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Transcription Factor 7-like 2 (TCF7L2) Gene Polymorphisms in Relation to the Risk of Type 2 Diabetes in three ethnicities

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

TRANSCRIPTION FACTOR 7-LIKE 2 (*TCF7L2*) GENE POLYMORPHISMS IN RELATION TO THE RISK OF TYPE 2 DIABETES IN THREE ETHNICITIES

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

DIETETICS AND NUTRITION

by

Ling Xu

2018

To: Dean Tomás R. Guilarte
Robert Stempel College of Public Health and Social Work

This dissertation, written by Ling Xu, and entitled Transcription Factor 7-like 2 (*TCF7L2*) Gene Polymorphisms in Relation to the Risk of Type 2 Diabetes in Three Ethnicities, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Vijaya Narayanan

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Juan P. Liuzzi

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Fatma G. Huffman, Major Professor

Date of Defense: June 8, 2018

The dissertation of Ling Xu is approved.

_____
Dean Tomás R. Guilarte
Robert Stempel College of Public Health and Social Work

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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2018

DEDICATION

I dedicate this dissertation to my family, especially my parents Baoling Wu and Zhihong Xu, and my parents-in-law Changfeng Wu and Mingyu Zhang. Without their unconditional support and love, I could not have today's achievements. Special thanks to my husband, Jilin Zhang, who always encourages me and supports me to pursue my goals. To my dear daughters, Ashlyn and Summer, who are my driving force to be an independent person, a role model and a super Mom they can be proud of. Finally, thank God for guiding me through such a great journey here!

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ABSTRACT OF THE DISSERTATION

TRANSCRIPTION FACTOR 7-LIKE 2 (*TCF7L2*) GENE POLYMORPHISMS IN RELATION TO THE RISK OF TYPE 2 DIABETES IN THREE ETHNICITIES

by

Ling Xu

Florida International University, 2018

Miami, Florida

Professor Fatma G. Huffman, Major Professor

Type 2 Diabetes (T2D) disproportionally affects ethnic minorities in the United States. The development of T2D involves complex interaction between environmental factors and genetic predisposition. The genetic associations of six single nucleotide polymorphisms (SNPs) in *TCF7L2* gene with the risk of T2D were evaluated in three high risk minority populations: Cuban Americans, Haitian Americans, and African Americans. For Cuban Americans, four SNPs (rs7901695, rs4506565, rs7903146 and rs11225537) were significantly associated with the risk of T2D after multivariable adjustment (p=0.018, p=0.016, p=0.014, and p=0.0008, respectively). Among controls, risk allele carriers of SNPs rs7901695, rs4506565 and rs7903146 had significantly higher fasting glucose level, compared to non-risk allele carriers. Additionally, a significant interaction between dietary fiber intake and SNP rs7903146 for the risk of T2D (p= 0.044) was found in Cuban Americans. Similarly, for SNP rs7901695, significant interaction was also found for fiber intake (p=0.014) as well as glycemic load (p=0.040). Subgroup analysis revealed that significant associations were only found within higher

intake groups of dietary factors for both SNPs. For Haitian Americans, SNPs rs11196205 (p=0.059) and rs7895340 (p=0.069) showed marginal significance for the risk of T2D. After stratification by gender, SNPs with marginal significance from the gender-combined analysis became statistically significant with the same trend for the risk of T2D when analysis were done in males: rs11196205 (p=0.034) and rs7895340 (p=0.024). For African Americans, SNP rs7903146 (p=0.065) showed a marginal significance with the risk of T2D in gender-combined analysis and a statistical significance (p=0.013) in males. Two additional SNPs rs7901695 and rs4506565 were found to be significantly associated with the risk of T2D in males. Risk allele carriers of these two SNPs had significantly higher mean level of the fasting glucose level, compared to non-risk allele carriers in controls. T2D related quantitative trait analysis also demonstrated that in controls, compared to non-minor allele carriers of SNP rs12255372, minor allele carriers had significantly higher means of BMI, diastolic blood pressure, numbers of components of Metabolic Syndrome, significantly lower mean values of HDL-cholesterol and adiponectin. Taken together, our studies demonstrated ethno-specific genetic associations between TCF7L2 gene and the risk of T2D and related phenotypes.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER I INTRODUCTION

Review of literature

Diabetes is a complex, multi-factorial metabolic disorder characterized by hyperglycemia resulting from impaired pancreatic beta cell function, decreased action of insulin on target tissues, or both [1]. Most individuals with diabetes suffer serious complications of chronic hyperglycemia, involved in nephropathy, neuropathy, retinopathy, and accelerated development of cardiovascular disease [2]. Diabetes is currently the fastest-growing epidemic and a significant public health issue globally. The International Diabetes Federation estimated that 425 million people live with diabetes in 2017 and this number is projected to rise to 629 million by 2045 [1]. Type 2 diabetes (T2D) accounts for 90% to 95% newly diagnosed diabetes. In the United States, approximately 30.3 million US people, or 9.4% of the U.S. population have diabetes and an estimated 33.9% of U.S. adults aged 18 years or older (84.1 million people) have prediabetes [2].

Diabetes has been subdivided into type 1 diabetes and type 2 diabetes. Over the past decades, the concept of diabetes has developed into considering type 1 diabetes and type 2 diabetes as two ends of a diabetes spectrum, with intermediates including maturity-onset diabetes of the young (MODY), latent autoimmune diabetes in adults (LADA), maternally inherited diabetes and deafness (MIDD), gestational diabetes mellitus (GDM), and neonatal diabetes [3]. T2D is the most prevalent form, accounting for approximately 90% of all diabetes cases [4]. The development of T2D is a progressive process caused by a complex interplay between environmental, epigenetic and genetic factors [3].

Several environmental variables such as age, gender, central obesity, low physical activity and an unhealthy diet consisting of high saturated fatty acids and/or trans fatty acids and low dietary fiber, have been identified as risk factors for type 2 diabetes [4-6]. Heritability of T2D is estimated to be between 30% and 80% based on twin and familial studies [7-11]; the longest follow-up studies yield the highest estimates of up to 80% heritability of T2D [3]. Recent genetic studies, especially high throughput genome-wide association studies (GWAS), have identified a multitude of variants associated with T2D. To date, at least 68 regions on human genes have shown replicated association with T2D, or T2D related traits (e.g. glucose tolerance and insulin resistance) [12]. However, one primary limitation would be that of modest effect sizes of common variants, especially those identified through recent GWAS for T2D and most identified SNPs (single nuclear polymorphisms) that confer only 10–20% additional risk of T2DM with few exceptions with high odds ratios [3]. To date, *TCF7L2* (Transcription factor 7-like 2) is one of the top diabetes candidate genes yielding high risk for T2D. Several genome-wide association studies and meta-analysis studies have repeatedly confirmed that variants in *TCF7L2* are strongly associated with the susceptibility of type 2 diabetes in Caucasians and some Asian populations, conferring as high as 40% additional risk of T2D [12-18].

TCF7L2 **gene and protein structure**

TCF7L2 gene product is a transcription factor that belongs to a family of T-cell factor /lymphoid enhancer factor (TCF/LET) [19]. Proteins of the TCF/LEF family contain an 80-amino-acid high mobility group (HMG) box. HMG boxes bind DNA as monomers in a sequence-specific manner. TCF/LEFs bind to specific DNA sequences referred as Wnt responsive elements (WRE: CCTTTGA/TA/T) [20, 21]. *TCF7L2* gene

spans a 215,863 bases region on chromosome 10q25.3 [22]. It has 17 exons, of which five exons (4, 13, 14, 15 and 16) are alternative yielding different slicing possibility [23]. Interestingly, the protein products detected by Western blotting in most tissues are 78 kDa and 55 kDa, suggesting the existence of undefined post-translational modifications [24]. Vacik et al [25] also found another brain-specific isoform in the mouse model. This form is a truncated isoform, defined as TCF7L2DN (dominant TCF7L2) that lacks β-catenin binding domain and that therefore acts as a potent dominant-negative Wnt antagonist.

TCF7L2, a Wnt signaling pathway effector

TCF7L2 together with β-catenin is a well-known biparticle transcription factor in the canonical Wnt pathway. The Wnt signaling pathway is a conserved pathway in animals and human beings, and its activity is ubiquitous and involved in numerous fundamental processes essential for embryonic development and normal adult homeostasis [26]. In the nucleus, β-catenin can bind members of the TCF/LEF family of transcription factors and recruit the transcriptional Kat3 co-activators p300 and/or CBP (CREB-binding protein) to transcribe Wnt target genes [27, 28]. Products of Wnt signal targeted genes have a plurality and diversity of biochemical functions including activation of cell cycle progression and proliferation, inhibition of apoptosis, cell differentiation, growth and migration cell cycle kinase regulation, cell adhesion, hormone signaling, and transcription regulation [29]. Mutations in Wnt genes or Wnt pathway components lead to specific developmental defects, and numerous human diseases, such as various types of cancer, Type 2 diabetes, obesity, bone density defect, coronary disease, etc. [1].

Intensive effects to the function of the Wnt signaling pathway on glucose and metabolic homeostasis have been made immediately after recognition of the significant association between certain single nucleotide polymorphisms in *TCF7L2* gene and the risk of T2D. Current findings revealed the potential role of TCF7L2 in the Wnt signaling pathway related to incretin hormone regulation, β-cell proliferation and apoptosis, and glucose production have been documented tissues [30-32]. In addition, more recent studies also revealed that potential role of TCF7L2 in adipogenesis and demonstrated variants in this gene could modify the risk of developing obesity and Metabolic Syndrome [33-36].

Wnt signaling pathway in incretin hormone regulation

Incretins are a group of metabolic hormones that stimulate a decrease in blood glucose levels. Two main incretin hormones are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which are mainly secreted from gastro-intestinal K- and L-cells, respectively [30, 37]. They could directly stimulate the pancreatic β-cell, accounting for some 25-70% of postprandial insulin secretion in healthy subjects [38]. In patients with T2D, however, this effect is greatly reduced or lost due to a combination of severely impaired or eliminated insulinotrophic effect of GIP and reduced meal stimulated GLP-1 secretion. In addition to incretin insulinotropic effect, GLP-1 also possesses “insulin-like” or insulinomimetic effect on pancreatic β-cells and extra-pancreatic tissues or cell lineages and exert protective effects in pancreatic β-cells function and survival [39].

Studies found that in intestinal L endocrine cells, expression of the proglucagon gene (*GCG*) and production of GLP-1 could be positively regulated by Wnt ligand, lithium, which mimics the activation of Wnt signaling while inhibition of glycogen synthase kinase-3beta, the major negative modulator of the Wnt pathway inhibiting *GCG* mRNA expression and GLP-1 synthesis [30, 40]. The regulatory role of Wnt signaling in gut *GCG* expression was further confirmed by using a constitutively active β-catenin mutant and dominant negative TCF7L2 [37, 40, 41]. Mechanistically, Wnt signaling is involved in the regulation of the proglucagon gene expression due to the binding of β-catenin/TCF7L2 to the G2 enhancer element of *GCG* promoter, demonstrated by quantitative chromatin immunoprecipitation [40]. Wnt signaling pathway is not only involved in GLP-1 expression, but also has effect on GIP transcription. Lithium or Wnt-3, as Win signal ligands, was shown to increase GIP transcription in intestinal endocrine K cells [42]. Thus, an alteration in Wnt signaling pathway caused by mutations on *TCF7L2* gene could potentially lead to an altered secretion of GLP-1 or GIP which, in turn, could have consequences for the insulin secretion following a meal. In support of this hypothesis, Lyssenko [43] found that in T2D patients with CT/TT genotypes of SNP rs7903146 showed increased mRNA levels of *TCF7L2*, associated with impaired insulin secretion and incretin effects. In parallel, Schafer [44] found that there is a significant reduction in GLP-1-induced insulin secretion in people with *TCF7L2* risk alleles rs7903146 and rs12255372. Færch [45] found that healthy carriers with risk T allele of alleles rs7903146 have lower fasting GIP concentrations and lower total GIP secretion during the a 3-h oral glucose tolerance test (OGTT). This study suggests Wnt signaling and its effectors TCF proteins are not only involved in the production of both GLP-1 and

GIP, but also in the production of GLP-1 receptor and GIP receptor. Shu [46] found that GLP-1 receptor and GIP receptor was significantly reduced in isolated human islets when siRNA treatment was used to induce TCF7L2 depletion in islets. A recent study demonstrated TCF7L2 also regulates expression of *GCG* in the brain using transgentic mice with a functional knockdown of TCF7L2 [47]. Transgenic mice showed reduced brain *GCG* expression, impaired glucose tolerance and insulin sensitivity when fed a high-fat diet (HFD). Taken together these studies suggested that TCF7L2 may be an important mediator of the GLP-1 signaling pathway, and indicate a central action of TCF7L2 in control of glucose homeostasis.

Wnt signaling pathway in β-cell proliferation and apoptosis

The loss of functional β-cells is the root cause for the development of diabetes. Thus, the regulation of β-cell proliferation, differentiation and apoptosis is critical to maintain normal β-cell function and to promote adaptive growth of β-cells when the need for insulin increases. Previous studies have demonstrated that the Wnt signaling pathway plays critical roles in regulating multiple developmental processes, including proliferation of organ-specific stem/progenitor cell populations, growth control and cell fate determination in diverse organs, and tissue patterning [48]. In one study, Schinner and colleagues [32] found that human fat-cell conditioned medium stimulated the proliferation of a pancreatic β-cell line and primary mouse islet cells and stimulated insulin secretion from pancreatic β-cells, but the stimulation can be blocked by the Wnt repressor, secreted frizzled related protein 1. In another study, Shu [49] found that over-expression of TCF7L2 could protect human or mouse pancreatic isolated islets from glucose- and cytokine-mediated apoptosis while in contrast, siRNA-mediated TCF7L2

depletion resulted in a significant increase in β-cell apoptosis and decrease in β-cell proliferation, associated with the attenuation of glucose-stimulated insulin secretion. Later on, Shu group [50] demonstrated TCF7L2 levels correlated with beta cell adaptive compensation in a HFD induced diabetic mouse model and *TCF7L2* expression was increased together with pancreatic duct cell proliferation and differentiation. In addition, they found increased TCF7L2 levels correlated with insulin-positive ductal epithelial cells in patients with type 2 diabetes and *TCF7L2* overexpression induced proliferation of pancreatic duct cells and islet-like cell cluster formation next to duct cells in human isolated exocrine tissue. Reports from other research groups also confirmed that TCF7L2 protein levels were correlated with β-cell and able to improve β-cell survival, and regeneration [51, 52]. Interestingly, Yao and colleagues [53] found that geniposide, a nature occurring compound from Gardenia Fruits, was found to promote β-cell survival by increasing β-cell proliferation and decreasing β-cell apoptosis in cultured mouse islets after challenge with diabetic stimuli through upregulating β-catenin/TCF7L2 pathway. Collectively, these evidences support the role of Wnt signal in the β-cell homeostasis.

TCF7L2's role in gluconeogenesis from liver

Blood glucose levels are maintained by the balance between glucose uptake by peripheral tissues and glucose secretion by the liver. Thus gluconeogenesis from liver plays a critical role in maintaining glucose homeostasis. Studies suggested TCF7L2 might be a potential negative regulator of gluconeogenesis in the liver. Norton et al [54] demonstrated that interfering in Wnt signaling by silencing *TCF7L2* led to increased basal levels of hepatic glucose production in a rat hepatic cell line, associated with the over-expression of genes that encodes key rate-limiting gluconeogenic enzymes including

fructose-1-6-bisphosphatase, phosphoenolpyruvate carboxykinase 1 and glucose 6-phosphatase. In line with this finding, Oh et al [55] found that hepatic *TCF7L2* knockdown caused an increase in plasma glucose levels, which was associated with glucose intolerance and elevated hepatic gluconeogenic gene expression while overexpression of a nuclear isoform of *TCF7L2* in HFD-fed mice improved glucose tolerance. In another study, Ip et al [56] utilized a transgenic mouse model named LTCFDN. In this mouse model, TCF7L2DN, as a negative Wnt regulator, was specifically produced in liver. LTCFDN mice exhibited progressive impairment in response to pyruvate challenge and hepatocytes displayed elevated gluconeogenic gene expression, gluconeogenesis, and loss of Wnt-3a-mediated repression of gluconeogenesis. These studies collectively supported Wnt signaling activation and *TCF7L2* expression negatively regulated hepatic gluconeogenesis and imply variants in *TCF7L2* gene may contribute to dysregulated glucose production in the liver. To support this hypothesis, Lyssenko et al [57] found that the risk T allele of SNP rs7903146 was also associated with enhanced rates of hepatic glucose production. In line with this founding, Pilgaard demonstrated that this risk allele was associated with elevated hepatic glucose production, even in patients undergoing a hyperinsulinemic clamp [58].

Association between *TCF7L2* and T2D

The association between *TCF7L2* gene and the risk of T2D was first discovered by deCODE group from an Iceland cohort study in 2006 [59]. They found that a microsatellite, DG10S478, located within the fourth intron region of the *TCF7L2* gene, was strongly associated with T2D susceptibility. Later on a few other polymorphisms in this gene have been identified and reported to be significantly associated with T2D, and

majority of the significant polymorphisms were located within a genome area of 92-kb base pairs where the fourth intron and left flanking of the fifth intron are located within [60, 61]. Multiple lines of studies suggested this SNP-containing interval might exert an important role in regulation of *TCF7L2* gene expression. Savic et al [62] demonstrated this region contains a strong regulatory enhancer by using bacterial artificial chromosome assay in a transgenic mice model. Kennell and Locke [63, 64] found that this region also contains a signal for alternative polyadenylation for production of a truncated, inhibitory TCF7L2 isoform lacking HMG DNA-binding domain. Moreover, another independent study identified that the fifth intron of the *TCF7L2* gene harbors a highly conserved promoter Ex1b-e for a dominant-negative isoform of TCF7L2 which lacks the β-catenin binding domain [65]. However, the exact mechanism of how the polymorphisms in this gene influence gene production is not determined. So far, the association between *TCF7L2* gene polymorphisms and T2D have been validated in several populations, with majority in populations of European ancestry [66, 67]. Generalization of results across multiple racial/ethnic groups helps confirm the relevance of some of these loci for the risk of T2D. Up to now, few existing studies have examined genetic predisposition to T2D in Hispanic populations. Most genetic studies were either carried in population of Mexican origin or treated Hispanic populations as a single group despite the diverse genetic and environmental background among Hispanic populations [68, 69]. Similarly, results from studies conducted in African Americans were sparse and inconsistent, partially due to genetic heterogeneity among Blacks [66].

Food and Gene Interaction on T2D

TCF7L2 has the largest effect among the various susceptibility genes on type 2

diabetes, such as KCNQ1, CDKN2B, FTO and HHEX/IDE [70]. However, inconsistent replication of results is often present when studying genetic associations, possibly due to ethnic diversity present in this complex interplay between genetic and environmental factors, especially dietary factors. The epidemic of T2D only dates back 50 years. It is quite clear that during this period the environmental factors have changed dramatically. This epidemic is highly possible not due to genetic changes since these take many generations to occur. A traditional high energy-burning lifestyle has been replaced by a sedentary lifestyle and consumption of an energy-dense diet, which is a common phenomenon worldwide, especially pronounced in the United States [3]. However, genetic variants could affect specific metabolic processes to make an individual more susceptible to the harmful effects of environmental factors such as high consumption of energy dense food and less physical activity [71].

