	
Yes n(%)	229(67.6)	110(32.4)	339	
No n(%)	189(59.2)	130(40.8)	319	
BMI	393(63.0)	231(37.0)	624	0.087
Obese/overweight	269(60.9)	173(39.1)	442	
Normal	124(68.1)	58(31.9)	182	
Family History of T2DM	418(63.2)	243(36.8)	661	**0.0001***
Yes	194(53.7)	167(46.3)	361	
No	224(74.7)	76(25.3)	300	

Data are presented as mean ± standard deviation of the *N* (%) or mean (SD). *p*-values: chi-square, Independent sample T test as appropriate; ***p*-value** < 0.05 was considered significant

5.2 Associations of Baseline Characteristics, Lifestyle, BMI, and Diseases with Genotypes

Table 3 shows the characterstics, lifestyle factors, BMI, and diseases with *FTO* rs1421085 and *FTO* rs17817499 genotypes. We observed a significant difference between rs17817499 alleles and age (*p-value* = 0.04) (Table 3). The proportion of *FTO* rs1421085 and *FTO* rs17817499 alleles in participants who do not exercise is not different than the proportion of participants who exercise less than once a week, once or twice per week, or three or more times per week. The difference between three *FTO* rs1421085 and rs17817499 alleles and exercise is not significant (*p-value* =0.799, *p-value* =0.511 respectively) (Table 3). As shown in Table 3, to assess the effect of the *FTO* variants on BMI, participants were classified in two BMI groups, first including four BMI categories (1) underweight, (2) normal, (3) overweight, and (4) obese. In this group, there was a high prevalence of overweight in the study sample with 40.18 % being overweight (BMI > 25 kg/m^2). However, the proportion of *FTO* rs1421085 and *FTO* rs17817499 SNPs were not significantly different between the proportion of four BMI categories (*p-value* =0.372, *p-value* =0.116 respectively). The second group of BMI was categorized into two categories (1) normal weight n=182 (29.2%) and (2) overweight obese n=442 (70.8%). No significant difference was noted among the *FTO* rs1421085 different genotypes and the two BMI categories (*p-value* =0.375) (Table 3) (Figure 2); while the difference between

overweight/obese and normal BMI and *FTO* rs17817499 was significant (*p-value* =0.012) (Table 3) (Figure 3 T2DM was categorized into three categories (diabetic 254 (35.2%), prediabetic 104 (14.4%), and healthy 364 (50.4%)) according to questionnaire & HbA1C test & medication. There was no significant association between *FTO* rs1421085 and *FTO* rs17817499 alleles and the prevalence of diabetes (i.e., whether the participants were diabetic, non-diabetic, or pre-diabetic) (*p-value* =0.720, *p-value* =0.866 respectively) (Table 3). Similarly, the intake of OHA was not significantly associated to *FTO* rs1421085 and rs17817499 alleles (*p-value* =0.817, *p-value* =0.622 respectively) (Table 3). We assessed the association of family history of T2DM. No significant difference was noted among the *FTO* rs1421085 different genotypes and family history of T2DM (*p-value* =0.059); while the association was significant between family history of T2DM and *FTO* rs17817499 (*p-value* =0.009) (Table 3).

Table 3 Association of baseline characteristics, lifestyle factors, BMI, and diseases with *FTO* rs141085 and *FTO* rs1781749 genotypes