Previous studies have demonstrated that differences in intake of calories, dietary fat, high glycermic index carbohydrates, and certain micronutrients are linked to type 2 diabetes [72]. Some recent explorations on such interplay between *TCF7L2* gene polymorphisms and dietary factors have shown promising results. In the Diabetes Prevention program, the risk allele T of *TCF7L2* rs7903146 showed a tendency towards being strongly associated with type 2 diabetes in the placebo group compared with the lifestyle-intervention group but the results did not reach statistical significance [73]. In the European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam cohort higher whole-grain intake was found to be protective against type 2 diabetes among SNP rs7903146 CC genotype carriers but not among T allele carriers [74]. In Nurses' Health Study, the *TCF7L2* risk allele was reported to have a stronger association with type 2

diabetes among individuals with higher dietary glycaemic load and glycaemic index [14]. In the Tübingen Lifestyle Intervention Program (TULIP) described an interaction between dietary fiber and the *TCF7L2* rs7903146 risk variant with regard to successful weight loss after a lifestyle intervention [75]. In another study from Sweden, Hindy et al. [76] used a cohort of nearly 25,000 initially nondiabetic Swedish adults to assess interactions between dietary fiber and the *TCF7L2* rs7903146 variant on T2D incidence. The authors reported a nominally statistically significant interaction between the *TCF7L2* SNP rs7903146 and dietary fiber intake in T2D risk. Another recent study found consumption of Mediterranean diet could modify the association between *TCF7L2* risk allele and weight gain, and suggested a beneficial effect of the Mediterranean diet in subjects with *TCF7L2* risk allele(s) [77]. Thus, it will be of great interest to investigate the interplay between dietary factors and genetic variants in modifying the risk to T2D development.

Diabetes and Ethnicity

Compared to the general population, African Americans are disproportionately affected by diabetes. 12.7% of African Americans aged 18 years or older have diabetes, compared to 7.4% of non-Hispanic whites in 2015 [2]. African Americans are almost twice as likely to be diagnosed with diabetes as non-Hispanic whites. In addition, they were more likely to suffer from complications of diabetes, such as high blood pressure end-stage renal disease and lower extremity amputations and were twice as likely as non-Hispanic Whites to die from complications of diabetes [78]. Because of the high prevalence of diabetes in the African American community, it has been suggested that

African Americans may be more susceptible to the disease compared with Whites through direct genetic propensity or gene–unfavorable environment interactions (e.g. high in fat, sugar, and salt and low in fruits and vegetables) [79]. Although associations between various SNPs in *TCF7L2* and the risk of T2D have been observed across ethnically diverse populations [66], results from studies conducted in African Americans have been sparse and inconsistent. Moreover, the ethnic diversity within 'Black' population is not yet explored.

In general, Hispanics are at high risk for developing type 2 diabetes and related cardio-metabolic abnormalities. According to CDC report, Hispanic populations have a higher age-adjusted rate of diagnosed diabetes (12.1%), compared to7.4% of non-Hispanic Whites [2]. Among Hispanic population, Cuban Americans represent the third largest minority group in the United States and has the highest proportion of the elderly population (26% for age 55 or older) compared to other Hispanic groups (11.9% for Mexicans and 16.1% for Puerto Rican) [70]. Although Cuban Americans (9.0%) have a lower prevalence of T2D than Mexicans (13.8%) and Puerto Ricans (12.0%) but still 1.22 times higher than non-Hispanic whites (7.4%) and a highest proportion of diabetes as the underlying cause of death (44%) as compared to other Hispanics groups (39% for Puerto Ricans and 37% for Mexican Americans) [34, 80]. By far, existing studies have examined genetic association with T2D in Hispanic subgroup, of which majority were carried on in populations of Mexican origin [66, 81, 82]. The current Cuban population resulted from the complex process of racial mixture from Asian, African, European and Native American [83]. The genetic heritage from these populations might play an important role

for the higher prevalence of T2D and T2D related complications in Cuban Americans. In addition, the disproportionally affected rates of T2D in Cuban Americans could be ascribed to complex interaction of genetic predisposition with acculturation related unhealthy diet behavior (e.g. higher sugar and low fiber) but few studies have been conducted in this area [16].

Haitian Americans are one of the fastest growing Caribbean immigrant populations in the United States. According to the United States Census, an estimated 929,074 Haitian Americans were living in the U.S. in 2013, of which approximately half of them reside in Florida, and nearly one third (~290,000) live in the Miami metro area [84]. Haitian Americans are one of the high-risk populations for T2D. Though official data for Haitian Americans are not available, due to being grouped with other Blacks, a small-scale study conducted in a Haitian community (Little Haiti) in the Miami-Dade County, FL, estimated a 33% prevalence of diabetes among Haitian immigrants [85]. Haitian Americans have specific characteristic in their genetic background and lifestyle which distinguish them from African Americans. Apart from being African descent, populations of Haitian Americans also have lineage from France, and Spain [86]. Haitian Americans have unique dietary pattern featured by two meals a day with starch predominantly as energy source. These features might partially explain why Haitian Americans have poorer glycemic control than African Americans and non-Hispanic Whites with diabetes [87]. In addition, another study on eating behavior showed that Haitian Americans and African Americans have significantly different healthy eating index (HEI) scores, and levels of physical activity [88]. Studies from our research group also suggested that Haitian Americans may have different genetic makeup than African Americans [29]. These

unique features distinguish Haitian Americans as a unique ethnic group in South Florida and but very often they were grouped with other Blacks with African origins for health research. Thus, translation and generalization of the results from genetic association conducted in African Americans to Haitian Americans should be done with caution and warrants further replication. By far, studies looking at health disparity in a separated Haitian American population is limited, including genetic studies. Further investigation is needed to expand current knowledge.

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Specific aims and hypotheses

The *TCF7L2* gene product is a high-mobility box-containing transcription factor which is a ubiquitous protein that belongs to a family of T-cell factor /lymphoid enhancer factor (TCF/LET) [1]. TCF7L2, together with β-catenin is a well-known bipartite transcription factor in the canonical Wnt pathway. Great efforts have been devoted to elucidating the potential role of TCF2L2 in the Wnt signaling pathway related to incretin hormone regulation, β-cell proliferation and apoptosis, and hepatic glucose production [2-4]. The involvement of TCF7L2 in all these pathway puts this gene in a critical role suggesting strong relationship of polymorphisms of *TCF7L2* with type 2 diabetes (T2D). So far, some SNP loci on this gene have proved to be associated with type 2 diabetes in various ethnic groups (e.g. Caucasian, East Asian, Indian, and Middle East) [5].

However, inconsistent replication of results is often present when studying genetic associations, due to ethnic diversity present in the complex interplay between genetic and environmental factors. Compared to the general population, African Americans are disproportionately affected by diabetes. 12.7% of African Americans aged 18 years or older have diabetes, compared to 7.4% of non-Hispanic whites in 2015 [6]. African Americans are almost twice as likely to be diagnosed with diabetes as non-Hispanic whites. Because of the high prevalence of diabetes in the African American community, it has been suggested that African Americans may be more susceptible to the disease compared with Whites through direct genetic propensity or gene–unfavorable

environment interactions (e.g. high in fat, sugar, and salt and low in poor in fruits and vegetables) [7]. Haitian Americans are usually grouped together with other populations of African origins in population based studies. However, Haitian Americans have specific characteristic in their genetic background and lifestyle which distinguish them from African Americans and may contribute inconsistent results in gene association study due to population stratification in Black people [8]. So far, only two studies from our group have investigated the genetic predisposition on diabetes (vitamin D receptor gene and PPARGC1A gene) in Haitian Americans. Further investigation is needed in this area to expand current knowledge. Similarly, Hispanic populations also have a high age-adjusted rate of diagnosed diabetes (12.1%) [6]. Although Cuban Americans (9.0%) has a lower prevalence of T2D than Mexicans (13.8%) and Puerto Ricans (12.0%) but still 1.22 times higher than non-Hispanic whites (7.4%) and a highest proportion of diabetes as the underlying cause of death (44%) as compared to other Hispanics groups (39% for Puerto Ricans and 37% for Mexican Americans [9, 10]. The current Cuban population resulted from the complex process of racial mixture from Asian, African, European and Native American [11]. Data showed that those minority groups have a higher diabetes rate compared to their white counterparts [12]. Thus, the genetic heritage from these populations might play an important role for the higher prevalence of T2D in Cubans Americans. In addition to possible genetic differences, studies suggest higher consumption of junk foods and low consumption in fruits and vegetables among Cuban Americans [13, 14]. All of these factors make Cuban Americans an interesting population to study. However, few studies have looked at genetic associations of Cuban Americans with T2D. Therefore, due to aforementioned gap in knowledge, this study is aimed to

assess six *TCF7L2* common polymorphisms (table 1): rs7903146, rs12255372, rs11196205, rs7901695, rs7895340 and rs4506565, in relation to T2D and related phenotypes in three race/ethnic populations (African Americans, Haitian Americans and Cuban Americans) at high risk for T2D and potential environmental modification factors. Three hypotheses were tested in this dissertation: 1) there would be a significant association between *TCF7L2* risk-conferring variants and type 2 diabetes prevalence in each of three ethnic populations; 2) *TCF7L2* risk-conferring genotypes would be significantly associated with impaired beta-cell function, insulin sensitivity and insulin resistance in each of three ethnic populations; 3) different environmental factors would modify associations between *TCF7L2* variants and the risk of type 2 diabetes among each of three ethnic populations.

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Table 1. Characteristics of studied SNPs in the *TCF7L2* gene

NCBI ref SNP number*	Chromosome nucleotide position†	Location	Polymorphisms	Minor allele	Minor allele frequency&		
					EUR	AFR	AMR
rs7901695	Chr. 10: 112994329	intron	T/C	C	0.34	0.44	0.26
rs4506565	Chr. 10: 112996282	intron	A/T	T	0.35	0.44	0.26
rs7903146	Chr. 10: 112998590	intron	C/T	T	0.32	0.26	0.24
rs7895340	Chr. 10: 113041766	intron	A/G	G	0.48	0.18	0.34
rs11196205	Chr. 10: 113047288	intron	C/G	G	0.49	0.18	0.35
rs12255372	Chr. 10: 113049143	intron	G/T	T	0.29	0.30	0.22

**National Center for Biotechnology Information (NCBI) reference single nucleotide polymorphism (SNP) number (http://www.ncbi.nlm.nih.gov/)* †*Genome Reference Consortium Human Build 38 patch release 7 (GRCh38.p7) used for nucleotide position (http://www.ncbi.nlm.nih.gov/SNP)* &*Information of Minor allele frequencies are obtained from 1000 Genomes Project (http://www.internationalgenome.org). Population descriptors: EUR: European; AFR: African; AMR: Ad Mixed American.*

CHAPTER II THE VARIANTS IN THE TCF7L2 GENE ARE ASSOCIATED WITH THE RISK OF TYPE 2 DIABETES IN CUBAN AMERICANS

Abstract

Objectives: Type 2 diabetes (T2D) is a complex and chronic metabolic disorder that involves complex interaction between environmental factors and genetic predisposition. TCF7L2 gene recently emerged as a top candidate gene for T2D. Our study is aimed to assess six common polymorphisms in TCF7L2 gene: rs7901695, rs4506565, rs7903146, rs11196205, rs7895340 and rs12255372, in relation to the risk of T2D and related phenotypes in Cuban Americans who lived in Miami-Dade and Broward counties, Florida.

Methods: We conducted a case-control study with 341 Cuban Americans (172 without T2D /169 with T2D). Unconditional logistic regression method was used to assess the association between six TCF7L2 polymorphisms and the risk of T2D in three genetic models adjusted by potential confounding factors such as age, sex, BMI, physical activities and calorie intake. Student's t-tests on continuous values of T2D related quantitative traits were compared between risk allele carriers and non-carriers in control subjects. In addition, linkage disequilibrium and haplotype analysis were performed using Haploview 4.2.

Results: Four TCF7L2 SNPs (rs7901695, rs4506565, rs7903146 and rs11225537) were significantly associated with the risk of T2D in a Cuban American population after multivariable adjustment. We also found that among non-diabetic control subjects, risk minor allele carriers of three TCF7L2 SNPs (rs7901695 (T>C), rs4506565 (A>T) and rs7903146 (C>T)) had significantly higher fasting glucose level and marginally higher

level of glycated hemoglobin (A1C), compared to non-risk allele carriers. Consistently, results from haplotype analysis showed that two haplotypes TAC and CTT, formed by aforementioned three TCF7L2 SNPs (rs7901695, rs450565 and rs7903146), were significantly associated with the risk of T2D (p=0.0094, and p=0.0044, respectively), with the frequency of TAC significantly lower and CTT significantly higher in subjects with T2D, compared to subjects without T2D.

Conclusions: The result of this study provides convincing evidence that variants in TCF7L2 gene confers strong susceptibility to T2D in the population studied.

Introduction

Type 2 diabetes (T2D) is a complex, multi-factorial metabolic disorder characterized by hyperglycemia resulting from impaired pancreatic beta cell function, decreased action of insulin on target tissues, or both [1]. Heritability of T2D is estimated to be between 30% and 80% based on twin and familial studies [2-6]. Recent genetic studies, especially high throughput genome-wide association studies (GWAS), identified a multitude of variants associated with T2D. *TCF7L2* (Transcription factor 7-like 2) is one of the top candidate genes yielding high risk for T2D. The *TCF7L2* gene product is a high-mobility box-containing group (HMG) transcription factor which is a ubiquitous protein that belongs to a family of T-cell factor/lymphoid enhancer factor [7]. TCF7L2, together with β-catenin is a well- known bipartite transcription factor in the canonical Wnt pathway. Studies suggested TCF7L2 involvement in incretin hormone regulation, β-cell proliferation, apoptosis, and hepatic glucose production via Wnt signal pathway [8-10].

Strong associations between SNPs in *TCF7L2* and the risk of T2D had been repeatedly detected in multi-ethnic groups, but few existing studies have examined this

association in Hispanic subgroup, of which majority were carried in populations of Mexican origin [11-13]. Cuban Americans represent the third largest minority group in the United States, and two thirds live in the state of Florida and over 50% lived in Miami-Dade County. Approximately 16% of Cuban Americans ages 45-75 years in the US have diabetes which is 1.3 times higher than non-Hispanic Whites [14]. The disproportionally affected rates of T2D in Cuban Americans could be partially ascribed to increased genetic predisposition but few studies have been addressed in this area. One genetic study conducted by our research group demonstrated that peroxisome proliferator-activated receptor-γ coactivator 1- α (*PPARGC1A*) SNP rs7656250 (T>C) was significantly associated with the risk of T2D (OR=6.87, p=0.02) in Cuban Americans [15].

A serial of meta-analyses on the association between SNPs in *TCF7L2* gene and the risk of T2D discovered that several polymorphisms, including SNP rs7901695, SNPs rs4506565 and rs7903146 (all of which are located within the fourth intron); SNPs rs7895340, rs11196205, and rs12255340 (all of which are located within the fifth intron), are consistently replicated by studies [12, 16-18]. However, the evidence to support the role of *TCF7L2* gene polymorphisms related to the risk of T2D in Cuban Americans is limited. Therefore, the purpose of this study was to examine six most studied SNPs in *TCF7L2* gene: rs7903146, rs12255372, rs11196205, rs7901695, rs7895340 and rs4506565 in relation to the risk of T2D and T2D related phenotypes in a Cuban American population.

Methodology

Subjects

This was a case-control study. The target population was Cuban American adults in Broward and Miami-Dade Counties, Florida. Approximately half of the participants had type 2 diabetes and the rest were free of diabetes. Participants were initially recruited from randomly generated mailing lists. The lists of addresses were purchased from Knowledge Base Marketing, Inc., Richardson, TX, U.S. Throughout a one-year period, ten thousand letters, along with an invitation flyer in both English and Spanish, were mailed to Cuban Americans. Of the participants who received letters, 4% (n = 388) responded. To ascertain T2D status, each participant was asked for the age of diagnosis and initial treatment modalities. Only 18 out of 388 subjects did not qualify for the study: for not being Cuban Americans (n= 2), age younger than 30 years (n= 9), and having other chronic illnesses (n=7). Validated questionnaires were used to collect information on demographics, dietary intake and physical activities [19-21]. Participants were instructed to fast at least 8 hours prior to their blood collection. A written consent was obtained for this study. Subjects with T2D were matched for age and gender with subjects without diabetes. This study was approved by the Institutional Review Board at Florida International University (FIU). Seven participants reported not having diabetes but were reclassified because their lab results classified them as having T2D according to American Diabetes Association (ADA) standards. These subjects were given their laboratory results and referred to their physicians. For the present study, we included 341 subjects (172 with T2D /169 without T2D) due to biospecimen availability.

Anthropometric measurements and biospecimen analyses

Height, weight, and blood pressure were measured using standard methodology [14]. Twenty ml of venous blood was collected from each participant after an overnight fast (at least 8 hours) by a certified phlebotomist. Glucose levels in serum were quantified using hexokinase method. Whole blood was used to measure glycated hemoglobin (A1C) using Roche Tina Quant method (Laboratory Corporation of America, Lab Corp, FL). Automatic chemical analyzer was employed to determine high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC). Serum insulin levels were determined using the Human Insulin ELISA kit from Millipore (St Charles, MZ, USA). The updated homeostatic model assessment (HOMA2) is based on the Oxford University HOMA2 calculator (www.ocdem.ox.ac.uk) and is used to calculate HOMA2 indexes: HOMA2_IR (homeostatic model assessment of insulin resistance), HOMA2_IS (homeostatic model assessment of insulin sensitivity) and HOMA2_B (homeostatic model assessment of β-cell function) [22].

Isolation of DNA and genotyping

Genomic DNA was isolated from the whole blood using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), according to the vender's recommended protocol. Quality and quantity of the isolated DNA was assessed by using 2000c nanodrop spectrophotometer (Thermo Scientific, USA). Genotyping for all six SNPs was performed by real-time PCR amplification on BioRad CFX96 real time PCR instrument (Hercules, CA, USA) using commercially available TaqMan allelic discrimination assays (LifeTech, Foster City, CA, USA). To ensure reproducibility and reliability of genotyping

method, 10% of the DNA samples was duplicated during genotyping. We obtained a 100% concordance between the genotyped duplicate samples for the single nucleotide polymorphism (SNP).

Statistical Analysis

Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-tailed, and the threshold for statistical significance was set at $P \leq 0.05$. Current sample size has 80% statistical power for significance threshold of 0.05. Genotype counts in each SNP was checked for Hardy-Weinberg equilibrium (HWE) in controls using the Chi-squared goodness-fit test. The distributions of the T2D related quantitative traits, such as HOMA2 index, FPG, A1C, etc., were markedly skewed, which were normalized, to a large extent, by their logarithmic transformation. Therefore, student's t-tests on continuous values of these quantitative traits were performed on logarithmically transformed values. Demographic and clinical information between cases and controls was compared using student's t-test for continuous variables, and Chi-squared test for categorical variables. All genetic associations were assessed by using the three genetic models: dominant, additive and recessive model. Logistic regression models were used to calculate unadjusted and adjusted odds ratio (OR) and 95% confidence intervals (CIs) to assess the relationship of all SNPs with binary outcome for case-control status (T2D=Yes/No) before and after adjusting for potential confounding factors such as age, sex, body mass index (BMI), physical activities and calorie intake. Linkage disequilibrium and haplotype analysis were performed using Haploview 4.2 (Cambridge, MA, USA) [23].

Results

This study included N=341 Cuban Americans (n=222 female and n=119 male) with (n=172) and without (n=169) T2D. Characteristics of the participants were presented as comparison of means±SD by diabetes status (Table 1). The mean of age among participants without diabetes was 62.98±10.85 and 65.05±11.95 for participants with diabetes. Compared to adults without diabetes, significantly higher values in the following attributes are found in adults with diabetes: waist circumference, body mass index, HDL-cholesterol, fasting plasma glucose, AIC (%), insulin, and HOMA2_IR, while adults with diabetes had significantly lower diastolic blood pressure, triglycerides, HOMA2_B, HOMA2_IS, total kcal intake and weekly physical activities.