	FTO rs1421085					*FTO* rs17817499				
	Among All	**TT**	**CT**	**CC**	***p***	**Among All**	**TT**	**GT**	**GG**	***p***
	***N* =721**	***N* = 184**	***N* = 370**	***N* =164**		***N* =721**	***N* = 215**	***N* = 369**	***N* = 137**	
					Characteristics and Lifestyle Factors					
Gender n (%)	***N*= 723**				0.261	***N*= 723**				0.412
Female	***N*= 424**	118 (27.8)	211 (49.8)	95 (22.4)		***N*= 424**	136 (32.1)	211 (49.8)	77 (18.1)	
Male	***N*= 299**	70 (23.4)	159 (53.2)	70 (23.4)		***N*= 299**	79 (26.4)	160 (53.5)	60 (20.1)	
Age (years) Mean (SD)	65.39(11.27)	66.58(11.475)	64.62(11.029)	65.78(11.511)	0.137	65.39(11.27)	66.88(11.295)	64.44(11.032)	65.62(11.688)	0.040*
Exercise n (%)	***N* = 694**				0.799					0.511
Do not exercise	***N* = 458**	111(25.9)	220 (51.4)	97 (22.7)		***N* = 458**	130 (30.4)	216 (50.5)	82 (19.2)	
Less than once a week	***N* = 34**	11 (32.4)	17 (50.0)	6 (17.6)		***N* = 34**	10 (29.4)	18 (52.9)	6 (17.6)	
Once or twice per week	***N* = 63**	13 (21.0)	31 (50.0)	18 (29.0)		***N* = 62**	12 (19.4)	35 (56.5)	15 (24.2)	
Three or more times per week	***N* = 176**	44 (25.9)	91 (53.5)	35 (20.6)		***N* = 170**	57 (33.5)	86 (50.6)	27 (15.9)	

				Body mass index					
Body mass index (BMI) (kg/m²)	*N = 637*				0.372	*N = 637*			0.116
Underweight	*N* = 13	6 (46.2)	5 (38.5)	2 (15.4)		*N* = 13	5 (38.5)	6 (46.2)	2 (15.4)
Normal	*N =182*	49 (26.9)	98 (53.8)	35 (19.2)		*N =182*	68 (37.4)	84 (46.2)	30 (16.5)
Overweight	*N = 256*	56 (21.9)	137 (53.5)	63 (24.6)		*N = 256*	61 (23.8)	142 (55.5)	53 (20.7)
Obese	*N* = 186	50 (26.9)	92 (49.5)	44 (23.7)		*N* = 186	52 (28.0)	97 (52.2)	37 (19.9)
2 BMI Categories (kg/m²)	*N = 624*				0.375	*N = 624*			**0.012***
Control: Normal	182	49 (26.9)	98 (53.8)	35 (19.2)		182	68 (37.4)	84 (46.2)	30 (16.5)
Case: Overweight/Obese	442	106 (24.0)	229 (51.8)	107 (24.2)		442	113(25.6)	239(54.1)	90(20.4)
				Diseases					
Type 2 Diabetes(T2D) n (%)	*N= 722*				0.720	*N= 722*			0.866
Yes	*N =254*	72(28.3)	124 (48.8)	58 (22.8)		*N =254*	78 (30.7)	133 (52.4)	43 (16.9)
No	*N = 364*	90 (24.7)	194 (53.3)	80 (22.0)		*N = 364*	105 (28.8)	187 (51.4)	72 (19.8)
Pre-diabetes	*N* = 104	25 (24.0)	52 (50.0)	27 (26.0)		*N* = 104	31 (29.8)	51 (49.0)	22 (21.2)
Hypertension	*N= 659*				0.521	*N= 659*			0.153
Yes	*N* =340	84(24.7)	173(50.9)	83(24.4)		*N* =340	95(27.9)	173(50.9)	72(21.2)
No	*N* =319	84(26.3)	169(53.0)	66(20.7)		*N* =319	98(30.7)	168(52.7)	53(16.6)
				Family History of T2DM					
Family History of T2DM n (%)	*N= 662*				0.059	*N= 662*			**0.009***
Yes	*N =361*	79(21.9)	201(55.7)	81(22.4)		*N =361*	89(24.7)	205(56.8)	67(18.6)
No	*N = 301*	88(29.2)	143(47.5)	70(23.3)		*N = 301*	103(34.2)	137(45.5)	61(20.3)
				Oral Hypoglycemic Agents (OHA)					
OHA	*N= 717*				0.817	*N= 659*			0.622
Yes	*N =217*	57(26.3)	108(49.7)	52(24.0)		*N* =340	63(29.0)	117(53.9)	37(17.1)
No	*N =500*	130(26.0)	260(52.0)	110(22.0)		*N* =319	152(30.4)	251(50.2)	97(19.4)

Data are presented as mean ± standard deviation of the *N* (%) or mean (SD). *p*-values: chi-square, one-way analysis of variance (ANOVA) as appropriate; ***p*-value** < 0.05 was considered significant