Table 2 shows the distribution of allele frequencies and the genotypes of three genetic models in studied population. The genotypic distribution for each of the SNPs was in agreement with the predicted Hardy-Weinberg equilibrium in subjects without T2D (controls). Results showed that for the SNP rs12255372 (G>T), additive model, recessive model and allele frequency demonstrated significant differences between subjects with T2D and controls (p=0.0005, p=0.0002 and p=0.0004, respectively); the risk genotype TT and the T allele of the SNP rs12255372 was significantly higher in subjects with T2D than in controls. Similar significant results were also found for SNP rs7903146 (C>T), rs7901695 (T>C), and rs4506565 (A>T). We didn't find any significant results for SNP rs11196205 and rs7895340.

Table 3 shows results from unconditional logistic regression with/without adjustment by covariates (BMI, age, gender, physical activity, and calories intake) in three genetic models. Consistent with the results from genotype distribution analyses, all

four above mentioned SNPs showed significant results in at least two genetic models. After multivariable adjustment, the results stayed significant. Of note, the significance increases from dominant model, to additive model and to recessive model, with recessive model showing the most significant results. For the SNP rs12255372 (G>T), the adjusted odds ratio (aOR) in the additive model was 3.24 (p=0.0026) for carrying the risk TT genotype, compared to subjects with GG genotype. For the SNP rs7903146 (C>T), the aOR in additive model was 2.57 (p=0.014) for carrying the risk TT genotype, compared to subjects with CC genotype. For SNP rs7901695 (T>C), the aOR in additive model was 2.31 (p=0.018) for carrying the risk CC genotype, compared to subjects with TT genotype. For the SNP rs4506565 (A>T), the aOR in additive model was 2.38 (p=0.016) for carrying the risk TT genotype compared to subjects with AA genotype. We didn't find any significant results for *TCF7L2* SNP rs11196205 and rs7895340, with or without multivariable adjustment.

Table 4 shows T2D related metabolic traits between non-risk allele carriers and risk allele carriers of SNP rs7903146, SNP rs7901695 and SNP rs4506565 in non-diabetic controls. For all three SNPs, the fasting glucose level was significantly lower in non-risk allele carriers, compared to the risk allele carriers (p=0.019, p= 0.013 and p=0.011, respectively). Higher level of A1C was also observed in allele carriers of all three SNPs with marginal significance (p=0.069 p=0.067, and p=0.089, respectively).

In the haplotype-based case-control analyses, two blocks were defined by using Four Gamete method (Figure 1) [24]. The first block was established by three consecutive SNPs: rs7901695, rs450565 and rs7903146. The second block was established by two consecutive SNPs: rs7895340 and rs11196205. SNPs within each block were in a strong

linkage disequilibrium. The haplotype association test identified two haplotypes TAC (frequency=56.4%, p=0.0094) and CTT (frequency=39.8%, p=0.0044) within the first block were strongly associated with the risk of T2D, with TAC haplotype significantly lower and CTT haplotype significantly higher in participants with T2D, when compared to controls. After performing permutation test (n=1000), the significance remained for the two haplotype combinations (p=0.045, and p=0.031, respectively)

Discussion

Our study found that out of 6 SNPs, four SNPs (rs7901695, rs4506565, rs7903146 and rs1125537) in *TCF7L2* gene were significantly associated with the risk of T2D in a Cuban American population. The association between the *TCF7L2* gene polymorphisms with T2D stayed significant after multivariable adjustments. In addition, we also found that in the non-diabetic controls, risk minor allele carriers of three *TCF7L2* SNPs (rs7901695 (T>C), rs4506565 (A>T) and rs7903146 (C>T) had significantly higher fasting glucose level and a marginally significantly higher A1C%, compared to non-risk allele carriers. To the best of our knowledge, this was the first study to validate six previously most studied SNPs in *TCF7L2* gene in relation to the risk of T2D and T2D related quantitative traits in a homogeneous Cuban American population.

TCF7L2 gene spans a 215,863 bases region on chromosome 10q25.3 [25], and has 17 exons, of which five are alternative yielding different slicing possibility [26]. The association between *TCF7L2* gene and the risk of T2D was first discovered by deCODE group from an Iceland cohort study in 2006 [27]. They found that a microsatellite, DG10S478, located within the fourth intron region of the *TCF7L2* gene, was strongly associated with T2D susceptibility. Later on a few other polymorphisms in this gene have

been identified to be significantly associated with the T2D. Most significant polymorphisms were located within a genome area of 92-kb base pairs where the fourth intron and left flanking of the fifth intron are located within [28, 29]. Multiple lines of studies suggested this SNP-containing interval might exert an important role in regulation of *TCF7L2* gene expression. Savic et al. demonstrated this region contains a strong regulatory enhancer by using bacterial artificial chromosome assay in a transgenic mice model [30]. Kennell and Locke research group found that this region also contains a signal for alternative polyadenylation for production of a truncated, inhibitory TCF7L2 isform lacking HMG DNA-binding domain [31, 32]. Moreover, another independent study identified that the fifth intron of the *TCF7L2* gene harbors a highly conserved promoter Ex1b-e for a dominant-negative isoform of TCF7L2 which lacks the beta-cat binding domain [33]. Studies proposed that the variants on *TCF7L2* gene might have direct or indirect effect on incretin hormone regulation, β-cell function, and hepatic glucose production [9, 34, 35]. However, the exact mechanism of how the polymorphisms in this gene influence gene production is not fully discovered.

Among *TCF7L2* gene association studies with T2D and T2D related phenotypes, SNP rs7903146, which is located within the fourth intron, is the most studied and consistently replicated [35, 36]. By far, there is only one GWAS study in Hispanic/Latino individuals included Cuban American as a subgroup and showed that SNP rs7903146 in *TCF7L2* gene reached genome-wide significance [11]. Consistently with this finding, the present study found SNP rs7903146 was significantly associated with the risk of T2D in Cuban Americans. We found the risk allele T was significantly higher in participants with T2D than controls, and the risk TT genotype carriers have a 157 % increased risk of

developing T2D compared to non-risk allele carriers under the additive model. To date, the exact mechanism through which the SNP rs7903146 influenced the glucose homeostasis is not fully understood. Previous function studies regarding this SNP showed that T2D patients with CT/TT genotypes had increased mRNA levels of *TCF7L2*, associated with impaired insulin secretion and incretin effects [37]. Lyssenko also found that the risk T allele of SNP rs7903146 was associated with enhanced rates of hepatic glucose production. Schafer et al. found that there is a significant reduction in glucagon-like peptide-1-induced insulin secretion in people with risk alleles of SNP rs7903146 [38]. In the present study, we found SNP rs7903146 risk allele carriers had a significantly higher fasting glucose and a higher A1C (marginally significant), compared to non-risk allele carriers in non-diabetic controls. The Population Architecture using Genomics and Epidemiology (PAGE) study found that the SNP rs7903146 risk allele T was marginally significantly associated with increased fasting glucose level in European Americans but no association was found in Mexican Americans [39]. Another recent evidence also linked SNP rs7903146 risk allele T to altered blood glucose levels [40]. They found that T allele carriers of SNP rs7903146 had increased mean nocturnal glucose level, compared to non-T allele carriers. In addition, a genome-wide association meta-analysis has identified SNP rs7903146 has a strong significant association with of AIC% in three isolated populations: the Orkney Isles in the north of Scotland, the Dalmatian islands of Vis, and Korčula in Croatia [41]. Together, those evidence suggested that SNP rs7903146 has a potential impact on glucose homeostasis, possibly through the pathways mentioned above and warrants further investigations in Cuban American population.

Another most validated SNP in *TCF7L2* gene is SNP rs12255372. Based on a recent data analysis, twenty-four (60.0%) of the 40 studies showed significant positive associations, with the summary OR of 1.33 (95% CI: 1.27–1.40) [42]. So far, few studies investigated this SNP with the risk of diabetes in Hispanic populations, and results are inconsistent. A study of Mexican Americans showed that SNP rs12255372 of *TCF7L2* was significantly associated with the risk of gestational diabetes and interacts with adiposity to alter insulin secretion [43], whereas another study in Venezuelans showed that no significant association between SNP rs12255372 and the risk of T2D [44]. Another study in Mexican children showed that SNP rs12255372 TT genotype, generally considered as risk-conferring genotype for T2D in most previous studies, was significantly associated with lower fasting plasma glucose and lower homeostasis model of insulin resistance (HOMA2-IR) [45]. In our study, SNP rs12255372 allele T yielded a significant association with the risk of T2D. The risk allele T was significantly higher ($p=0.0004$) in patients with T2D compared to controls, and TT genotype carriers had 224% higher risk of developing T2D compared to non-risk allele carriers under the additive model. Based on the present study, results from our study indicated the independent contribution of SNP rs12255372 to the risk of T2D in the Cuban American population and suggested potential population stratification effect and consequently biased results when treating the heterogeneous Hispanic population as a single population for genetic studies [46].

In addition, we also identified two other SNPs rs4506565 and SNP rs7901695 that are significantly associated with the risk of T2D in Cuban Americans. The minor alleles in both SNPs (rs4506565 (A>T) and rs7901695 (T>C)) were significantly higher in

subjects with T2D than controls, and the homozygous minor allele genotype of SNP rs4506565 carriers have a 138 % increased risk of developing T2D compared to non-risk allele carriers under the additive model while 131% increased risk for participants with the homozygous minor allele genotype of SNP rs7901695. Previous studies in Caucasian and Asian populations observed significant association between these two SNPs and the risk of T2D [12]. To our best knowledge, only one genetic study on SNP rs7901695 and one on SNP rs4506565 confirmed their association to the risk of gestational diabetes in the Hispanic/Latino population [12, 42]. The missing association between these two SNPs and T2D in the Hispanic population could be due to different genetic architecture of sub-Hispanic groups. The relationship between these two SNPs and the risk of T2D is yet to be determined in Cuban Americans. Two functional investigations showed that SNP rs7901695 risk alleles is significantly associated with the increased proinsulin: insulin ratio and suggested this risk variants detrimental influence on beta-cell functions [36, 47]. In line with these findings, our study demonstrated that the fasting glucose level is significantly higher, as well as A1C level marginally significantly higher in risk-allele carriers of SNP rs7901695, compared to the non-risk allele carriers in participants without T2D. In our study, SNP rs4506565 located in a strong linkage disequlibrium with SNP rs7901695 (r^2=0.99). As expected, similar significant results were found for SNP rs4506565. The association between SNP rs4506565 and the risk of T2D have been previously reported in some studies, but few attempts have been made to explore the underlying mechanism for this association, which warrants further investigation [48-50]. One study conducted in Indian patients with T2D who were treated with sulfonylureas suggested that carriers with SNP rs4506565 TT genotype has a higher success rate of

treatment under the recessive model [51]. In our study, results from pairwise linkage disequilibrium analysis showed that these two SNPs (rs7901695 and rs4506565) are in a strong linkage disequilibrium with SNP rs7903146 (r^2=0.863). Two haplotypes TAC and CTT, formed by these three *TCF7L2* SNPs (rs7901695, rs450565 and rs7903146), were strongly associated with the risk of T2D (p=0.0094, and p=0.0044, respectively) in our studied population, with the frequency of TAC significantly lower and CTT significantly higher in subjects with T2D, compared to controls. The results of the haplotype analysis were consistent with the results from individual SNP analysis that C allele of SNP rs7901695, T allele of SNP rs4506565 and T allele of SNP rs7903146 were the risk alleles for T2D in our studied population.

Our study has several strengths. T2D is a heterogeneous disorder with contributions from peripheral insulin resistance and β-cell dysfunction. In the present study, in addition to the association analyses of *TCF7L2* SNPs in relationship to the risk of T2D, we also investigated some T2D related quantitative intermediate phenotypes (e.g. fasting glucose level and A1C) in control subjects. The consistent results from both analyses validated our findings. More than half of Cuban Americans who live in the United States are geographically concentrated in Miami-Dade and Broward Counties, Florida [52]. In this study, both cases and controls were selected from the same population pool and geographic area, with information on ethnicity up to two generations. Also, over 95% of our participants were white Cuban Americans. Together, these make our sample a relatively homogenous which consequently reduced the possibility of gene admixture with other ethnicities and the false positive rate due to population stratification [53]. This study also has some limitations. When we conducted the analyses on T2D related

quantitative traits in control subjects, due to the relatively small sample size in control group, the genetic variants with modest effect on the T2D related quantitative traits might be omitted. As the study was focused on the one gene, the influence of gene-gene interactions was not considered. Due to all of our participants recruited from Miami metropolitan area, the generalization of our results to other Cuban Americans residing outside this area should be made with caution and need further validation.

Conclusions

We found that of the six SNPs, four SNPs (rs7901695, rs4506565, rs7903146 and rs1125537) in the *TCF7L2* gene were significantly associated with the risk of T2D in Cuban Americans living in Broward/Miami-Dade counties, Florida. After multivariable adjustment, the association between the *TCF7L2* gene polymorphisms with the risk of T2D remained significant. We also found that among non-diabetic controls, risk minor allele carriers of SNP rs7901695 (T>C), SNP rs4506565 (A>T) and SNP rs7903146 (C>T) have significantly higher fasting glucose level and a marginally significantly higher A1C% level, compared to non-risk allele carriers. In addition, we identified two haplotypes TAC and CTT, formed by three *TCF7L2* SNPs (rs7901695, rs450565 and rs7903146), were strongly associated with the risk of T2D ($p=0.0094$, and $p=0.0044$, respectively), with the frequency of TAC significantly lower and CTT significantly higher in subjects with T2D, compared to controls. Validation and generalization of the results from previous genetic studies of other ethnic populations provide important public and clinical implications in T2D prevention and treatment in Cuban American population.

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Table 1. Anthropometric and demographic characteristics of all participants

	Without T2D (n=172)	With T2D (n=169)	*p** value
Age (Years)	62.98±10.85	65.05±11.95	0.095
Female (N (%))	115 (66.86%)	107 (63.31%)	0.49
BMI (kg/m2)	29.58±1.18	30.92±1.22	0.026
Waist circumference (cm)	99.54±1.13	104.16±1.14	0.0011
SBP (mm of Hg)	129.76±1.15	131.91±1.12	0.23
DBP (mm of Hg)	81.55±1.12	79.15±1.12	0.016
Total cholesterol (mg/dl)	187.65±1.26	190.66±1.22	0.49
Triglycerides (mg/dl)	112.6±1.67	93.04±1.56	0.0003
HDL-C (mg/dl)	47.14±1.32	53.7±1.28	<.0001
LDL-C (mg/dl)	109.55±1.51	113.77±1.32	0.32
FPG (mmol/L)	95.51±1.14	135.42±1.49	<.0001
A1C (%)	5.89±1.07	7.51±1.22	<.0001
Insulin	12.37±1.83	16.14±1.95	0.0007
HOMA2_B	115.48±1.52	70.06±2.22	<.0001
HOMA2_IR	1.56±1.78	2.14±2.00	<.0001
HOMA2_IS	64.07±1.77	46.6±2.00	<.0001
kcal	2235.68±1.41	1993.01±1.47	0.0039
PA_MET_wk	21.91±4.22	13.45±4.62	0.0075

*Values are unadjusted mean ± SD for continuous variables or N for categorical variables. * Derived from t-test for means or chi-square test for percentage differences between cases and controls. BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood; HDL-C= high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol;FPG= fasting plasma glucose; A1C= hemoglobin A1C; PA_MET_wk= physical activity measured by metabolic equivalents (MET) per week*

Table 2. Distribution of allele frequencies and the genotypes of three genetic models in our studied population.

SNPs	Number (%)		*p** values
rs12255372	Without T2D n (%)	With T2D n (%)	
Dominant Model			
GG	74 (43.27)	55 (32.54)	0.054
GT+TT	97 (56.73)	114 (67.46)	
Additive Model			
GG	74 (43.02）	55 (32.54)	
GT	80 (46.51)	70 (41.42)	0.0005
TT	17 (9.88)	44 (26.04)	
Recessive Model			
GG+GT	154 (90.06)	125 (73.96)	0.0002
TT	17 (9.88)	44 (26.04)	
Allele Model			
G	228 (66.67)	180 (53.25)	0.0004
T	114 (33.33)	158 (46.75)	
rs11196205	Without T2D n (%)	With T2D n (%)	
Dominant Model			
CC	60 (34.88)	59 (34.91)	0.92
GC+GG	112 (65.12)	110 (65.09)	
Additive Model			
CC	60 (34.88)	59 (34.91)	
GC	75 (43.60)	82 (48.52)	0.46
GG	37 (21.51)	28 (16.57)	
Recessive Model			
CC+GC	135 (78.49)	141 (83.43)	0.063
GG	37 (21.51)	28 (16.57)	
Allele Model			
C	195 (56.69)	200 (59.17)	0.51
G	149 (43.31)	138 (40.83)	
rs7903146	Without T2D n (%)	With T2D n (%)	
Dominant Model			
CC	76 (44.19)	58 (34.32)	0.079
CT+TT	96 (55.81)	111 (65.68)	
Additive Model			
CC	76 (44.19)	58 (34.32)	0.018
CT	73 (42.44)	69 (40.83)	

TT	23 (13.37)	42 (24.85)	
Recessive Model			
CC+CT	149 (86.63)	127 (75.15)	0.010
TT	23 (13.37)	42 (24.85)	
Allele Model			
C	225 (64.84)	185 (54.73)	0.0044
T	119 (35.16)	153 (45.27)	
rs7901695	Without T2D n (%)	With T2D n (%)	
Dominant Model			
TT	68 (39.53)	55 (32.54)	0.22
TC+CC	104 (60.47)	114 (67.46)	
Additive Model			
TT	68 (39.53)	55 (32.54)	
TC	75 (43.60)	65 (38.46)	0.028
CC	29 (16.86)	49 (28.99)	
Recessive Model			
TT+TC	143 (83.14)	120 (71.01)	0.011
CC	29 (16.86)	49 (28.99)	
Allele Model			
T	211 (61.34)	175 (51.78)	0.012
C	133 (38.66)	163 (48.22)	
rs7895340	Without T2D n (%)	With T2D n (%)	
Dominant Model			
AA	58 (33.72)	56 (33.14)	0.91
GA+GG	114 (66.28)	113 (66.86)	
Additive Model			
AA	58 (33.72)	56 (33.14)	
GA	73 (42.44)	85 (50.30)	0.18
GG	41 (23.84)	28 (16.57)	
Recessive Model			
AA+GA	131 (76.16)	141 (83.43	0.12
GG	41 (23.84)	28 (16.57)	
Allele Model			
A	189 (54.94)	197 (58.28)	0.38
G	155(45.06)	141(41.72)	
rs4506565	Without T2D n (%)	With T2D n (%)	
Dominant Model			
AA	69 (40.12)	54 (31.95)	0.14
AT+TT	103 (59.88)	115 (68.05)	
Additive Model			

AA	69 (40.12)	54 (31.95)	
AT	74 (43.02)	66 (39.05)	0.025
TT	29 (16.86)	49 (28.99)	
Recessive Model			
AA+AT	143 (83.14)	120 (71.01)	0.011
TT	29 (16.86)	49 (28.99)	
Allele Model			
A	212 (61.63)	174 (51.48)	0.0075
T	132 (38.37)	164 (48.52)	