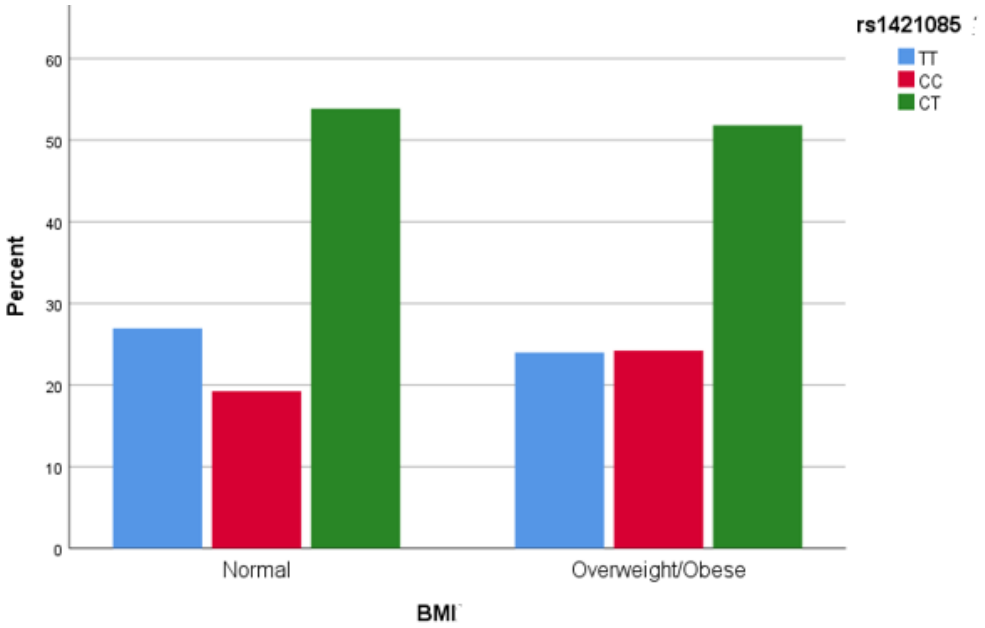

Figure 2 Distribution of *FTO* rs1421085 genotypes by BMI (normal vs overweight/obese)

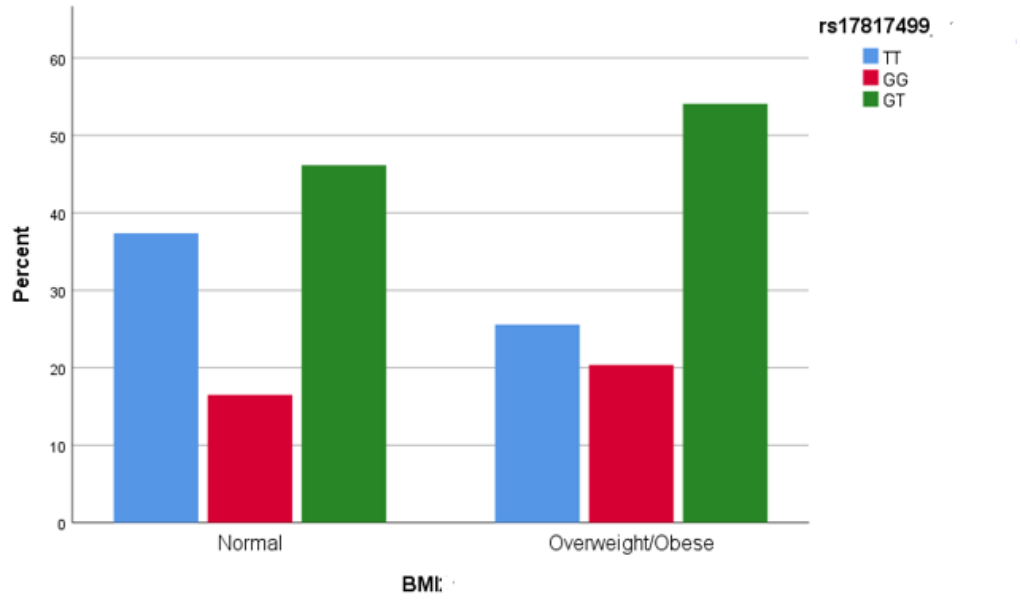

Figure 3 Distribution of *FTO* rs17818499 genotypes by BMI (normal vs overweight/obese)

5.3 Association of *FTO* variants rs17817499, rs1421085 and BMI

A logistic regression analysis was conducted to predict BMI using age, gender, T2DM, and *FTO* rs17817499, rs1421085 as predictors. *FTO* variants rs17817499 GG and GT were significantly associated with overweight and obese compared to normal weight BMI after adjusting for age, gender, and T2DM (*p*-value 0.01 and 0.0001 respectively). *FTO* variant rs1421085 CT genotype showed a significant association with BMI after adjusting for age, gender, and T2DM (*p*-value 0.003). Gender, age, T2DM were not a significant predictor in both unadjusted and adjusted model (*p*-value >0.05; (OR 1.016, 95%CI (0.711-1.451), p = 0.930), (OR 0.990, 95%CI (0.973-1.007), p = 0.235), (OR 1.392, 95%CI (0.958-2.023), p = 0.083); respectively) (Table 4).

We assessed the association of genotype of *FTO* variants rs17817499 and rs1421085 with BMI in Table 5. We performed logistic regression analysis for *FTO* variant rs17817499 GT and GG genotypes with TT as a reference genotype. We observed that the GG genotype and GT genotype showed 1.8- and 1.71-folds higher risk for overweight/obesity respectively compared to TT genotype unadjusted $p < 0.05$ (Table 5). The GT and GG genotypes combined had 1.73 times significantly higher risk for overweight/obesity compared to TT genotypes (unadjusted OR 1.73(1.201-2.511), p= 0.003). The association of *FTO* variant rs17817499 (TT vs GG, TT vs GT, TT vs GT + GG) remained significant regardless of the adjustment for age, gender and T2DM (OR 1.82, 95%CI (1.090-3.045), p = 0.022), (OR 1.681, 95%CI (1.133-2.493), p = 0.010), (OR 1.722, 95%CI (1.188-2.497), p = 0.004); respectively. The highest risk of overweight and obesity was detected among GG carriers in comparison to those with TT

genotypes as shown in Table 5. Similarly, we conducted logistic regression analysis for *FTO* variant rs1421085 CT and CC genotypes with TT as a reference genotype. We observed that the association of *FTO* variant rs1421085 genotypes (TT vs CC, TT vs CT, TT vs CT + CC) did not have a significant association with BMI even after adjusting for age, gender and T2DM (OR 1.408, 95%CI (0.844-2.349), p = 0.190), (OR 1.079, 95%CI (0.712-1.636), p = 0.720), (OR 1.165, 95%CI (0.784-1.732), p = 0.450); respectively) (Table 5).

Table 4 Logistic regression analysis to predict variables related to BMI

Exposure	**Unadjusted OR & 95% CI**	***p*-value**	**Adjusted OR & 95% CI**	**[a]*p*-value**
Age		0.116	0.990(0.973-1.007)	0.235
Gender	0.975 (0.689-1.381)	0.88	1.016(0.711-1.451)	0.930
T2DM	1.375(.954-1.982)	0.087	1.392 (0.958-2.023)	0.083
rs17817499		**0.011**		
rs17817499 GG				**0.010**
rs17817499 GT				**0.0001**
rs1421085		0.376		
rs1421085 CC				0.062
rs1421085 CT				**0.003**

Abbreviation: BMI Body mass index, T2DM Type 2 diabetes mellitus, OR Odds ratio, CI confidence interval

[a]*p*-value from logistic regression models adjusted for age, gender, T2DM, and *FTO* rs17817499, rs1421085. **p*-value < 0.05 was considered significant