***** *Derived from chi-square test.*

Table 3. Unconditional logistic regression with/without adjustment by covariates in three genetic models

rs12255372	Unadjusted OR (95% CI)	*p** values	Adjusted OR (95% CI)	*p*[&] values
Dominant Model				
GG	Ref	0.042	reference	0.040
GT+TT	1.58 (1.02~2.46)		1.61 (1.02~2.54)	
Additive Model				
GG	reference	0.0007	reference	0.0026
GT	1.18 (0.73~1.89)		1.24 (0.76~2.02)	
TT	3.48 (1.80~6.74)		3.24 (1.65~6.36)	
Recessive Model				
GG+GT	reference	0.0002	reference	0.0008
TT	3.19 (1.74~5.85)		2.88 (1.55~5.3)	
rs7903146	Unadjusted OR (95% CI)	*p** values	Adjusted OR (95% CI)	*p*[&] values
Dominant Model				
CC	reference	0.063	reference	0.031
CT+TT	1.52 (0.98~2.35)		1.65 (1.05~2.6)	
Additive Model				
CC	reference	0.020	reference	0.014
CT	1.24 (0.77~1.99)		1.35 (0.83~2.21)	
TT	2.39 (1.30~4.42)		2.57 (1.37~4.84)	
Recessive Model				
CC+CT	reference	0.0078	reference	0.0076
TT	2.14 (1.22~3.75)		2.19 (1.23~3.9)	
rs7901695	Unadjusted OR (95% CI)	*p** values	Adjusted OR (95% CI)	*p*[&] values
Dominant Model				
TT	reference	0.18	reference	0.11
TC+CC	1.36 (0.87~2.11)		1.46 (0.92~2.31)	
Additive Model				
TT	reference	0.029	reference	0.018
TC	1.07 (0.66~1.74)		1.14 (0.69~1.89)	
CC	2.09 (1.17~3.73)		2.31 (1.26~4.22)	
Recessive Model				
TT+TC	reference	0.0083	reference	0.0052
CC	2.01 (1.2~3.39)		2.15 (1.26~3.68)	
rs4506565	Unadjusted OR (95% CI)	*p** values	Adjusted OR (95% CI)	*p*[&] values

Dominant Model				
AA	reference	0.12	reference	0.069
AT+TT	1.43 (0.92~2.23)		1.53 (0.97~2.44)	
Additive Model				
AA	reference	0.027	reference	0.016
AT	1.14 (0.70~1.85)		1.21 (0.73~2.00)	
TT	2.16 (1.21~3.86)		2.38 (1.3~4.36)	
Recessive Model				
AA+AT	reference	0.0083	reference	0.0052
TT	2.01 (1.2~3.39)		2.15 (1.26~3.68)	

**P value and Odds ratios (OR) with 95% confidence interval (CI) were calculated using unconditional logistic models, and [&]further adjusted by BMI, gender, age, physical activity and calorie intake.*

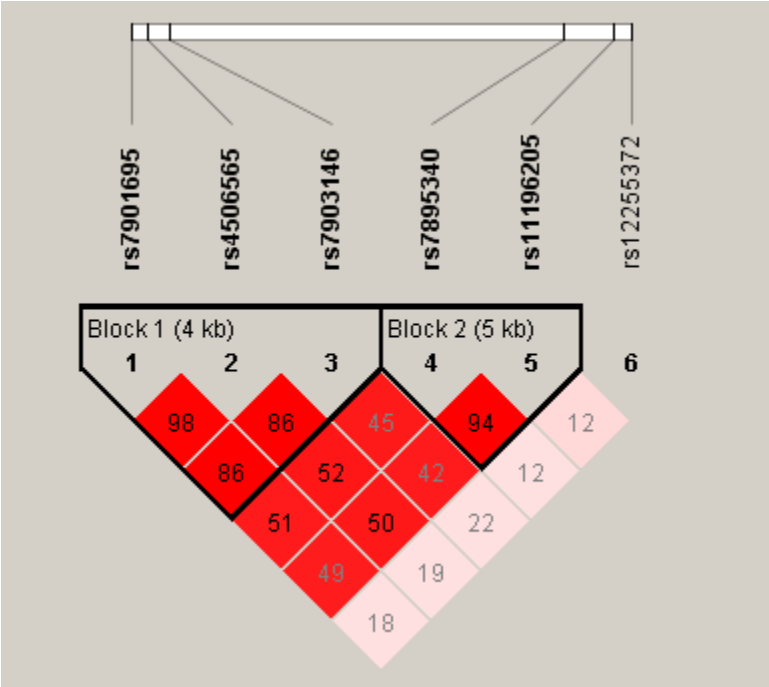
Table 4. Comparison of T2D related metabolic traits between non-risk allele carriers and risk allele carriers of SNPs rs7903146, rs7901695 and rs4506565 in non-diabetic controls.

rs7903146	CC (N=76)	CT/TT (N=96)	*p** values
BMI (kg/m2)	29.54±1.18	29.61±1.18	0.92
Waist circumference (cm)	99.47±1.11	99.6±1.14	0.94
SBP (mm of Hg)	128.56±1.15	130.71±1.14	0.43
DBP (mm of Hg)	81.82±1.13	81.33±1.11	0.73
Total cholesterol (mg/dl)	187.75±1.26	187.58±1.25	0.98
Triglycerides (mg/dl)	114.02±1.69	111.5±1.65	0.78
HDL-C (mg/dl)	47.14±1.33	47.14±1.31	1.00
LDL-C (mg/dl)	107.93±1.59	110.83±1.45	0.68
FPG (mmol/L)	93±1.16	97.48±1.12	0.019
A1C (%)	5.82±1.07	5.93±1.07	0.069
Insulin	12.07±1.78	12.61±1.87	0.66
HOMA2_B	119.15±1.53	112.77±1.5	0.41
HOMA2_IR	1.48±1.71	1.63±1.82	0.30
HOMA2_IS	67.41±1.7	61.55±1.81	0.32
rs7901695	TT (N=68)	TC/CC (N=104)	*p** values
BMI (kg/m2)	29.38±1.18	29.71±1.18	0.67
Waist circumference (cm)	99.2±1.11	99.77±1.14	0.76
SBP (mm of Hg)	127.98±1.15	130.93±1.15	0.28
DBP (mm of Hg)	81.13±1.13	81.82±1.11	0.62
Total cholesterol (mg/dl)	188.56±1.26	187.07±1.25	0.83
Triglycerides (mg/dl)	111.52±1.66	113.3±1.67	0.84
HDL-C (mg/dl)	48.17±1.33	46.49±1.31	0.42
LDL-C (mg/dl)	107.97±1.62	110.58±1.44	0.71
FPG (mmol/L)	92.62±1.16	97.42±1.12	0.013
A1C (%)	5.82±1.07	5.93±1.07	0.067
Insulin	11.75±1.81	12.79±1.84	0.41
HOMA2_B	118.42±1.56	113.67±1.48	0.54
HOMA2_IR	1.45±1.72	1.65±1.8	0.16
HOMA2_IS	69.09±1.72	60.97±1.79	0.18
rs4506565	AA (N=69)	AT/TT (N=103)	*p** values
BMI (kg/m2)	29.33±1.18	29.75±1.18	0.58
Waist circumference (cm)	99.27±1.11	99.73±1.14	0.79
SBP (mm of Hg)	127.78±1.14	131.1±1.15	0.23
DBP (mm of Hg)	81.15±1.13	81.81±1.11	0.63
Total cholesterol (mg/dl)	189.05±1.26	186.74±1.25	0.73
Triglycerides (mg/dl)	113.53±1.69	111.99±1.66	0.86

HDL-C (mg/dl)	47.86±1.33	46.67±1.31	0.56
LDL-C (mg/dl)	108.13±1.61	110.5±1.44	0.74
FPG (mmol/L)	92.61±1.16	97.48±1.12	0.011
A1C (%)	5.82±1.07	5.93±1.07	0.089
Insulin	11.79±1.8	12.77±1.85	0.43
HOMA2_B	118.75±1.56	113.41±1.48	0.49
HOMA2_IR	1.45±1.72	1.64±1.8	0.18
HOMA2_IS	68.8±1.71	61.06±1.8	0.19

Values are unadjusted mean ± SD for continuous variables or N for categorical variables.
** From t-test. P<0.05 considered significant. BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood; HDL-C= high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; FPG= fasting plasma glucose; A1C= hemoglobin A1C;*

Figure 1. Haploview plot showing linkage disequilibrium (LD) with r^2 values and block structure for six selected SNPs of *TCF7L2* gene.



CHAPTER III INTERACTION BETWEEN *TCF7L2* POLYMORPHISM AND GLYCEMIC LOAD, DIETARY CARBOHYDRATE AND FIBER INTAKE ON THE RISK OF TYPE 2 DIABETES AND ITS ASSOCIATED QUANTITATIVE TRAITS IN CUBAN AMERICANS

Abstract

Objectives: Type 2 diabetes (T2D) is a multifaceted and chronic metabolic disorder that involves complex interaction between environmental factors and genetic predisposition. *TCF7L2* gene recently emerged as a top candidate gene for T2D. Studies suggested the *TCF7L2* involvement in incretin hormone regulation, β-cell function, and hepatic glucose production. Differences in dietary factors, particularly carbohydrate (CHO) intake that influences glycemic regulation, may modify some genetic association with T2D. Few studies investigated such interactions in relation to the risk of T2D and none has been done in Cuban Americans. We hypothesized that significant interaction exists between *TCF7L2* common polymorphisms SNP rs7903146 (C>T) and SNP rs7901695 (T>C) in *TCF7L2* gene and dietary factors in relation to the risk of T2D and related phenotypes in Cuban Americans.

Methods: We genotyped *TCF7L2* SNPs rs7903146 and rs7901695 from 341 Cuban Americans (169 with and 172 without T2D). Logistic regression method was used to assess the association between *TCF7L2* SNPs genotypes and the risk of T2D in a dominant model. Interaction was tested by including the product of genotype*dietary factors in the model. Subgroup analyses were conducted by strata (high vs low) of dietary factors including glycemic load (GL), carbohydrate, and dietary fiber intake.

Results: We found a significant interaction between dietary fiber intake and SNP rs7903146 polymorphism for the risk of T2D (p= 0.044). For subgroup analyses, SNP rs7903146 significantly associated with the risk of T2D (odds ratio (OR) = 2.43, p= 0.010) only for the subgroup of a higher dietary fiber intake, while no significant results were found for the subgroup with a lower dietary fiber intake. For SNP rs7901695, significant interaction was also found for fiber intake (p=0.014) as well as glycemic load (p=0.040). Similarly, significant associations were only found within higher intake groups of higher fiber (OR=2.45, p=0.011) and GL (OR=2.18, p=0.037) for SNP rs7901695. Additionally, consistent results were found for T2D related quantitative traits for both SNPs in control subjects. For SNP rs7903146, risk allele carriers had significant higher levels of A1C% compared to non-risk allele carriers in all higher intake groups. For SNP rs7901695, risk allele carriers had significantly higher means of A1C%, HOMA2_IR (homeostatic model assessment of insulin resistance) and ghrelin and significantly lower means of HOMA2_IS (homeostatic model assessment of insulin sensitivity) for all three higher intake groups. For lower intake groups of CHO and GL, compared to non-risk allele carriers, risk allele carriers for both SNPs had significant higher level of fasting blood glucose and significantly lower level of HOMA2_B (homeostatic model assessment of β-cell function).

Conclusions: Carbohydrate quality and quantity, and fiber content of the diet could modify the risk of T2D associated with *TCF7L2* SNPs rs7903146 and rs7901695 in Cuban Americans. Higher ORs for developing T2D was observed for risk allele carriers of both SNPs when reporting higher intake of CHO, dietary fiber or GL. Investigation of the mechanisms through which *TCF7L2* SNPs-diet interactions affect the risk of T2D

might yield new insights into the understanding of etiology of diabetes and have implications for prevention of T2D in individuals carrying *TCF7L2* risk allele(s) though dietary modification.

Introduction

Diabetes is a complex, multi-factorial metabolic disorder characterized by a relative deficiency in insulin action, characterized by hyperglycemia due to insulin resistance or diminished capacity by the pancreas to produce insulin [1]. Currently, diabetes is the fastest-growing epidemic and the International Diabetes Federation estimated that 425 million people live with diabetes in 2017 and this number is projected rise to 629 million by 2045 [2]. Type 2 Diabetes (T2D) is the most prevalent form, accounting for approximately 90% to 95% of all diagnosed cases and currently ranks seventh among the leading causes of death in the United States [3]. Heritability of T2D was estimated to be between 30% and 80% based on twin and familial studies [4-8]. High throughput genome-wide association studies (GWAS) have identified a number of variants associated with the risk of T2D but most reported variants have small to moderate effects for the development of T2D [9]. Some investigators speculated that the genetic predisposition to T2D would work synergically with the environmental facilitators, such as poor dietary, inactivity and obesity, to contribute to the development of T2D but few studies have been conducted in the area of interaction between these two [1].

TCF7L2 has emerged as one of the top candidate genes for T2D in the past decade since its first discovery by Decode group [10]. The *TCF7L2* gene product is a high-mobility box-containing group (HMG) transcription factor which is a ubiquitous protein

that belongs to a family of TCF/lymphoid enhancer factor [11]. Studies suggested TCF7L2 plays an important role in glucose homeostasis via regulation of the production of incretin hormone, β-cell functions and hepatic glucose production via Wnt signal pathway [12-14]. Insulin is secreted in response to elevated postprandial blood glucose that is directly linked to the quantity and quality of intake of carbohydrate, which could be mathematically determined by food's glycemic load. On the other hand, dietary fiber is an important factor to influence postprandial glucose response via slowing down or reducing the absorption of glucose [15]. It is biologically possible that dietary carbohydrate intake with different glycemic loads and fiber contents may modify the association between risk-conferring SNPs in *TCF7L2* gene and the risk of T2D. Several previous studies have demonstrated such modification effect of food-gene interaction on the risk of T2D [16-19].

Association of *TCF7L2* SNPs with the risk of T2D have been replicated in several populations. However, inconsistent replication of results is often present when studying genetic associations, possibly due to the ethnic diversity at the complex interplay between genetic and environmental factors. In the United States, Hispanics are often grouped together for research purpose, though many ethnicities exist within the Hispanic population and studies have demonstrated ethnic variations in many aspects such as disease mortality, health outcomes, behaviors, risk factors, demographics, and social determinants within Hispanic populations [20]. Among Hispanic population, Cuban Americans represent the third largest minority group in the United States and also have the highest proportion of the elderly population (26% for age 55 or older) compared to other Hispanic groups (11.9% for Mexicans and 16.1% for Puerto Rican) [21]. Although

Cuban Americans (9.0%) have a lower prevalence of T2D than Mexicans (13.8%) and Puerto Ricans (12.0%) but still 1.22 times higher than non-Hispanic whites (7.4 %) and a highest proportion of diabetes as the underlying cause of death (44%) as compared to other Hispanic groups (39% for Puerto Ricans and 37% for Mexican Americans [22, 23]. By far, existing studies have examined genetic association with T2D in Hispanic subgroup, of which majority were carried on populations of Mexican origin [24-26]. The disproportionally affected rates of T2D in Cuban Americans could be ascribed to increased genetic predisposition and its interaction with acculturation related unhealthy diet behavior (e.g. higher sugar and low fiber) but few studies have addressed this area [27]. In the present study, we hypothesized that there is a gene-diet interaction between different dietary intakes, particularly glycemic load (GL), carbohydrate (CHO) and fiber intakes, and variants in *TCF7L2* gene in incident type 2 diabetes.

Methodology

Subjects

This was a case-control study. The target population was Cuban American adults in Broward and Miami-Dade Counties, Florida. Approximately half of the participants had type 2 diabetes and the rest were free of diabetes. Participants were initially recruited from randomly generated mailing lists. The lists of addresses were purchased from Knowledge Base Marketing, Inc., Richardson, TX, U.S. Throughout a one-year period, ten thousand letters, along with an invitation flyer in both English and Spanish, were mailed to Cuban Americans. Of the participants who received letters, 4% (n = 388) responded. Interested participants were interviewed on the phone. The study purpose was

explained and preliminary demographics (age, sex and ethnicity) of the respondents were recorded to determine eligibility.

To ascertain T2D status, each participant was asked for the age of diagnosis and initial treatment modalities. Only 18 out of 388 subjects did not qualify for the study: for not being Cuban Americans (n= 2), age younger than 30 years (n= 9), and having other chronic illnesses (n=7). If a subject was determined to be eligible, then their participation was requested at the Human Nutrition Laboratory at Florida International University (FIU). Validated questionnaires were used to collect information on demographics and physical activities [28]. Participants were instructed to refrain from smoking, consuming any food and beverages except water, and doing any unusual exercise for at least 8 hours prior to their blood collection. Subjects with T2D were matched for age and gender with subjects without diabetes. This study was approved by the Institutional Review Board at FIU. The purpose and protocol of the study were explained to the subjects, and their written consent, either in Spanish or English, was obtained prior to the commencement of the study. Seven participants reported not having diabetes but were reclassified because their lab results classified them as having T2D according to American Diabetes Association (ADA) standards. These subjects were given their laboratory results and referred to their physicians. For the data analysis, subjects with missing genotyping information were excluded from analysis. In total, we included data from subjects with (n=172) and without (n=169) T2D who were 30 years and older.

Anthropometric measurements and medical assessment

A SECA balance scale was used to measure both height and weight (Seca Corp, US) which were later used to calculate body mass index (BMI) in kg/height in m^2.

Additionally, waist circumference (WC) to the nearest 0.1 cm was measured horizontally with a non-stretchable measuring tape placed midway between the 12th rib and iliac crest at minimal respiration and was used to determine central obesity (male=102 cm/ female=88 cm). Blood pressure (BP) measurement was repeated two times then averaged using a random zero sphygmomanometer (Tycos 5090-02 Welch Allyn Pocket Aneroid Sphygmomanometer, Arden, NC, USA) and a stethoscope (Littmann Cardiology, 3M, St Paul, MN, USA) in participants after a 15-minute rest while sitting. Presence of hypertension was established either systolic BP $\geq$ 140 mm Hg, diastolic BP $\geq$ 90 mm Hg or they were using antihypertensive medication.

Assessment of dietary intake

Dietary intake was determined using the semi-quantitative food frequency questionnaire (FFQ; 97GP 2006 version copyrighted at Harvard University, Boston, MA, U.S.), developed by Walter C. Willett, which has been validated by Nath and Huffman for Cuban Americans [29, 30]. On the FFQ, participants self-report average consumption of specified amounts of various foods and vitamins over the past year. Daily servings for food groups were calculated by summing frequency factors for all related food items. Macronutrient intakes were calculated by multiplying frequency of consumption by the nutrient value of the food item obtained from the Harvard University Food Composition Database. The Glycemic Load (GL) was calculated by multiplying the CHO content of one serving by the glycemic index value of the food and beverage. We then multiplied this GL value by the frequency of consumption and summed the products of all food and beverage items to produce the dietary GL.

Biospecimen analyses

Data for serum biomarkers were available from our parental database. Briefly, twenty ml of venous blood was collected from each participant after an overnight fast (at least 8 hours) by a certified phlebotomist. Glucose levels in serum were quantified using hexokinase method. Whole blood was used to measure A1C using Roche Tina Quant method (Laboratory Corporation of America, Lab Corp, FL, U.S.). Automatic chemical analyzer was employed to determine high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC). Serum insulin levels were determined using the Human Insulin ELISA kit from Millipore (St Charles, MZ, U.S.). The level of acylated ghrelin was assayed with fasted blood plasma by Enzyme Immunometric Assay (Cayman Chemical Inc., MI, US). The manufacturer's protocol was followed to perform this Sandwich ELISA assay.

Determination of beta cell function (HOMA2-β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS)

HOMA2 (Homeostatic model assessment 2) is based on the Oxford University HOMA2 calculator (www.ocdem.ox.ac.uk) [31] and is used to estimate beta cell function (HOMA2-β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS). The HOMA2 computer model accounts for variations in hepatic and peripheral glucose resistance (Rudenski, Matthews, Levy, & Turner, 1991). The computer model can be used to determine β-cell function (%B) insulin sensitivity (%S), and the insulin resistance index from paired fasting plasma glucose and radioimmunoassay insulin across a range of 1-2,200 pmol/l for insulin, and 1-25 mmol/l for glucose [31].

Isolation of DNA and Genotyping

Genomic DNA was isolated from the whole blood using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), according to the vender's recommended protocol. Quality and quantity of the isolated DNA was assessed by using 2000c nanodrop spectrophotometer (Thermo Scientific, USA). Genotyping for all six SNPs was performed by real-time PCR amplification on BioRad CFX96 real time PCR instrument (Hercules, CA, USA) using commercially available TaqMan allelic discrimination assays (LifeTech, Foster City, CA, USA). To ensure reproducibility and reliability of genotyping method, 10% of the DNA samples was duplicated during genotyping. We obtained a 100% concordance between the genotyped duplicate samples for the single nucleotide polymorphism (SNP).

Statistical Analysis

Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). All statistical tests were two-tailed, and the threshold for statistical significance was set at $P \leq 0.05$. Genotype counts in each SNP was checked for Hardy-Weinberg equilibrium (HWE) in controls using the Chi-squared goodness-fit test. The distributions of the T2D related quantitative traits, such as HOMA2 indexes, BMI, WC, et al, were markedly skewed, which were normalized, to a large extent, by their logarithmic transformation. Therefore, formal statistical tests on continuous values of these quantitative traits were performed on logarithmically transformed values. Demographic and clinical information between cases and controls was compared using student's t-test for continuous variables, and Chi-squared test for categorical variables. All genetic associations were assessed by using the dominant genetic model (noncarrier=0,

heterozygous and homozygous=1). Unconditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CIs) to assess the relationship of all SNPs with binary outcome for case-control status (T2D=Yes/No). Dietary factors including CHO intake, dietary fiber intake and glycemic load were dichotomously divided by median values. Gene-diet interactions on the risk of T2D was tested by including the product of genotype*dietary factor in the logistic model. Study subjects were further divided into two groups by strata of dietary factors. Within each category of dietary factors, logistic regression models were utilized to calculate odds ratios (OR) and 95% confidence intervals (CIs), adjusted for potential confounding factors such as age, sex, BMI, physical activities and calorie intake. To evaluate the relationship between SNPs and T2D related quantitative traits, the student's t-test methods was used again to compare the mean levels of T2D related quantitative traits such as age, sex, BMI, physical activities and calorie intake between risk allele carriers and non-carriers in control subjects, stratified by dietary factors. Quanto (http://biostats.usc.edu/Quanto.html) was used to calculate the statistical power for the gene-diet interaction with incident T2D. Assuming an OR of 0.90 for higher fiber intake and an OR of 1.5 for *TCF7L2* risk-conferring genotype (0.30 allele frequency, dominant model) on T2D incidence, at significant level of 0.05 (two sides), we had 80% power to detect an interaction of OR of at least 2.4 in T2D incidence.

Results

This study included N=341 Cuban Americans (n=222 female and n=119 male) with (n=172) and without (n=169) T2D. Characteristics of the participants were presented as

comparison of means ±SE by diabetes status (Table 1). The mean of age among participants without diabetes was 62.98±0.83 and 65.05±0.92 for participants with diabetes; where the majority were female in both groups. Compared to adults without diabetes, adults with diabetes had significantly higher means of BMI, WC, fasting plasma glucose, A1C (%), insulin, and HOMA2_IR, and significantly lower HOMA2_B, HOMA2_IS, CHO, GL and calorie intake.

Table 2 and Table 3 shows the interactions of *TCF7L2* SNP rs7903146 and rs7901695 with carbohydrate, fiber and GI intake on the risk T2D and results from logistic regression for all subjects and stratified by dietary factors, respectively. Overall, the risk-conferring genotype of SNP rs7903146 was significantly associated with the risk of T2D with the dominant model. Compared to non-risk allele carriers as reference, the adjusted odds ratio (OR) for risk allele carriers is 1.65 (CI: 1.05-2.60, p=0.031). A significant interaction was also found between dietary fiber intake and SNP rs7903146 polymorphism in relation to the risk of T2D (p= 0.044). For subgroup analysis by strata of dietary fiber, SNP rs7903146 (OR = 2.43, p = 0.010) was significantly associated with the risk of T2D only for the subgroup of a higher dietary fiber intake (dietary fiber intake ≥ median), while no significant result was found for the subgroup with a lower dietary fiber intake. Similar trends were also found when conducting subgroup analysis by strata of CHO and GL for SNP rs7903146. For SNP rs7901695, no significant association were found in relation to the risk of T2D in all subjects (p=0.11) by the dominant model. After including the gene X diet interaction term in the model, significant interaction was found for fiber intake (p=0.014) and glycemic load (p=0.040). In accordance to SNP rs7903146, significant results were only found within higher intake groups. In higher fiber intake

group and higher GL group, compared to non-risk carriers, the multi-variable adjusted OR for risk allele carriers were 2.45 (CI: 1.23-4.90, p=0.011) and 2.18 (CI: 1.05-4.54, p=0.037), respectively.

Table 4 and table 5 shows T2D related metabolic traits between non-risk allele carriers and risks allele carrier of SNPs rs7903146 and rs7901695 by strata of dietary factors in controls, respectively. Consistent results were found for higher intake groups of dietary factors for both SNPs. For SNP rs7903146, risk allele (T) carriers has significant higher levels of A1C% compared to non-risk allele carriers in all higher intake groups. For SNP rs7901695, risk allele (C) carriers had significantly higher means of A1C%, HOMA2_IR and ghrelin and significantly lower means of HOMA2_IS than non-risk allele carriers in all three higher intake groups. For lower intake groups of CHO and GL, compared to non-risk allele carriers, risk allele carriers for both SNPs has significant higher level of fasting blood glucose and significantly lower level of HOMA2_B. In addition, we found in our studied population, the investigated three dietary variables are highly correlated with each other, the Pearson correlation coefficient between each two dietary factors are 0.99 ($p<0.0001$) for CHO intake and GL, 0.74 ($p<0.0001$) for CHO intake and dietary fiber intake, and 0.66 ($p<0.0001$) for dietary fiber intake and GL. Similar trends found across the dietary factors could be partially due to the significant correlation amongst them.

Discussion

SNPs in the *TCF7L2* gene has the largest effect among the various susceptibility genes on type 2 diabetes and have been widely replicated associated with T2D [32]. In

addition, several studies revealed that variation in *TCF7L2* are associated with several glucose homeostasis related phenotypes such as impaired insulin secretion and incretin effects, reduced GLP-1-induced insulin, beta cell function, insulin sensitivity and resistance, and hepatic glucose production [33-35]. Considering insulin is regulated in response to the variation of blood glucose level and secretion of incretin hormones may vary by dosage of carbohydrates [36], dietary carbohydrate might modify the association between *TCF7L2* SNPs and the risk of T2D. In the present study, we found significant interactions between two *TCF7L2* SNPs (rs7903146 and rs7901695), dietary fiber and glycemic load in relation to the risk of T2D in Cuban Americans. Subgroup analysis by strata of dietary factors revealed that the increased risks were only significant among higher intake participants, compared to participants who reported lower intake of dietary factors.

Polymorphism rs7903146 is the most studied SNP in *TCF7L2* gene. Repeated replications of association with this SNP in relation to the risk of T2D have been reported in several populations, including Hispanic population [37]. Consistent with previous findings, our research replicated the association in Cuban Americans. Importantly, we also identified a significant interaction between this SNP and the dietary fiber intake ($p=0.044$) on the risk of T2D. The odds ratio increased from 1.65 (CI:1.05-2.60, $p=0.031$) for all subjects to 2.43 (CI:1.24-4.79, $p=0.010$) when analysis was conducted in the subgroup of higher fiber intake. Our result was in accordance with a previous study. Hindy et al. performed an investigation on a cohort of nearly 25,000 initially nondiabetic Swedish adults to assess interactions between dietary fiber and the *TCF7L2* rs7903146 variant on T2D incidence [18]. The authors reported the elevated risk of T2D with

rs7903146 increased with higher intake of dietary fiber. Two other studies also found significant interactions between whole grain intake and rs7903146 on the risk of T2D [15, 38]. Both studies demonstrated that the protective effect from whole grain intake against the development of T2D was negated by the risk T allele of rs7903146. Higher fiber intake has been associated with increased plasma short-chain fatty acids (SCFAs), which are byproduct of fiber fermentation in large intestine. It has been demonstrated that SCFAs can stimulate incretin hormone secretion from L-cells via G-protein-coupled receptor FFAR2 [39]. Thus, it could be speculated that risk allele carriers of rs7903146 have decreased protective effect from SCFAs induced incretin hormone secretion as rs7903146 has been previously reported with impaired incretin response [40, 41]. Though the interaction was not statistically significant between rs7903146 and the other two dietary factors (CHO intake, GL) for the risk of T2D, similar trend were identified when analysis by strata of these two dietary factors were conducted. For both dietary factors, the odds ratio for the risk of T2D increased from lower intake groups (OR=1.26, p=0.50 for CHO intake; OR=1.27, p=0.49 for GL) to higher intake groups (OR=2.08, p=0.040 for CHO intake; OR=2.08, p=0.041for GL). Findings from the present study were similar as a previous report from Nurses' Health Study. Researchers found a significant interaction between GL and a variant (rs12255373) in *TCF7L2* gene on the risk of T2D and the risk allele was reported to have a stronger association with type 2 diabetes among individuals with higher dietary glycemic load and glycemic index [19]. In the present study, we also found that in controls, risk allele carriers of rs7903146 had significantly higher A1C% level when reporting a higher intake of carbohydrate or GL. These results tentatively suggested that the risk T allele of rs7903146 might affect

specific metabolic processes to make an individual more susceptible to the harmful effects of environmental factors such as high consumption of carbohydrate dense food, as previous studies on the underlying mechanism demonstrated subjects with risk *TCF7L2* genotype exhibited substantially reduced insulin response to glucose, impairing β-Cell function and hepatic glucose production [35, 42]. In contrast to higher intake group of CHO or GL, in lower intake group of controls, significantly lower beta cell (HOMA2-B) founds were found in risk allele carriers of both studied SNPs, compared to non-risk allele carriers. It is biologically possible that deficit within genes might be more manifesting under the condition that the compensatory mechanism for the regulation of high blood glucose level hasn't get involved yet. Further investigation need to be performed to confirm this finding.

SNP rs7901695 is another most investigated SNP on *TCF7L2* gene. Significant associations were not only found between this SNP and the risk of T2D, recent studies also demonstrated significant associations with gestational and type 1 diabetes [26, 43]. In accordance with previous findings, our research replicated the association between rs7901695 and the risk of T2D in Cuban Americans. Consistent with the finding for rs7903146, a similar significant interaction with dietary fiber intake ($p=0.014$) was found for rs7901695, as well as the interactions with GL ($p=0.040$). To our best knowledge, this is the first report on the significant interactions between SNP rs7901695 and dietary factors on the risk of T2D. Results of logistic regression by strata of dietary factors (GL and dietary fiber intake) showed that ORs of developing T2D in relation to the risk genotype of rs7901695 substantially increased from lower intake group (OR=0.92, $p=0.80$ for fiber intake; OR=0.97, $p=0.93$ for GL) to higher intake group with significant

results only found in higher intake groups (OR=2.45, p=0.011 for fiber intake; OR=2.18, p=0.037 for GL). For the T2D related quantitative traits in control subjects, in addition to the finding that significantly higher A1C% level was found in risk allele carriers than that in non-carriers within higher intake groups of three dietary factors, HOMA2-IR and HOMA2_IS were also significantly different between risk allele carriers and non-carriers with HOMA2-IR significantly higher and HOMA2_IS significantly lower for risk allele carriers. HOMA model was recognized as robust clinical and epidemiological tool in descriptions of the pathophysiology of beta cell functions and insulin sensitivity [31]. Our results can roborate the findings from previous studies. An earlier investigation demonstrated that rs7901695 were significantly associated with insulin sensitivity by intravenous glucose tolerance tests in non-diabetic subjects [44]. A recent functional study also revealed the involvement of *TCF7L2* gene in whole-body glucose intolerance and hepatic insulin resistance [27]. Collectively, those evidence suggested the involvement of *TCF7L2* rs7901695, or SNPs in the adjacent region in the linkage disequilibrium with rs7901695, in the pathology of T2D in Cuban Americans, especially for people who has higher intake of CHO and/or dietary fiber.

Another novel finding from the present study is the significant difference on acylated ghrelin levels between rs7901695 risk allele carriers and non-risk allele carriers in controls when reporting higher intake of CHO, dietary fiber or GL. Similar trends was also found for rs7903146 but didn't reach significance. Ghrelin, also known as "the hunger hormone", was recognized as orexigenic hormone to stimulate food intake. The main site for ghrelin secretion is in stomach and pancreas, and to a lesser extent in the hypothalamus [45]. Previous studies by animal models and in vitro indicated ghrelin

plays an important role in glucose homeostasis via negatively influencing insulin secretion and insulin sensitivity in peripheral tissues but the mechanism between ghrelin and insulin resistance varied within humans [46-48]. Two more recent studies provided new insights regarding the role of ghrelin in incretin hormone secretion. Both studies suggested ghrelin could be a potential positive regulator for GLP-1 secretion [49, 50]. The present study found significantly higher ghrelin level in risk allele carriers of rs7901695, compared to non-risk allele carriers in controls of higher intake group of dietary factors. Considering accumulated evidence suggesting the involvement of *TCF7L2* in mediating function of the incretin hormones, the higher ghrelin level found in risk allele carriers could be the compensatory mechanism for people with defective incretin effects [13, 51, 52]. Further study on the crosstalk between incretins and ghrelin need to be further investigated.

Several limitations need to be considered when interpreting the results from the present study. Due to the relatively small sample size, the present study may not have enough statistical power to detect the significant interaction. To maximize the sample size within each category of dietary factors and genotype, dichotomy for dietary factors and dominant model was selected for the present study. Another limitation is the validity of dietary variable. Dietary intake was self-reported, it is possible that dietary intake may be over or underestimated. Food frequency questionnaires are not optimal for absolute intake but can provide population estimates and trend. The FFQ used in the present study has been previously validated in this population [53]. Finally, due to the nature of case-control study, the present study cannot provide information on causality. We acknowledge that our data on gene-diet interaction are preliminary and replication is required to

conform our findings. Our study also has some significant strengths. This is the first study to investigate the gene-diet interaction in Cuban Americans. Both cases and controls from the present study were selected from the same population pool and geographic area and over 95% of our participants were white Cuban Americans. Together, these make our sample a relatively homogenous in terms of genetic, life style factors and diet, which consequently reduced the possibility of gene admixture with other ethnicities and the false positive rate due to population stratification [54].

Conclusion

In conclusion, carbohydrate quality and quantity, and fiber content of the diet could modify the risk of T2D associated with *TCF7L2* SNPs rs7903146 and rs7901695 in Cuban Americans. Higher ORs for developing T2D was observed for risk allele carriers of both SNPs when reporting higher intake of CHO, dietary fiber or GL. Investigation of the mechanisms by which *TCF7L2* SNPs-diet interactions affect the risk of T2D might yield new insights into the understanding of etiology of diabetes and have implications for prevention of T2D in individuals carrying *TCF7L2* risk allele(s) though dietary modification.

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Table 1. Anthropometric, T2D related quantitative traits and dietary intake in all participants

	Without T2D (n=172)	With T2D (n=169)	*p** value
Age (Years)	62.98±0.83	65.05±0.92	0.095
Female (N (%))	114 (66.67%)	107 (63.31%)	0.52
BMI (kg/m2)	29.67±1.01	30.88±1.02	0.025
WC (cm)	99.63±1.13	104.16±1.14	0.0014
FPG (mmol/L)	95.58±1.01	135.64±1.03	<.0001
A1C (%)	5.87±1.01	7.54±1.02	<.0001
Insulin	12.33±1.05	16.12±1.06	0.0007
HOMA2_B	115.58±1.51	70.11±1.07	<.0001
HOMA2_IR	1.57±1.05	2.14±1.06	<.0001
HOMA2_IS	64.07±1.05	46.53±1.06	<.0001
CHO (g/day)	284.29±1.03	225.88±1.03	<.0001
GL	152.93±1.03	116.75±1.04	<.0001
Dietary fiber (g/day)	21.12±1.04	19.89±1.04	0.28
Calories (Kcal/day)	2230.54±1.03	1998.20±1.03	0.0039

*Values are unadjusted mean ± SE for continuous variables or N for categorical variables. *Derived from t-test for means or chi-square test for percentage differences between cases and controls. BMI= body mass index; WC= waist circumference; FPG= fasting plasma glucose; A1C= hemoglobin A1C; CHO= carbohydrate intake(g/day); GL= daily glycemic load.*

Table 2. Interactions of *TCF7L2* SNP rs7903146 with carbohydrate, fiber and GL intake on the risk T2D and logistic regression for all subjects and stratified by dietary factors

TCF7L2 SNP rs7903146 X dietary factors					
	Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p**	*p&* for interaction
All Subjects					
CC	76 (44.19)	58 (34.32)	reference	0.031	NA
CT+TT	96 (55.81)	111 (65.68)	1.65 (1.05-2.60)		
SNP X CHO					0.13
Low (< Median)					
CC	27 (40.30)	40 (38.10)	reference	0.50	
CT+TT	40 (59.70)	65 (61.90)	1.26 (0.64-2.46)		
High (≥ Median)					
CC	48 (46.60)	18 (28.13)	reference	0.04	
CT+TT	55 (53.40)	46 (71.88)	2.08 (1.04-4.18)		
SNP X Fiber					0.044
Low (< Median)					
CC	33 (40.74)	38 (40.86)	reference	0.62	
CT+TT	48 (59.26)	55 (59.14)	1.18 (0.61-2.25)		
High (≥ Median)					
CC	42 (47.19)	20 (26.32)	reference	0.01	
CT+TT	47 (52.81)	56 (73.68)	2.43 (1.24-4.79)		
SNP X GL					0.17
Low (< Median)					
CC	28 (42.42)	41 (38.68)	reference	0.49	
CT+TT	38 (57.58)	65 (61.32)	1.27 (0.65-2.45)		
High (≥ Median)					
CC	47 (45.19)	17 (26.98)	reference	0.041	
CT+TT	57 (54.81)	46 (73.02)	2.08 (1.03-4.20)		

**p value and Odds ratios (OR) with 95% confidence interval (CI) were calculated using unconditional logistic models, adjusted by BMI, gender, age, physical activity and calorie intake. &p for interaction was calculated by including the product of genotype*diet factor in the unconditional logistic model. CHO= carbohydrate intake(g/day); GL= daily glycemic load;*

Table 3. Interactions of *TCF7L2* SNP rs7901695 with carbohydrate, fiber and GL intake on the risk T2D and logistic regression for all subjects and stratified by dietary factors

TCF7L2 SNP rs7901695 X dietary factors					
	Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p**	*p&* for interaction
All Subjects					
TT	68 (39.53)	55 (32.54)	reference	0.11	NA
TC+CC	104 (60.47)	114 (67.46)	1.46 (0.92~2.31)		
SNP X CHO					0.086
Low (< Median)					
TT	24 (35.82)	39 (37.14)	reference	0.85	
TC+CC	43 (64.18)	66 (62.86)	1.07 (0.54-2.10)		
High (≥ Median)					
TT	43 (41.75)	16 (25.00)	reference	0.086	
TC+CC	60 (58.25)	48 (75.00)	1.87 (0.92-3.83)		
SNP X Fiber					0.014
Low (< Median)					
TT	28 (34.57)	37 (39.78)	reference	0.8	
TC+CC	53 (65.43)	56 (60.22)	0.92 (0.48-1.77)		
High (≥ Median)					
TT	39 (43.82)	18 (23.68)	reference	0.011	
TC+CC	50 (56.18)	58 (76.32)	2.45 (1.23-4.90)		
SNP X GL					0.040
Low (< Median)					
TT	24 (36.36)	41 (38.68)	reference	0.93	
TC+CC	42 (63.64)	65 (61.32)	0.97 (0.49-1.90)		
High (≥ Median)					
TT	43 (41.35)	14 (22.22)	reference	0.037	
TC+CC	61 (58.65)	49 (77.78)	2.18 (1.05-4.54)		

**p value and Odds ratios (OR) with 95% confidence interval (CI) were calculated using unconditional logistic models, adjusted by BMI, gender, age, physical activity and calorie intake. $p^{\&}$ for interaction was calculated by including the product of genotype*diet factor in the unconditional logistic model. CHO= carbohydrate intake(g/day); GL= daily glycemic load;*

Table 4. Comparison of T2D related metabolic traits between non-risk allele carrier and risk allele carrier of SNP rs7903146 by strata of dietary factors in subjects without T2D (controls).