Table 5 Association of *FTO* SNPs rs1421085 and rs17817499 with BMI

Genotype	**Unadjusted OR (95% CI)**	***p*-value**	**Adjusted OR (95% CI)**	**[a]*p*-value**
rs17817499 TT vs GG	1.805(1.083-3.010)	**0.024***	1.822(1.090-3.045)	**0.022***
rs17817499 TT vs GT	1.712(1.59-2.529)	**0.007***	1.681(1.133-2.493)	**0.010***
rs17817499 TT vs GT + GG	1.737(1.201-2.511)	**0.003***	1.722(1.188-2.497)	**0.004***
rs1421085 CC vs TT	1.413(0.848-2.354)	0.199	1.408(0.844-2.349)	0.190
rs1421085 TT vs CT	1.080(0.715-1.633)	0.714	1.079(0.712-1.636)	0.720
rs1421085 TT vs CT +CC	1.168(0.788-1.731)	0.440	1.165(0.784-1.732)	0.450

Abbreviation: BMI Body mass index, OR Odds ratio, CI confidence interval

[a]*p*-value from logistic regression models adjusted for age, gender, and T2DM

**p*-value < 0.05 was considered significant

5.4 Association of *FTO* variants rs17817499, rs1421085 and T2DM

A logistic regression analysis was performed to assess potential predictors of T2DM which were age, gender, BMI, and *FTO* rs17817499, rs1421085. Age, gender, BMI, and *FTO* variants rs17817499, rs1421085 were not a significant predictor for T2DM in both unadjusted and adjusted model (*p*-value >0.05) (Table 6).

We assessed the association of genotypes of *FTO* gene polymorphisms rs17817499 and rs1421085 with T2DM in Table 7. We conducted logistic regression analysis for *FTO* variant rs17817499 GT and GG genotypes with TT as a reference genotype and for *FTO* variant rs1421085 CT and CC genotypes with TT as a reference genotype.

The association of *FTO* variant rs17817499 (TT vs GG, TT vs GT, TT vs GT + GG) remained non-significant even after adjusting for age, gender, and BMI (OR 0.719, 95%CI (0.439-1.177), p = 0.189), (OR 0.975, 95%CI (0.666-1.428), p = 0.897), (OR 0.886, 95%CI (0.617-1.272), p = 0.512); respectively (Table 7). Similarly, we found that the *FTO* variant rs1421085 genotypes (TT vs CC, TT vs CT, TT vs CT + CC) did not have a significant association with T2DM even after adjusting for age, gender, and BMI (OR 0.516, 95%CI (0.781-2.014), p = 0.348), (OR 0.244, 95%CI (0.563-1.240), p = 0.372), (OR 0.818, 95%CI (0.563-1.189), p = 0.293) respectively (Table 7).

Table 6 Logistic regression analysis to predict variables associated with T2DM

Exposure	**Unadjusted OR & 95% CI**	***p*-value**	**Adjusted OR & 95% CI**	**[a]*p*-value***
Age		0.535	1.007(0.992-1.023)	0.356
Gender	1.030(0.755-1.405)	0.851	1.092(0.786-1.522)	0.602
BMI	1.375(0.954-1.982)	0.087	1.384(0.953-2.010)	0.088
rs17817499		0.580		0.157
rs1421085		0.508		0.270

Abbreviation: T2DM Type 2 diabetes mellitus, BMI Body mass index, OR Odds ratio, CI confidence interval

[a]*p*-value from logistic regression models adjusted for age, gender, BMI, rs17817499, and rs1421085

**p*-value < 0.05 was considered significant

Table 7 Association of *FTO* SNPs rs1421085 and rs17817499 with T2DM

Genotype	**Unadjusted OR (95% CI)**	***p*-value**	**Adjusted OR (95% CI)**	**[a]*p*-value**
rs17817499 TT vs GG	0.798(0.506-1.258)	0.330	0.719(0.439-1.177)	0.189
rs17817499 TT vs GT	0.974(0.687-1.383)	0.884	0.975(0.666-1.428)	0.897
rs17817499 TT vs GT + GG	0.924(0. 663-1.290)	0.643	0.886(0.617-1.272)	0.512
rs1421085 TT vs CC	.866(0.561-1.337)	0.516	1.254(0.781-2.014)	0.348
rs1421085 TT vs CT	0.805(0.559-1.160)	0.244	0.835(0.563-1.240)	0.372
rs1421085 TT vs CT +CC	0.823(0.582-1.162)	.269	0.818(0.563-1.189)	0.293

Abbreviation: T2DM Type 2 diabetes mellitus, OR Odds ratio, CI confidence interval

[a]*p*-value from logistic regression models adjusted for age, gender, and BMI

**p*-value < 0.05 was considered significant

Chapter Six

Discussion

6.1 Summary of Results

To our knowledge, this is the first study conducted in Lebanon that evaluates the association of *FTO* SNPs rs1421085 and rs17817449 with both overweight/obesity and risk of T2D. It is important to know more about this relation especially that it has been shown that the association of *FTO* SNPs, obesity and T2D are ethnicity and region dependent (Bressler et al., 2010).