				***TCF7L2* rs7903146**			
CHO (High (≥ Median))	CC	CT+TT	p value	**CHO (Low (< Median))**	CC	CT+TT	p value
FPG (mmol/L)	94.61±1.02	97.21±1.01	0.27	FPG (mmol/L)	89.60±1.03	98.17±1.02	0.0093
A1C (%)	5.80±1.01	6.03±1.01	0.0039	A1C (%)	5.85±1.01	5.81±1.01	0.67
Insulin	11.88±1.09	13.31±1.09	0.40	Insulin	12.39±1.11	11.76±1.10	0.74
HOMA2_B	111.07±1.06	117.73±1.06	0.94	HOMA2_B	137.02±1.1	106.32±1.07	0.029
HOMA2_IR	1.73±1.08	1.39±1.08	0.053	HOMA2_IR	1.67±1.11	1.52±1.11	0.56
HOMA2_IS	71.76±1.08	57.99±1.08	0.058	HOMA2_IS	60.26±1.11	66.10±1.11	0.56
AG	77.07±1.16	117.74±1.19	0.074	AG	61.42±1.20	67.71±1.21	0.72
Fiber (High (≥ Median))	CC	CT+TT	p value	**Fiber (Low (< Median))**	CC	CT+TT	p value
FPG (mmol/L)	93.53±1.02	97.89±1.02	0.093	FPG (mmol/L)	91.94±1.03	97.35±1.02	0.062
A1C (%)	5.82±1.01	5.99±1.01	0.044	A1C (%)	5.81±1.01	5.88±1.01	0.40
Insulin	11.24±1.09	12.82±1.09	0.33	Insulin	13.19±1.11	12.43±1.10	0.71
HOMA2_B	110.84±1.06	112.55±1.06	0.85	HOMA2_B	132.52±1.09	112.37±1.07	0.13
HOMA2_IR	1.36±1.09	1.65±1.08	0.092	HOMA2_IR	1.67±1.11	1.62±1.11	0.83
HOMA2_IS	73.83±1.09	60.92±1.08	0.093	HOMA2_IS	59.70±1.10	62.05±1.11	0.80
AG	67.24±1.18	105.95±1.23	0.095	AG	75.36±1.18	81.09±1.18	0.76
GL (High (≥ Median))	CC	CT+TT	p value	**GL (Low (< Median))**	CC	CT+TT	p value
FPG (mmol/L)	94.75±1.02	97.39±1.01	0.26	FPG (mmol/L)	89.56±1.03	97.95±1.02	0.0099
A1C (%)	5.80±1.01	6.01±1.01	0.0085	A1C (%)	5.84±1.01	5.82±1.01	0.86
Insulin	11.81±1.81	13.17±1.09	0.41	Insulin	12.50±1.11	11.86±1.11	0.75
HOMA2_B	110.17±1.06	116.42±1.05	0.49	HOMA2_B	137.84±1.10	107.36±1.07	0.035
HOMA2_IR	1.38±1.08	1.71±1.08	0.051	HOMA2_IR	1.68±1.11	1.53±1.12	0.57

HOMA2_IS	72.32±1.08	58.59±1.08	0.056	HOMA2_IS	59.85±1.11	65.64±1.12	0.57
AG	75.08±1.16	118.40±1.19	0.055	AG	64.69±1.20	66.00±1.21	0.94

*Values are unadjusted mean ± SE. * Derived from t-test for means. CHO= carbohydrate intake(g/day); GL= daily glycemic load; FPG= fasting plasma glucose; A1C= hemoglobin A1C; AG= acylated ghrelin. CHO= carbohydrate intake(g/day); GL= daily glycemic load.*

Table 5. Comparison of T2D related metabolic traits between non-risk allele carrier and risk allele carrier of SNP rs7901695 by strata of dietary factors in subjects without T2D (controls).

				***TCF7L2* rs7901695**			
CHO (High (≥ Median))	TT	TC+CC	p value	**CHO (Low (< Median))**	TT	TC+CC	p value
FPG (mmol/L)	94.14±1.02	97.33±1.01	0.21	FPG (mmol/L)	89.40±1.03	97.87±1.02	0.011
A1C (%)	5.80±1.01	6.01±1.01	0.015	A1C (%)	5.81±1.01	5.83±1.01	0.84
Insulin	11.63±1.1	13.39±1.09	0.30	Insulin	11.95±1.13	12.04±1.10	0.97
HOMA2_B	109.86±1.07	118.06±1.05	0.38	HOMA2_B	136.92±1.11	107.65±1.07	0.043
HOMA2_IR	1.34±1.09	1.74±1.08	0.021	HOMA2_IR	1.65±1.12	1.53±1.11	0.65
HOMA2_IS	74.39±1.09	57.58±1.07	0.024	HOMA2_IS	60.79±1.12	65.50±1.11	0.65
AG	72.85±1.14	117.08±1.19	0.034	AG	59.38±1.24	68.39±1.19	0.62
Fiber (High (≥ Median))	TT	TC+CC	p value	**Fiber (Low (< Median))**	TT	TC+CC	p value
FPG (mmol/L)	93.02±1.02	98.03±1.02	0.066	FPG (mmol/L)	91.63±1.03	97.09±1.02	0.10
A1C (%)	5.80±1.01	6.00±1.01	0.024	A1C (%)	5.81±1.01	5.87±1.01	0.50
Insulin	11.02±1.1	12.92±1.09	0.24	Insulin	12.82±1.13	12.70±1.09	0.95
HOMA2_B	110.23±1.07	112.93±1.05	0.77	HOMA2_B	132.66±1.10	113.81±1.06	0.17
HOMA2_IR	1.32±1.09	1.66±1.08	0.043	HOMA2_IR	1.65±1.12	1.63±1.10	0.95
HOMA2_IS	75.90±1.09	60.32±1.08	0.045	HOMA2_IS	60.46±1.11	61.48±1.10	0.91
AG	60.21±1.15	110.93±1.23	0.016	AG	78.23±1.21	78.75±1.16	0.98
GL (High (≥ Median))	TT	TC+CC	p value	**GL (Low (< Median))**	TT	TC+CC	p value
FPG (mmol/L)	94.1±1.02	97.67±1.01	0.16	FPG (mmol/L)	89.46±1.03	97.38±1.02	0.018
A1C (%)	5.80±1.01	6.00±1.01	0.012	A1C (%)	5.82±1.01	5.83±1.01	0.96
Insulin	11.38±1.10	13.30±1.08	0.21	Insulin	12.42±1.13	11.96±1.10	0.82
HOMA2_B	108.25±1.06	117.44±1.05	0.31	HOMA2_B	140.64±1.11	108.17±1.07	0.031

HOMA2_IR	1.31±1.09	1.75±1.07	0.0089	HOMA2_IR	1.72±1.12	1.52±1.11	0.45
HOMA2_IS	76.07±1.09	57.34±1.07	0.010	HOMA2_IS	58.36±1.12	66.08±1.11	0.46
AG	72.74±1.14	116.47±1.19	0.035	AG	59.53±1.24	68.88±1.19	0.61

*Values are unadjusted mean ± SE. * Derived from t-test for means. CHO= carbohydrate intake(g/day); GL= daily glycemic load; FPG= fasting plasma glucose; A1C= hemoglobin A1C; AG= acylated ghrelin. CHO= carbohydrate intake(g/day); GL= daily glycemic load.*

CHAPTER IV VARIANTS IN *TCF7L2* IN RELATION TO THE RISK OF TYPE 2 DIABETES AND RELATED METABOLIC TRAITS: DIFFERENCES AMONG POPULATIONS WITH AFRICAN ORIGINS IN SOUTH FLORIDA.

Abstract

Objectives: In the United States, Blacks are disproportionately affected by diabetes. 12.7% of non-Hispanic Blacks aged 18 years or older have diabetes, compared to 7.6% of non-Hispanic whites in 2015. To date, *TCF7L2* (transcription factor 7-like 2) confers the strongest statistical evidence for the association with the risk of type 2 diabetes (T2D). However, inconsistent replication of results is often present when studying genetic associations in Blacks, partially due to the subgroup heterogeneity within Blacks in the context of complex interaction between genetic predisposition and environmental factors. The primary purpose of this study was to examine six most studied SNPs in *TCF7L2* gene: rs7903146, rs12255372, rs11196205, rs7901695, rs7895340 and rs4506565 in relation to the risk of T2D and related metabolic quantitative traits in individuals with and without T2D in adults of African American (AA) and Haitian American (HA) from South Florida.

Methods: We conducted a case-control study with 245 AAs (119 without T2D /126 with T2D) and 240 HAs (117 without T2D /123 with T2D) and. Unconditional logistic regression method was used to assess the association between six *TCF7L2* polymorphisms and the risk of T2D in the dominant model adjusted by potential confounding factors. Subgroup analysis was conducted by strata of gender. Student's t-tests on continuous values of T2D related quantitative traits were compared between risk

allele carriers and non-carriers in control subjects. In addition, linkage disequilibrium and haplotype analysis were performed using Haploview 4.2**.**

Results: Adjusted logistic regression demonstrated that SNP rs7903146 (OR=2.01, p=0.065) minor allele showed a marginal significance with the increased risk of T2D in AAs; minor alleles of SNP rs11196205 (OR=0.58, p=0.059) and rs7895340 (OR=0.59, p=0.069) showed marginal significance with the protective effect for the risk of T2D in HAs. After stratification by gender, for both ethnicities, SNPs with marginal significance from the gender-combined analyses became statistically significant with the same trend for the risk of T2D when analyses were done in males: SNPs rs7903146 (OR=2.86, p=0.013) for AAs, rs11196205 (OR=0.35, p=0.033) and rs7895340 (OR=0.33, p=0.024) for HAs. In addition, SNP rs7901695 and rs4506565 (OR=2.69, p=0.025) were found to be significantly associated with the risk of T2D in AA males. In addition, T2D related quantitative trait analysis showed that compared to non-minor allele carriers (TT) of SNP rs12255372, minor allele carriers had significantly higher means of BMI (p= 0.036), DBP (p=0.0099), numbers of components of Mets (p< 0.0001.) and significantly lower mean values of HDL-C (p=0.020) and adiponectin (p=0.017) in AA non-diabetic controls.

Conclusions: The present study demonstrated ethno-specific genetic association between variants in *TCF7L2* gene and the risk of T2D and related metabolic traits among Haitian Americans and African Americans. Our results suggested potential issues regarding population stratification within Blacks. The lack of genetic association studies in Blacks, especially for Haitian Americans, warrants further replication study with larger samples.

Introduction

Diabetes is a significant public health issue globally. The International Diabetes Federation estimated that 425 million people live with diabetes in 2017 and this number is projected to rise to 629 million by 2045 [1]. Type 2 diabetes (T2D) accounts for 90% to 95% newly diagnosed diabetes. In the United States, approximately 30.3 million US people, or 9.4% of the U.S. population had diabetes and an estimated 33.9% of U.S. adults aged 18 years or older (84.1 million people) had prediabetes [2]. African Americans are disproportionately affected by diabetes. 12.7% of African Americans aged 18 years or older have diabetes, compared to 7.4% of non-Hispanic whites in 2015 [2]. African Americans are almost twice as likely to be diagnosed with diabetes as non-Hispanic whites. In addition, they were more likely to suffer from complications of diabetes, such as high blood pressure end-stage renal disease and lower extremity amputations and were twice as likely as non-Hispanic Whites to die from complications of diabetes [3].

Because of the high prevalence of diabetes in the African American community, it has been suggested that African Americans may be more susceptible to the disease compared with Whites through direct genetic propensity or gene–unfavorable environmental interactions (e.g. high fat and sugar intake) [4, 5]. Strong genetic component to T2D have been discovered by family history and concordance studies [6-10]. Recent genetic studies, especially high throughput genome-wide association studies (GWAS), have identified a multitude of variants associated with T2D. To date, *TCF7L2* (transcription factor 7-like 2) confers the strongest statistical evidence for the association with the risk of T2D. *TCF7L2* spans a 215,863 bases region on chromosome 10q25.3,

and has 17 exons, of which five are alternative yielding different slicing possibility [11, 12]. The *TCF7L2* gene product is a high-mobility box-containing transcription factor which is a ubiquitous protein that belongs to a family of TCF/lymphoid enhancer factor [13]. TCF7L2, together with β-catenin is a well-known bipartite transcription factor in the canonical Wnt pathway. Efforts to elucidate the potential role of TCF7L2 in the Wnt signaling pathway related to incretin hormone regulation, β-cell proliferation and apoptosis, and glucose production have been documented [14-16]. The involvement of TCF7L2 in all these pathway puts this gene in a critical role suggesting strong relationship of polymorphisms of *TCF7L2* with type 2 diabetes (T2D). In addition, more recent studies also revealed that potential role of TCF7L2 in adipogenesis and demonstrated variants in this gene could modify the risk of developing obesity and Metabolic Syndrome [17-20].

Although associations between various SNPs in *TCF7L2* and T2D were observed across ethnically diverse populations, results from studies conducted in African Americans have been inconsistent [21]. Currently in the United States, all Blacks, regardless of ancestry or country of origin, have been grouped as one category for research purpose. This practice could mask the true association among diverse subgroups within Black population due to genetic heterogeneity. Beyond genetic variation, environmental factors, especially diet pattern, lifestyle, and physical activity among subgroups of Blacks may modify the risk of T2D for people carrying deleterious genes based on “Thrift Gene” theory and consequently complicated the results from genetic association studies in Black people [22, 23]. Haitian Americans are one of the fastest growing Caribbean immigrant populations in the United States. According to the U.S.

Census, an estimated 929,074 Haitian Americans were living in the U.S. in 2013, of which approximately half of them reside in Florida, and nearly one third (~290,000) live in the Miami metro area [24]. Previous studies indicated that Haitian Americans have specific characteristic in their genetic background, eating patterns and lifestyles which distinguish them from African Americans [25]. Haitian Americans represent a unique ethnic group in South Florida but very often they were grouped with other Blacks with African origins for health research. Thus, translation and generalization of the results from genetic association conducted in African Americans to Haitian Americans should be done with caution and warrants further replication. Studies from our research group also suggested Haitian Americans may have different genetic makeup than African Americans [26]. By far, studies looking at health disparity in a separated Haitian American population is limited, including genetic studies. The primary purpose of this study was to examine six most studied SNPs in *TCF7L2* gene: rs7903146, rs12255372, rs11196205, rs7901695, rs7895340 and rs4506565 in relation to the risk of T2D and related metabolic quantitative traits in individuals with and without T2D in African Americans and Haitian Americans from South Florida.

Methodology

Samples for this study were from participants of an earlier cross-sectional study of N = 485 participants: Haitian American (HA) (N = 240), and African American (AA) (N = 245). These samples were collected from 2008–2010, of which 249 subjects with type 2 diabetes and 236 who had not developed type 2 diabetes were recruited. By study design, approximately half of each racial/ethnic minority in this study had type 2 diabetes and the

rest were free of diabetes. Participants were initially recruited by random selection from randomly generated mailing lists. The lists of addresses were purchased from Knowledge Base Marketing, Inc., Richardson, TX, USA. Approximately 7,550 letters were mailed to African Americans with and without T2D. Of the participants who received letters, 4% responded. Because we did not have a similar mailing list database for Haitian Americans, we recruited these participants from community-based sources: (a) local diabetes educators and community health practitioners in Miami-Dade and Broward Counties; (b) Florida International University (FIU) faculty, staff, and students; (c) several active adult apartment complexes; and (d) print advertisements and radio advertisements. Interested participants were interviewed by phone, at which time the study purpose was explained and information on age, self-identified ethnicity, gender, self-reported T2D status (confirmed by fasting plasma glucose (> 126 mg/dL) and/or A1c (>6.5%), years since diagnosis, and initial treatment modalities (oral glucose-lowering drugs and insulin)) were obtained. If a subject was determined to be eligible, then his or her participation was requested at the Human Nutrition Laboratory at FIU. Participants were instructed to fast at least 8 hours prior to their blood collection. Written consent in English, or Creole was obtained from the participants on their first visit to the laboratory. Validated questionnaires were used to collect information on demographics, dietary intake and physical activities [27-29]. A total of 12 participants (HA = 8; and AA= 4) who reported not having diabetes were reclassified in the study as having T2D according to American Diabetes Association (ADA) standards. These participants were provided with a copy of their laboratory results and referred to their physicians.

Measurements

Anthropometric measurements and biospecimen analyses

Data for serum biomarkers were available from our parent database. Briefly, height, weight, and blood pressure were measured using standard methodology with detailed description in our previous study [30]. Twenty ml of venous blood was collected from each participant after an overnight fast (at least 8 hours) by a certified phlebotomist. Fasting plasma glucose (FPG) levels in serum were quantified using hexokinase method. Whole blood was used to measure A1C (glycated hemoglobin) using Roche Tina Quant method (Laboratory Corporation of America, Lab Corp, FL, USA). Automatic chemical analyzer was employed to determine high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC). Serum insulin levels were determined using the Human Insulin ELISA kit from Millipore (St Charles, MZ, USA). Adiponectin was measured in the PI's lab using an enzyme-linked immunosorbent assay (ELISA) (Linco Research Inc, St. Charles, MO, USA). The updated homeostatic model assessment (HOMA2) was based on the Oxford University HOMA2 calculator (www.ocdem.ox.ac.uk) and was used to estimate beta cell function (HOMA2-β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS) [24]. The presence of Metabolic Syndrome (MetS) and number of MetS components (Yes or No) was determined based on the National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP-ATP III) [31].

Isolation of DNA and genotyping

Genomic DNA was isolated from the whole blood using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), according to the vender's recommended protocol. Quality

and quantity of the isolated DNA was assessed by using 2000c nanodrop spectrophotometer (Thermo Scientific, USA). Genotyping for all six SNPs was performed by real-time PCR amplification on BioRad CFX96 real time PCR instrument (Hercules, CA) using commercially available TaqMan allelic discrimination assays (LifeTech, Foster City, CA). To ensure reproducibility and reliability of genotyping method, 10% of the DNA samples were duplicated during genotyping. We obtained a 100% concordance between the genotyped duplicate samples for the single nucleotide polymorphism (SNP).