The *FTO* rs17817449 variant showed a significant association with BMI, while *FTO* rs1421085 did not (Table 1 and Table 3). Results of the logistic regression showed a significant association between *FTO* variants rs17817499-GG and rs17817499-GT, and rs1421085-CT genotypes after adjustment (Table 4). On the other hand, logistic regression analysis for *FTO* variant rs1421085 having TT as a reference genotype showed no significant association with BMI (Table 5). While *FTO* rs17817499 genotypes were all significantly correlated with BMI, the highest risk was observed among *FTO* rs17817499-GG carriers (Table 5). Several GWAS studies emphasize the strong association between the *FTO* rs17817499 (G>T) and obesity phenotype (Dina et al., 2007; Prakash et al., 2011). Other studies, in European (Balkau et al., 2007) and American populations (Bressler et al., 2010), showed a significant association between *FTO* rs1421085 and obesity. A study conducted in Pakistan revealed a significant association of the rs1421085 with overweight/obese phenotype with heterozygous CT genotype of

FTO rs1421085 was found to increase the risk of being overweight/obese by 1.583 times. CT genotype was observed to influence metabolic parameters; however, was not significantly associated with FBG and HOMA–IR since in the study Rana et al., did not include diabetic overweight, and obese individuals (Rana S., & Bhatti A., 2020).

No significant association was observed between rs17817499, rs1421085, and T2DM even after adjustment (Table 7). The lack of association of *FTO* variant rs1421085 genotypes with BMI and T2DM after adjustments are not in line with most of the studies in the literature. Studies conducted on the French population found an association between rs1421085 and increase IR, HOMA-IR (Rabjou). Similarly, studies in Tunis and Egypt confirmed the association of *FTO* variant rs1421085 with impaired fasting glucose and risk of T2DM (Abdel Rahman et al., 2018; Elouej et al., 2016). In the literature, the *FTO* rs17817499 variant also observed contradicting results on its association with T2DM. In Lebanon, 19 GWAS T2D risk variants study showed a significant association between *FTO* rs17817449 and T2DM in the unadjusted model; however, this significant association was lost after adjusting for confounders such as BMI (Almawi et al., 2013). BMI is usually considered as a confounder of T2DM risk (Yang et al., 2017); however, in our study the associations of T2DM with *FTO* variants were not significant with and without adjustment for BMI, thus the lack of associations was BMI-independent.

The role of *FTO* and obesity is well established in the literature, but the role of *FTO* in T2DM is less clear. Our study showed an association with BMI but not with T2DM. This is in line with several studies conducted in Europe (Do, Bailey et al., 2008; Scott et al., 2007).

Previously, GWAS studies suggested an association between *FTO* SNPs with obesity and T2D (Frayling et al., 2007); however, studies have shown that these relationships are dependent on several factors such as location, environment, race, and ethnicity. In Lebanon, a GWAS concluded that Lebanon has more a European genetic affinity than Middle Eastern one; this might explain the potential similarity of association of *FTO* variants to BMI but not T2DM which was also identified in the European population (Do, Bailey et al., 2008; Ghassibe-Sabbagh et al., 2014; Scott et al., 2007). In addition, a GWAS in Quebec, Canada also found an association between *FTO* variants rs17817449 and rs1421085 with measures of adiposity and increase the risk of diabetes however, the association was lost after adjusting for BMI (Do, Bailey et al., 2008). The lack of association between T2DM and *FTO* after adjusting for BMI, suggests that the effect of *FTO* on diabetes is mediated by the adiposity mechanism.