Statistical analysis

Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The sample size of the study had sufficient statistical power (>80%) to detect odds ratio of 1.5 or more, for matched case and control at significance threshold of 0.05 [26]**.** All statistical tests were two-tailed, and the threshold for statistical significance was set at $P \leq 0.05$. Genotype counts in each SNP was checked for Hardy-Weinberg equilibrium (HWE) in controls using the Chi-squared goodness-fit test. The distributions of the metabolic quantitative traits, such as lipid profiles, HOMA2 index, FPG, A1C, etc., were markedly skewed, which were normalized, to a large extent, by their logarithmic transformation. Therefore, t-tests on continuous values of these quantitative traits were performed on logarithmically transformed values. Demographic and clinical information between cases and controls were compared using student's t-test for continuous variables, and Chi-squared test for categorical variables. Dominant genetic model (noncarrier=0, heterozygous and homozygous=1) was selected for genetic association analysis, due to a small sample size for homozygotes of minor alleles. Logistic regression models were

used to calculate odds ratios (OR) and 95% confidence intervals (CIs) to assess the relationship of all SNPs with binary outcome for case-control status (T2D=Yes/No), and model was adjusted by potential confounding factors such as age, sex, BMI, physical activities and calorie intake. Spearman's rank-order correlation were applied to evaluate the correlation coefficient between continuous variables and discrete variables. Haplotype analysis and linkage disequilibrium (LD) coefficients were calculated using Haploview 4.2 (Cambridge, MA, USA) [32].

Results

General characteristics

This study included N=240 Haitian Americans (n=123 with T2D and n=117 without T2D) and N= 245 African Americans (n=126 with T2D and n=119 without T2D). Characteristics of the participants were presented as comparison of means ± SD by diabetes status for both ethnic groups (Table 1a and 1b). Briefly, individuals with T2D (cases) were older than those without T2D (controls) in both Haitian Americans and African Americans. Compared to individuals without T2D, individuals with T2D for both ethnic groups had significantly higher mean values of waist circumference, fasting plasma glucose, A1C (%), as well as higher percentage of MetS and higher number of MetS components and significantly lower mean values of HOMA2_B. Significant differences on mean values of BMI, SBP, TG and indexes of HOMA2_IR and HOMA2_IS and adiponectin between cases and controls were only present in African Americans whilst significant differences on mean values of insulin, kcal (calorie intake/day) and PA_MET_wk (weekly physical activity) were only present in Haitian Americans.

Distribution of *TCF7L2* polymorphisms

All cases and controls were genotyped for the six SNPs. None of the 6 *TCF7L2* SNPs showed any deviation from Hardy-Weinberg equilibrium in controls. Table 2 shows genotype distribution of all 6 *PPARGC1A* SNPs in the case-control sample for both ethnicities. Genotype distribution and allele frequency were not significantly different between subjects with diabetes and subjects without diabetes. Linkage disequilibrium analyses showed both similarity and difference between two ethnic groups. Two haplotype blocks with the same combinations for both ethnic groups were defined by using Four Gamete method (Figure 1a and 1b) [33]. The first block was established by three consecutive SNPs: rs7901695, rs450565 and rs7903146. The second block was established by three consecutive SNPs: rs7895340, rs11196205 and rs12255372. SNPs within each block were in a strong linkage disequilibrium. When looking pairwise LD, compared to African Americans, Haitian American tend to have more distinguished block as defined by low value of r^2 (r^2=0.22 for HA and r^2=0.66 for AA, respectively) between two haplotype blocks.

Correlations between *TCF7L2* polymorphisms and type 2 diabetes

Table 3a and 3b shows results from unconditional logistic regression by the dominant genetic model with adjustment for covariates (BMI, age, gender, physical activity, and calories intake) for African Americans and Haitian Americans, respectively. Though we didn't find any significant associations with the risk of T2D which was consistent with the results from genotype distribution analyses, SNP rs7903146 (OR=1.71, p=0.065) minor allele showed a marginal significance with the increased risk of T2D in African Americans; minor alleles of SNP rs11196205 (OR=0.58, p=0.059) and

rs7895340 (OR=0.59, p=0.069) showed marginal significance with the protective effect for the risk of T2D in Haitian Americans. Effect modification of sex on *TCF7L2* SNPs association with T2D was also explored by stratification by sex adjusted for age, BMI, physical activity, and calories intake. Interestingly, for both ethnic groups, significant associations were only found in male subjects. Of note, for both ethnic groups, SNPs with marginal significance from the gender-combined analyses became statistically significant with the same trend for the risk of T2D when analyses were done in males: SNPs rs7903146 (OR=2.86, p=0.013) for African Americans, rs11196205 (OR=0.35, p=0.034) and rs7895340 (OR=0.33, p=0.024) for Haitian Americans. In addition, SNP rs7901695 (OR=2.69, p=0.025) was found to be significantly associated with the risk of T2D in males of African American. Same result was also found for rs4506565 due to its strong LD with rs7903146 (D=1, r^2=0.99).

Correlations between *TCF7L2* polymorphisms and metabolic traits

Comparison of metabolic traits between non-risk allele carriers and risk allele carriers were conducted in subjects without T2D (controls). For African Americans, the fasting glucose level was significantly higher in non-risk allele carriers of SNPs rs4566565 and rs7901695, compared to the risk allele carriers (p=0.017). The difference stayed significant after covariates adjustment (BMI, age, gender, physical activity, and calories intake) for both SNPs. In addition, for African Americans, we identified consistent trends in metabolic traits when comparing minor allele carriers with non-carriers of SNP rs12255372 in non-diabetic controls (table 4). Compared to non-carriers, minor allele carriers had significantly higher means of BMI (*p*= 0.036), DBP (p=0.0099), numbers of components of MetS (p< 0.0001.) and significantly lower mean values of

HDL-C (p=0.020) and adiponectin (p=0.017). Results stayed significant after covariates adjustment. Spearman's rank-order correlation also demonstrated that serum level of adiponectin was negatively associated with numbers of components of Mets (correlation coefficient = -0.22, p= 0.014) in African American non-diabetic controls.

Discussion

Type 2 diabetes are highly prevalent and lead to significant morbidity and mortality. This is particularly true for African Americans. Compared to non-Hispanic whites, African American adults are 80 percent more likely to have been diagnosed with diabetes; 4.2 times more likely to be diagnosed with end stage renal disease; 3.5 times more likely to be hospitalized for lower limb amputations and twice as likely as non-Hispanic Whites to die from complications of diabetes [34]. Efforts have been made to understand the genetic variation that underlies T2D in African Americans and other individuals of more recent African descent. However, inconsistent replication of results is often present when studying genetic associations in African Americans. Studies suggested potential issues regarding racial designations as a common practice to group people but very often ignoring ethnic and sociocultural diversity [35]. *TCF7L2* is one of the top candidate genes for T2D and recent studies also reveal its association with obesity and metabolic syndrome [17-20]. The present study demonstrated ethno-specific genetic association with T2D and related metabolic traits among Haitian Americans and African Americans and suggested potential issues regarding population stratification within Blacks.

TCF7L2 is a transcription factor involved in the Wnt signaling pathway. Functional studies revealed that Wnt signaling pathway plays important role in glucose homeostasis

[14-16]. Variants within intron 4 and intron 5 of the *TCF7L2* were the most studied SNPs and by far yielded the strongest signal with the risk of T2D [21, 36-38]. Several studies led the way in investigating the role of variations in *TCF7L2* for the risk of T2D in populations of African descent. One hospital-based case–control study conducted in Kumasi, Ghana found a significant association of *TCF7L2* SNP rs7903146 with T2D; the risk allele confers 1.39-fold increased risk for type 2 diabetes [39]. In line with this finding, another study in Cameroonians confirmed such association though sample size is limited (n=74) [40]. Research in individuals of North African (Moroccan) also demonstrated reproducible association between SNP rs7903146 and the risk of T2D [41]. Several investigations in African Americans also demonstrated similar trends as seen in Africans. The Atherosclerosis Risk in Communities (ARIC) study found that African Americans with the risk allele at SNP rs7903146 had a higher risk of T2D, especially for individual with adverse metabolic syndrome (e.g. high BMI and low HDL) [42]. Similarly, a study of an African-American population enriched for nephropathy showed that 5 SNPs (rs7903146, rs7901695, rs7895340, rs11196205, and rs12255372) in *TCF7L2* were significantly associated with T2D, among which rs7903146 and rs7901695 demonstrated most significant associations [42]. In a multi-ethnic youth study, researchers found that each copy of risk allele of SNP rs7903146 was significantly associated with a 1.97-fold increased odd for type 2 diabetes in African Americans [43]. Consistent with these findings, the results from the present study showed that SNP rs7903146 (OR=1.71, p=0.065) minor allele was marginally significant with the risk of T2D in African Americans. The estimates of allele frequencies and LD pattern among our studied individuals were consistent with other published reports in African Americans,

which suggested representativeness of our samples [42-44]. In addition, we found that when conducting gender-specific analysis, SNP rs7903146 (OR=2.86, p=0.013) along with two additional SNPs (rs7901695 and rs4506565) were significantly associated with the risk of T2D in males whilst no such significant association were observed in females. Recent evidence from heritability studies and GWASs also demonstrated that common genetic variation influences many diseases and medically relevant traits in a sex-dependent manner [46]. The results from our study suggested gender-dependent effect on the risk association for variants in *TCF7L2*.

However, our results were different from those that failed to identify such association. One study to investigate two *TCF7L2* SNPs s7903146 and rs12255372 in relation to the risk of T2D, glucose homeostasis traits and gene expression did not find any significance in African Americans [47]. Researchers for the Diabetes Prevention Program performed Cox regression model to predict the progression to type 2 diabetes in individual with impaired glucose intolerance. Homozygotes of risk T allele at rs7903146 conferred excess risk of incident diabetes only in Hispanics but not in African Americans [48]. The discrepancy among African Americans could be due to different study design, unadjusted analysis complicated by confounding factors, especially life style and eating pattern, and genetic heterogeneity within Blacks. Of note, two previous studies yielding significant association with *TCF7L2* gene did adjust the ancestry markers for association analysis [42, 43]. To date, SNP rs7903146 was most significantly associated with T2D, with association observed across diverse populations. Our study contributed additional evidence to confirm such association in African Americans.

Haitians Americans are one of those high-risk populations for T2D. Though official data for Haitian Americans are not available, due to being grouped with other Blacks for health studies, a small-scale study conducted in a Haitian community (Little Haiti) in the Miami-Dade County, FL, estimated a 33% prevalence of diabetes among Haitian immigrants [49]. By far, only one study conducted in African-Caribbeans investigated the association between variants (SNPs rs7903146 and rs12255372) in *TCF7L2* and the risk of T2D and found no significant association (p=0.17) [50]. Consistent with this finding, we didn't find any significant association of these two SNPs with T2D. The minor allele frequency of *TCF7L2* SNPs rs7903146 and rs12255372 and LD pattern between these two SNPs in the present study were also comparable to the finding in aforementioned research, which further validated our study. In addition, we found that minor alleles of SNPs rs11196205 (OR=0.58, p=0.059) and rs7895340 (OR=0.59, p=0.069) showed marginal significance with the protective effect for the risk of T2D in Haitian Americans. Modification effect by sex on association with T2D was also noticed in Haitian American. The trend observed in Haitian Americans was similar as that found in African Americans. Both SNPs rs11196205 and rs7895340 with marginal significance in combined gender analysis demonstrated significant association with the risk of T2D in male subjects (OR=0.35, p=0.034; OR=0.34, p=0.024, respectively). This finding agreed with a previous study which showed that minor alleles of SNPs rs7895340 and rs1196205 were significantly associated with reduced risk of T2D in African Americans but contrast with finding from studies in other populations [42, 51, 52]. Due to limited statistical power, replication on such association is warranted for further validation, especially in gender-specific investigation. Collectively, the results from the present study

demonstrated different risk association of *TCF7L2* SNPs between Haitian Americans and African Americans. Previous studies suggested Haitian Americans may have specific characteristics which distinguish them from African Americans. Apart from African descent, populations of Haitian Americans also have genetic heritage from France, and Spain [53]. Haitian Americans have a unique dietary pattern featured by two meals a day with starch predominantly as energy source. One study on eating behavior showed that Haitian Americans and African Americans have significantly different healthy eating index (HEI) scores, and levels of physical activity [54]. These unique features of Haitian Americans in their genetic background and lifestyles may suggest Haitian Americans should be independently and separately evaluated from the African Americans.

The metabolic syndrome is a cluster of factors which requires the presence of least three of the five following components: central obesity, high blood pressure, elevated triglycerides, low high-density lipoprotein cholesterol (HDL), or fasting hyperglycemia [31]. Metabolic syndrome increased the risk of T2D and is prevalent in patients with T2D [55, 56]. Studies also suggested that metabolic syndrome and T2D present an altered lipid metabolism with increased triglyceride levels and postprandial dyslipidemia [57-59]. As such, it has been speculated that T2D and Metabolic Syndrome may share some common pathology and mediators including genetic predisposition in energy metabolism and substrate utilization. In line with this hypothesis, the T2D-related genetic variants showed pleiotropic effects to multiple MetS-related traits, which included blood pressure, obesity, lipids profiles and blood glucose [20, 59-62]. From the present study, consistent trends in metabolic traits were found when comparing minor allele carriers with non-carriers of SNP rs12255372 in African Americans non-diabetic controls. Compared to non-carriers

of SNP rs12255372, minor allele carriers had significantly higher mean values of BMI (p= 0.036), DBP (p=0.010), components of Mets (p=0.035) and significantly lower mean values of HDL-C (p=0.02). Two previous studies which investigated the association between SNP rs12255372 and metabolic traits demonstrated minor allele in this SNP was significantly and positively associated with dyslipidemia [19, 63]. Functional studies suggested TCF7L2 may involve in adipogenesis and adipocyte differentiation via measuring the main adipokines present in mature adipocytes [63-65]. In the present study, we additionally found minor allele carriers of SNP rs12255372 had significantly reduced adiponectin level (p=0.017) compared to non-carriers in African American non-diabetic controls. Adiponectin is an important adipocytokine secreted exclusively by adipocytes and plays an important role in glucose and lipid homeostasis, inflammation and oxidative stress [66]. Various studies suggest that circulation adiponectin levels are negatively associated with waist circumference, triglycerides, insulin resistance and arterial blood pressure whilst positively associated with plasma HDL level [66]. In line with these findings, adiponectin level was significantly associated with reduced components of Mets in our study (p=0.014). Collectively, the consistent trends in the association between SNP rs12255372, metabolic syndrome traits and serum adiponectin level suggested SNP rs12255372 could be the potential genetic variant with pleiotropic effects on multiple MetS-related traits and warrants replication by a larger sample and further investigation in functional mechanism.

We must be cautious when extrapolating our results since there are some limitations. One limitation was the relatively small sample size which may have decreased the statistical power to detect significant associations. The cross-sectional design of the study

precluded the establishment of cause and effect. Further study is needed to investigate the nature of the associations found in the present study. In addition, generalization of the results from the present study may be made with caution due the selection bias caused by recruiting method. Haitian Americans, and African Americans may not be representative of the same ethnicities residing in other parts of Florida or USA. In this study, the heterogeneity within the African American and Haitian American population cannot be ruled out and thus residual confounding is still a concern. However, both cases and controls were selected from the same population pool and geographic area, with information on ethnicity up to two generations, for each respective ethnicity. When conducting genetic association in individuals with African origin, a common practice is to include ancestry marker to adjust the genetic heterogeneity. However, as T2D is a complex disease with strong environmental influence. Merely adjusting genetic heterogeneity while ignoring the environmental variations (e.g. culture, lifestyle, eating pattern, et al) may mask the true association in Black people with diverse ethnicities. As seen in studies of Samoa and American Samoa, same genetic pool with distinct eating behaviors yielded different genetic association with chronic diseases [67, 68].

Conclusion

This study demonstrated ethno-specific associations of *TCF7L2* SNPs with the risk T2D and metabolic traits in African Americans and Haitian Americans and suggested that the two ethnic groups may have different genetic risk factors under the complex interactions between genetic and environmental factors.

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Table 1a. Anthropometric, demographic and metabolic characteristics of Haitian Americans

	Without T2D (n=117)	With T2D case (n=123)	*p** value
Age (Years)	54.08±11.01	58±9.99	0.0042
Female (N (%))	59 (50.43%)	71 (57.72%)	0.26
BMI (kg/m2)	28.41±1.18	29.11±1.2	0.28
Waist circumference (cm)	95.16±1.13	98.93±1.13	0.015
SBP (mm of Hg)	142.84±1.18	146.03±1.18	0.30
DBP (mm of Hg)	89.51±1.15	90.48±1.15	0.56
Triglycerides (mg/dl)	92.05±1.59	99.40±1.57	0.19
Total cholesterol (mg/dl)	194.79±1.21	188.05±1.25	0.19
HDL-C (mg/dl)	51.33±1.3	51.71±1.33	0.83
LDL-C (mg/dl)	119.51±1.34	111.29±1.37	0.07
FPG (mmol/L)	97.55±1.18	144.82±1.5	<.0001
A1C (%)	5.93±1.09	8.09±1.32	<.0001
Insulin	8.58±1.8	6.44±2.06	0.0008
HOMA2_B	90.09±1.59	37.7±2.4	<.0001
HOMA2_IR	1.18±1.7	1.18±1.75	0.98
HOMA2_IS	85.24±1.71	85.04±1.75	0.97
Number of MetS components	1.85±1.00	3.04±0.94	<.0001
Presence of MetS (N (%))	30 (25.64%)	87 (70.73%)	<.0001
Adiponectin	14.51±1.76	13.35±1.75	0.25
kcal	1673.55±1.76	1442.6±1.76	0.043
PA_MET_wk	23.85±5.4	12.56±5.02	0.0047

Values are unadjusted mean ± SD for continuous variables or N for categorical variables.
** Derived from t-test for means or chi-square test for percentage differences between cases and controls. BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood; HDLC= high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol;FPG= fasting plasma glucose; A1C= hemoglobin A1C; MetS= Metabolic Syndrome; PA_MET_wk= physical activity measured by metabolic equivalents (MET) per week*

Table 1b. Anthropometric, demographic and metabolic Characteristics of African Americans

	Without T2D (n=119)	With T2D case (n=126)	*p** value
Age (Years)	50.74±8.54	54.35±9.84	0.0025
Female (N (%))	59 (49.8%)	65 (51.59%)	0.75
BMI (kg/m2)	30.36±1.23	34.76±1.26	<.0001
Waist circumference (cm)	100.47±1.15	112.33±1.17	<.0001
SBP (mm of Hg)	130.86±1.14	139.77±1.16	0.0003
DBP (mm of Hg)	86.86±1.15	89.27±1.13	0.11
Triglycerides (mg/dl)	104.56±1.6	121.91±1.65	0.014
Total cholesterol (mg/dl)	186.18±1.22	184.55±1.26	0.75
HDL-C (mg/dl)	49.08±1.30	47.29±1.33	0.29
LDL-C (mg/dl)	110.34±1.36	104.23±1.54	0.24
FPG (mmol/L)	94.73±1.14	135.64±1.48	<.0001
A1C (%)	5.85±1.07	7.44±1.26	<.0001
Insulin	10.01±2.01	11.40±2.39	0.20
HOMA2_B	106±1.54	57.15±2.17	<.0001
HOMA2_IR	1.36±1.91	1.67±1.93	0.016
HOMA2_IS	73.65±1.91	59.50±1.92	0.014
Number of MetS components	1.95±1.24	3.40±0.97	<.0001
Presence of MetS (N (%))	35 (29.41%)	107 (84.72%)	<.0001
Adiponectin	19.46±1.71	23.45±1.88	0.014
kcal	2258.15±1.93	1965.10±1.86	0.09
PA_MET_wk	19.28±6.91	16.94±5.66	0.61

*Values are unadjusted mean ± SD for continuous variables or N for categorical variables. * Derived from t-test for means or chi-square test for percentage differences between cases and controls. BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood; HDLC= high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; FPG= fasting plasma glucose; A1C= hemoglobin A1C; MetS= Metabolic Syndrome; PA_MET_wk= physical activity measured by metabolic equivalents (MET) per week*