6.2 Mechanisms of *FTO* on obesity

6.2.1 Biochemical function of *FTO*

In the present study, we observed a significant association between *FTO* rs17817449 genotypes and increased risk of overweight and obesity after adjustment. It is known that the *FTO* gene plays a vital role in regulating body weight; however, the mechanisms behind it are still not very clear. To better understand the role of *FTO* on obesity, it is crucial to comprehend the biochemical characteristic of *FTO*. *FTO* encodes RNA adenosine demethylase enzyme mainly for the 6-methyladenosine (m6A) substrate which is a common modification of RNA. Studies have shown the role of m6A in post transcription regulation and RNA-splicing. The m6A RNA demethylase affects nucleic acid and differentiates metabolism-related cells. (Chang, Park, Park, Shon, & Park, 2018;

Fawcett & Barroso, 2010; Jia et al., 2011). Studies have demonstrated the function of m6A in the regulation of mRNA splicing in adipocytes. In other words, the *FTO*-dependent function of m6A can affect gene expression that is related to metabolism and obesity (Zhao, X. et al. 2014).

6.2.2 The Role of *FTO* on adipogenesis

In the present study, we found that rs17817499 variant of the *FTO* gene was highly correlated with overweight and obesity in the Lebanese population. There are several mechanisms that explain how the *FTO,* which is greatly expressed in adipose tissues, affects fat mass and adipogenic capacity (Frayling et al., 2007). As previously noted, there is an *FTO*- dependent m6A demethylation that regulates the mRNA splicing and adipogenesis. *FTO* does that by regulating the capability of Serine and Arginine Rich Splicing Factor 2 (SRSF2) thus regulating mRNA splicing. *FTO* controls the splicing of an important regulatory factor known as the Runt-related transcription factor 1 (RUNX1T1) which is a target of SESF2 (Merkestein et al., 2015; Zhao, X. et al. 2014). RUNX1T1 has two isoforms. The short isoform (S) is a pro-adipogenic where its over-expression in 3R3-L1 cells leads to the stimulation of adipogenesis. However, the long isoform (L) decreases adipogenesis (Deng et al., 2018; Merkestein et al., 2015; Merkestein & Sellayah, 2015). Studies on mice showed the difference between the overexpression and the deletion/mutation (i.e., knockout (KO)) *FTO* in Mouse Embryonic Fibroblats (MEF). The MEF of the KO *FTO* mice had a lower adipogenic capacity; however, overexpression of *FTO* leads to enhanced adipogenesis. Analysis revealed that this was due to lower mRNA levels of PPARγ, C/EBPα, PL1N1, and FABP4 genes that have a great role in adipogenesis. On the contrary, overexpression of *FTO*

promotes obesogenic adipogenesis (Fischer et al., 2009; Merkestein et al., 2015; Merkestein & Sellayah, 2015). In addition, Merkestein et al., (2015) demonstrated that *FTO* acts in the early stages of adipogenesis which is during a mitotic clonal expansion (MCE). The latter is required for adipocyte differentiation that happens within 48 hours after adipogenic stimulation. Furthermore, Merkestein et al., (2015) discovered that *FTO* KO prevented adipogenesis only earlier to MCE, but the *FTO* overexpression enhanced MCE as well as the expression of C/EBPα and PPARγ which are leading regulators of adipogenesis. These findings verify that *FTO* has a role in early adipogenesis during MCE. Proposed mechanisms include controlling the splicing of an important adipogenicity-related transcription factor and reducing mRNA factors for essential regulators of adipogenesis.

6.2.3 The effect on modified food craving and appetite

Another mechanism that may explain the significant role of *FTO* on obesity is its effect on craving and appetite. Obesity's multifactorial causes include genetics and psychological factors (i.e., eating behaviors, food cravings, and appetite).

The "obesity risk" *FTO* gene is expressed in the hypothalamus which is involved in the regulation of energy expenditure and food intake (Abdella, El Farssi, Broom, Hadden, & Dalton, 2019). Many studies show the influence of *FTO* on food behavior through different mechanisms such as the food reward mechanism. On a neurochemical level, animal studies revealed that deficient *FTO* expression demonstrated almost the same effect as missing the midbrain dopamine D2-receptors (DRD2) (Hess et al., 2013). Thus, reward signaling may be altered because of the *FTO* gene inactivation decreasing dopamine signaling ability (Melhorn et al., 2018). Another mechanism of how the *FTO*

gene affects food craving is via modified *ad libitum* food intake (Dang et al., 2018). A study conducted in Nashville on seventy-eight healthy adults assessed the correlation of *FTO* rs9939609 with food cravings, using a self-reported questionnaire. Results showed that *FTO* rs9939609-A carriers had a higher total food cravings score compared to TT homozygotes and were more likely to experience negative states and emotions before or during food cravings or consumption (Dang et al., 2018). Similarly, *FTO* rs9939609 AA carriers were shown to have greater preference for calorie-dense food. This was demonstrated in a study where higher-risk individuals showed increased postprandial trigger in satiety-related parts of the brain when exposed to visual cues of "fattening" food (Mehta et al., 2012; Melhorn et al., 2018). Those higher risk individuals also had a disrupted circulating concentration (i.e., reduced post-prandial suppression) of the orexigenic gut hormone, ghrelin (Huang et al., 2014; Karra et al., 2013). These findings suggest that the *FTO* gene may increase food cravings through dopamine-dependent regulatory mechanisms as well as nervous and hormonal mechanisms that lead to an impaired satiety response and overeating.

The latter studies on *FTO* rs9939609 variant might also propose a mode of action or mechanism that might be applicable for *FTO* rs1421085and rs17817449. Thus, further studies are needed to assess the exact mechanism of the role of *FTO* variants on adiposity.

6.3 Limitations, Strengths, and Future Implications

The present study included some limitations that should be noted. First, its cross-sectional retrospective observational design does not allow to infer causation. Second, the included sample size is modest. Third, only two variants of the *FTO* gene were included; while there might be other variants of the same gene that influence BMI and diabetes. Fourth,

BMI is not the most reliable marker to assess overweight and obesity; percent body fat, skinfold thickness, and waist circumference could have been coupled with BMI for most accurate results (Vasan et al., 2012). Finally, some studies suggest an effect of the diet on *FTO* variants where diet appears to modify the association of *FTO* variants and obesity. A cross-sectional study in four major ethnocultural groups- Caucasians, East Asian, South Asian, and others, suggested that high dietary protein intake may have a protective effect against the risk of *FTO* gene outcome on BMI and waist circumference (Merritt, Jamnik, & El-Sohemy, 2018). In the present study, we did not adjust for dietary factors. Most of the limitations are attributed to the fact that this study is a secondary data analysis that is retrospectively assessed; consequently, we do not have control over the study design, sample size, and available variables.

Despite the mentioned limitations, the present study carries many strengths. To our knowledge, this is the first study in Lebanon to assess the relation of *FTO* variants rs1421085 and rs17817449 on BMI and type 2 diabetes in a strictly Lebanese population. We corrected for major confounders when we conducted the logistic regression analysis. Future work could use a greater sample size, include other FTO variants prospective design to validate and expand on the present findings. Further research could also evaluate the impact of diet characteristics and our obesogenic environment on the gene and its polymorphisms as well as the predisposition to increased BMI in the Lebanese population.

Chapter Seven

Conclusion

In conclusion, the present study showed a significant association between *FTO* rs17817499 and overweight and obesity in the Lebanese population. Our results which are reported for the first time in Lebanon, provide insights about risk factors and chronic disease prevention for Middle Eastern populations. We showed that when analyzing the genotypes of the *FTO* variants rs17817499 with BMI, homozygous GG genotype carriers had the highest risk of overweight/obesity compared to the TT genotype. Heterozygous genotype CT of the *FTO* rs1421085 may be associated with overweight/obesity; however, further studies are needed to clarify this association.

The studied *FTO* variants were not significantly associated with T2DM, highlighting that the role of the *FTO* gene in T2DM is less clear than its influence on obesity. Consequently, further research is needed to assess the relation between *FTO* SNPs and T2DM, and detect, if possible, the mechanism behind this association. Finally, there is a complex relationship between diet, exercise, environment, race, ethnicity, geographic location, and genes; thus, it is challenging to assess the full picture. Future Longitudinal, randomized, culture-specific studies with a larger sample size are needed to clarify the relationship between several *FTO* SNPs and body weight as well as T2DM.

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