Table 2. Genotype distribution and minor allele frequency of all 6 *TCF7L2* SNPs in the case-control samples for both ethnicities

TCF7L2 SNPs	Haitian American			African American		
rs12255372	Without T2D n (%)	With T2D n (%)	*p** value	Without T2D n (%)	With T2D n (%)	*p** value
GG	52 (44.83%)	68 (55.28%)		55 (46.22%)	64 (50.79%)	
GT	55 (47.41%)	47 (38.21%)	0.27	52 (43.70%)	57 (45.24%)	0.17
TT	9 (7.76%)	8 (6.50%)		12 (10.08%)	5 (3.97%)	
MAF (%)						
T	31.47%	25.61%	0.51	31.93%	26.59%	0.19
rs11196205						
CC	70 (59.83%)	84 (68.29%)		74 (62.18%)	76 (60.32%)	
GC	40 (34.19%)	32 (26.02%)	0.37	38 (31.93%)	42 (33.33%)	0.76
GG	7 (5.98%)	7 (5.69%)		7 (5.88%)	8 (6.35%)	
MAF (%)						
G	23.08%	18.70%	0.55	21.85%	23.02%	0.98
rs7903146						
CC	58 (49.57%)	63 (51.22%)		57 (47.90%)	49 (38.89%)	
CT	49 (41.88%)	49 (39.84%)	0.95	51 (42.86%)	67 (53.17%)	0.27
TT	10 (8.55%)	11 (8.94%)		11 (9.24%	10 (7.94%)	
MAF (%)						
T	29.49%	28.86%	0.35	30.67%	34.52%	0.36
rs7901695						
TT	36 (30.77%)	32 (26.02%)		40 (33.61%)	35 (27.78%)	
TC	52 (44.44%)	62 (50.41%)	0.62	58 (48.74%)	62 (49.21%)	0.46
CC	29 (24.79%)	29 (23.58%)		21 (17.65%)	29 (23.02%)	
MAF (%)						
C	47.01%	48.78%	0.94	42.02%	47.62%	0.21
rs7895340						
AA	69 (58.97%)	82 (66.67%)		71 (59.66%)	76 (60.32%)	
GA	41 (35.04%)	34 (27.64%)	0.44	41 (34.45%)	42 (33.33%)	0.98
GG	7 (5.98%)	7 (5.69%)		7 (5.88%)	8 (6.35%)	
MAF (%)						
G	23.50%	19.51%	0.50	23.11%	23.02%	0.98
rs4506565						
AA	35 (29.91%)	32 (26.02%)		40 (33.61%)	35 (27.78%)	
AT	52 (44.44%)	61 (49.59%)	0.70	57 (47.90%)	62 (49.21%)	0.52
TT	30 (25.64%)	30 (24.39%)		22 (18.49%)	29 (23.02%)	
MAF (%)						
T	47.86%	49.19%	0.88	42.44%	47.62%	0.25

** Derived from chi-square test; MAF= minor allele frequencies*

Table 3a. Unconditional logistic regression by the dominant genetic model with adjustment for covariates for African Americans.

African Americans (all subjects)					
SNPs		Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p** value
rs12255372	GG	56 (46.22%)	64 (50.79%)	1 (reference)	0.15
	GT+TT	64 (53.78%)	62 (51.43%)	0.66 (0.38-1.17)	
rs11196205	CC	74 (62.18%)	76 (60.32%)	1 (reference)	0.77
	GC+GG	45 (37.82%)	50 (39.68%)	1.09 (0.61-1.94)	
rs7903146	CC	57 (47.90%)	49 (38.89%)	1 (reference)	0.065
	CT+TT	62 (52.10%)	77 (61.11%)	1.71 (0.97-3.04)	
rs7901695	TT	40 (33.61%)	35 (27.78%)	1 (reference)	0.20
	TC+CC	79 (66.39%)	91 (72.22%)	1.49 (0.81-2.73)	
rs7895340	AA	71 (59.66%)	76 (60.32%)	1 (reference)	0.99
	GA+GG	48 (40.34%)	50 (39.68%)	1.00 (0.56-1.76)	
rs4506565	AA	40 (33.61%)	35 (27.78%)	1 (reference)	0.20
	AT+TT	79 (66.39%)	91 (72.22%)	1.49 (0.81-2.73)	
AA (Gender=Female)					
SNPs		Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p** value
rs12255372	GG	26 (44.07%)	32 (49.23%)	1 (reference)	0.50
	GT+TT	33 (55.93%)	33 (50.77%)	0.74 (0.32-1.74)	
rs11196205	CC	39 (66.10%)	38 (58.46%)	1 (reference)	0.51
	GC+GG	20 (33.90%)	27 (41.54%)	1.34 (0.56-3.19)	
rs7903146	CC	24 (40.68%)	28 (43.08%)	1 (reference)	0.89
	CT+TT	35 (59.32%)	37 (56.92%)	0.94 (0.40-2.19)	
rs7901695	TT	15 (25.42%)	20 (30.77%)	1 (reference)	0.49
	TC+CC	44 (74.58%)	45 (69.23%)	0.72 (0.29-1.81)	
rs7895340	AA	37 (62.71%)	38 (58.46%)	1 (reference)	0.72
	GA+GG	22 (37.29%)	27 (41.54%)	1.17 (0.50-2.76)	
rs4506565	AA	15 (25.42%)	20 (30.77%)	1 (reference)	0.49
	AT+TT	44 (74.58%)	45 (69.23%)	0.72 (0.29-1.81)	
AA (Gender=Male)					
SNPs		Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p** value
rs12255372	GG	29 (48.33%)	32 (52.46%)	1 (reference)	0.22
	GT+TT	31 (51.67%)	29 (47.54%)	0.61 (0.28-1.34)	
rs11196205	CC	35 (58.33%)	38 (62.30%)	1 (reference)	0.74
	GC+GG	25 (41.67%)	23 (37.70%)	0.88 (0.40-1.93)	

rs7903146	CC	33 (55.00%)	21 (34.43%)	1 (reference)	0.013
	CT+TT	27 (45.00%)	40 (65.57%)	2.86 (1.25-6.54)	
rs7901695	TT	25 (41.67%)	15 (24.59%)	1 (reference)	0.025
	TC+CC	35 (58.33%)	46 (75.41%)	2.69 (1.14-6.37)	
rs7895340	AA	34 (56.67%)	38 (62.30%)	1 (reference)	0.61
	GA+GG	26 (43.33%)	23 (37.70%)	0.82 (0.37-1.79)	
rs4506565	AA	25 (41.67%)	15 (24.59%)	1 (reference)	0.025
	AT+TT	35 (58.33%)	46 (75.41%)	2.69 (1.14-6.37)	

**P value and odds ratios (OR) with 95% confidence interval (CI) were calculated using unconditional logistic models adjusted by BMI, gender, age, physical activity and calorie intake; AA= African American.*

Table 3b. Unconditional logistic regression by the dominant genetic model with adjustment for covariates for Haitian American.

Haitian Americans (all subjects)					
SNPs		Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p** value
rs12255372	GG	52 (44.83%)	68 (55.28%)	1 (reference)	0.28
	GT+TT	64 (55.17%)	55 (44.72%)	0.74 (0.44-1.27)	
rs11196205	CC	70 (59.83%)	84 (68.29%)	1 (reference)	0.059
	GC+GG	47 (48.75%)	39 (31.71%)	0.58 (0.33-1.02)	
rs7903146	CC	58(49.57%)	63 (51.22%)	1 (reference)	0.99
	CT+TT	59 (50.43%)	60 (48.78%)	1.00 (0.59-1.70)	
rs7901695	TT	36 (30.77%)	32 (26.02%)	1 (reference)	0.34
	TC+CC	81 (69.23%)	91 (73.98%)	1.33 (0.74-2.40)	
rs7895340	AA	69 (58.97%)	82 (66.67%)	1 (reference)	0.069
	GA+GG	48 (41.03%)	41 (33.33%)	0.59 (0.34-1.04)	
rs4506565	AA	35 (29.91%)	32 (26.02%)	1 (reference)	0.44
	AT+TT	82 (70.09%)	91 (73.98%)	1.26 (0.70-2.28)	
HA (Gender=Female)					
SNPs		Without T2D N (%)	With T2D N (%)	OR (95% CI)	*p** value
rs12255372	GG	25 (42.37%)	39 (54.93%)	1 (reference)	0.20
	GT+TT	34 (57.63%)	32 (45.07%)	0.63 (0.30-1.29)	
rs11196205	CC	36 (61.02%)	45 (63.38%)	1 (reference)	0.69
	GC+GG	23 (38.98%)	26 (36.62)	0.86 (0.42-1.79)	
rs7903146	CC	27 (45.76%)	45 (63.38%)	1 (reference)	0.082
	CT+TT	32 (54.24%)	26 (36.62%)	0.53 (0.26-1.09)	
rs7901695	TT	15 (25.42%)	22 (30.99%)	1 (reference)	0.56
	TC+CC	44 (74.58%)	49 (69.01%)	0.78 (0.35-1.76)	
rs7895340	AA	36 (61.02%)	43 (60.56%)	1 (reference)	0.84
	GA+GG	23 (38.98%)	28 (39.44%)	0.93 (0.45-1.9)	
rs4506565	AA	14 (23.73%)	22 (30.99%)	1 (reference)	0.41
	AT+TT	45 (76.27%)	49 (69.01%)	0.71 (0.31-1.60)	
HA (Gender=Male)					
SNPs		Without T2D N (%)	With T2D n N (%)	OR (95% CI)	*p** value
rs12255372	GG	27 (47.37%)	29 (55.77%)	1 (reference)	0.80
	GT+TT	30 (52.63%)	23 (44.23%)	0.90 (0.39- 2.08)	
rs11196205	CC	34 (58.62%)	39 (75.00%)	1 (reference)	0.034
	GC+GG	24 (41.38%)	13 (25.00%)	0.35 (0.14-0.92)	
rs7903146	CC	31 (53.45%)	18 (34.62%)	1 (reference)	0.052
	CT+TT	27 (46.55%)	34 (65.38%)	2.35 (0.99-5.56)	

rs7901695	TT	21 (36.21%)	10 (19.23%)	1 (reference)	0.052
	TC+CC	37 (63.79)	42 (80.77%)	2.62 (0.99-6.91)	
rs7895340	AA	33 (56.90%)	39 (75.00%)	1 (reference)	0.024
	GA+GG	25 (43.10%)	13 (25.00%)	0.33 (0.12-0.86)	
rs4506565	AA	21 (36.21%)	10 (19.23%)	1 (reference)	0.052
	AT+TT	37 (63.79)	42 (80.77%)	2.62 (0.99-6.91)	

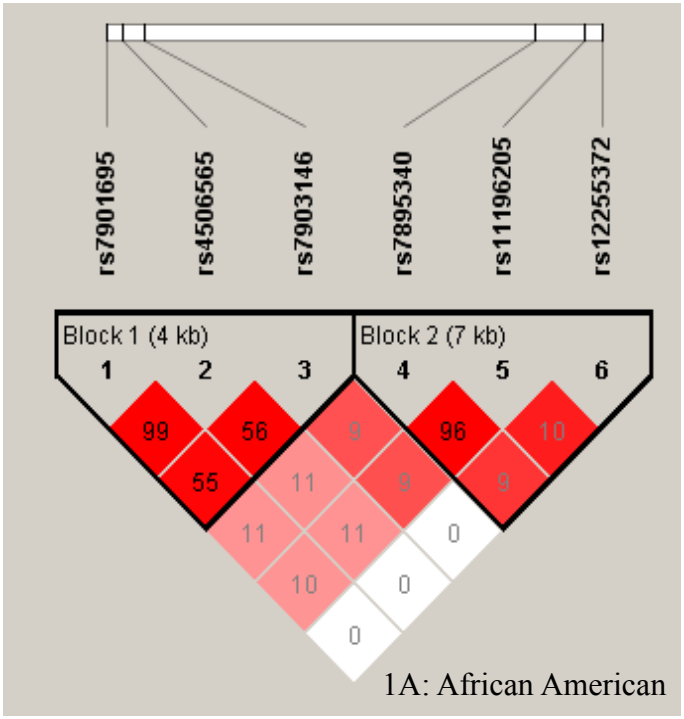
**P value and odds ratios (OR) with 95% confidence interval (CI) were calculated using unconditional logistic models adjusted by BMI, gender, age, physical activity and calorie intake; HA= Haitian American.*

Table 4. Comparison of T2D related metabolic traits between non-risk allele carriers and risk allele carriers of SNP rs12255372 in African Americans without T2D.

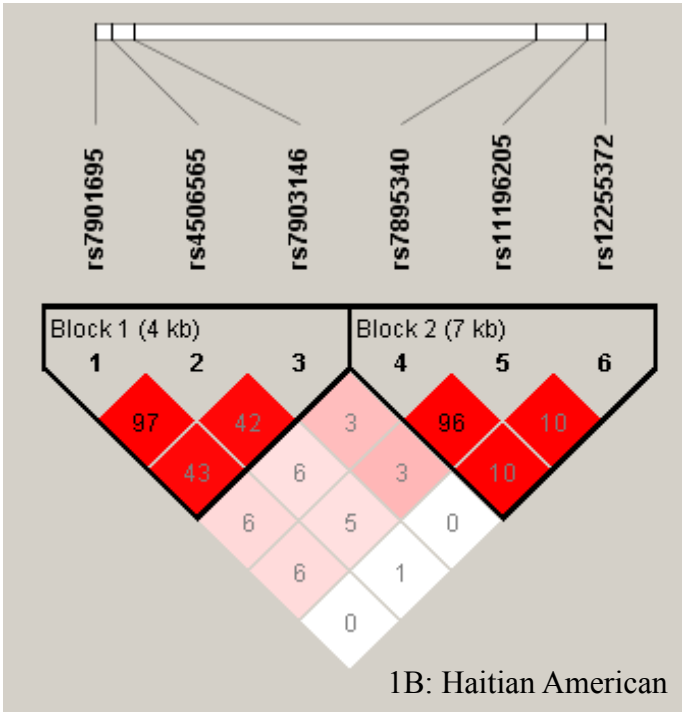
Variables	GG (N=56)	GT+TT (N=64)	*p** value
BMI (kg/m2)	29.09±1.22	31.5±1.23	0.036
Waist circumference (cm)	98.52±1.15	102.18±1.15	0.16
SBP (mm of Hg)	127.57±1.12	133.74±1.16	0.054
DBP (mm of Hg)	83.81±1.14	89.57±1.15	0.010
Triglycerides (mg/dl)	99.20±1.62	109.42±1.57	0.26
Total cholesterol (mg/dl)	183.79±1.24	188.27±1.2	0.51
HDL-C (mg/dl)	52.11±1.31	46.61±1.27	0.02
LDL-C (mg/dl)	105.43±1.39	114.76±1.32	0.13
FPG (mmol/L)	93.16±1.13	96.10±1.15	0.19
A1C (%)	5.87±1.07	5.83±1.07	0.57
Insulin	9.30±2.06	10.67±1.95	0.29
HOMA2_B	105.00±1.65	106.84±1.45	0.83
HOMA2_IR	77.97±1.9	70.2±1.92	0.39
HOMA2_IS	1.28±1.9	1.43±1.92	0.38
Number of MetS components	1.69±1.02	2.17±1.38	0.035
Adiponectin	22.07±1.67	17.46±1.72	0.017

*Values are unadjusted mean ± SD for continuous variables or N for categorical variables. * Derived from t-test for means or chi-square test for percentage differences between cases and controls. BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood; HDLC= high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol;FPG= fasting plasma glucose; A1C= hemoglobin A1C; MetS= Metabolic Syndrome; PA_MET_wk= physical activity measured by metabolic equivalents (MET) per week*

Figure 1. Haploview plot showing linkage disequilibrium (LD) with r2 values and block structure for six selected SNPs of *TCF7L2* gene for African American (1A) and Haitian American (1B).



1A: African American



1B: Haitian American

CHAPTER V STRENGTHS AND LIMITATIONS

This study has several strengths. T2D is a heterogeneous disorder with contributions from peripheral insulin resistance and β-cell dysfunction. In the present study, in addition to the association analyses of *TCF7L2* SNPs in relationship to the risk of T2D, we also investigated some T2D related biomarkers (FPG, A1C%) and quantitative intermediate phenotypes (e.g. HOMA2 indexes) in three high risk race/ethnic populations. We also investigated the differences within the Black race with regards to genetic influences on T2D as well as associated phenotypes. Findings from our study provided valuable information for the ethno-specific genetic association with T2D and related metabolic traits and suggested potential issues regarding population stratification within Blacks which need to be further validated. Additionally, besides genetic factors, environmental influences, such us dietary factors and gender effect were taken into consideration on the genetic association with the risk of T2D, which might shed some light on how genetic variants could modify specific metabolic processes to make an individual more susceptible (or less susceptible) to the harmful (or preventive) effects of environmental factors. The case-control candidate gene study has the advantage of looking closely at polymorphisms of one single gene and its association with the disease. This study could contribute in genotype determination of the candidate gene (*TCF7L2*) polymorphisms with some biological importance to T2D.

This study has some limitations. Due to the relatively small sample size in each race/ethnic population, the genetic variants with modest effect on the T2D might be omitted. As the study focused on the genetic associations of only one gene (*TCF7L2*), the

influence of gene-gene interactions was not considered. The effects of polymorphisms on the regulation of *TCF7L2* gene expression were not quantified, so direct relationship with the gene product could not be established. The participants in the study were self-reported ethnicities; resulting in possible genetic heterogeneity. Confounding due to population stratification can be controlled by ancestry-informative markers to avoid false associations could be used to address this problem. Nevertheless, both cases and controls of our studied population were selected from the same population pool and geographic area, with information on ethnicity up to two generations, which to some extend mitigate the heterogeneity effect. Additionally, due to the nature of case-control study, there might be a recall bias on dietary intake information.

CHAPTER VI SUMMARY AND PROSPECTIVE

Summary

The correlations among *TCF7L2* polymorphisms, type 2 diabetes and its related quantitative traits were examined in Cuban American, Haitian American and African American population of South Florida. Additionally, the genetic associations of *TCF7L2* polymorphisms with T2D were also explored for the effects of environmental influences such as gender and dietary factors.

The main objective of this study was to examine the correlations of *TCF7L2* gene polymorphisms with T2D in three ethnicities which are at high risk of T2D. For Cuban Americans, we were able to see associations between polymorphisms of this gene with the risk of T2D and some related quantitative traits. Due to few existing studies examining this association in Hispanic subgroup, of which majority were carried in populations of Mexican origin, replication is lacking in Cuban Americans, the third largest minority group in the United States. This research filled this knowledge gap and confirmed the implication of *TCF7L2* in T2D development reported earlier in other race/ethnic populations [1-3]. The epidemic of T2D only dates 50 years. It is quite clear that during this period the environmental factors have changed dramatically. A traditional high energy-burning lifestyle has been replaced by a sedentary lifestyle and consumption of an energy-dense diet, a common phenomenon worldwide, especially pronounced in the United States [4]. Studies in Cuban Americans also suggested higher consumption of junk foods and low consumption in fruits and vegetables during the acculturation [5, 6]. Some investigators speculated that the genetic predisposition to T2D would work synergically with the environmental facilitators, especially poor dietary behavior, to

contribute to the development of T2D [7]. Thanks to the validated food frequency questionnaire used in the present study, we were able to perform further analysis on the interaction between *TCF7L2* polymorphisms and some dietary factors on the risk of T2D in Cuban American [8]. Such gene-diet associations reported earlier by other studies were validated by our findings. Our study yielded new insights into the understanding of etiology of diabetes and have implications for prevention of T2D in individuals carrying *TCF7L2* risk allele(s) through dietary modification.

Inconsistent results often present in genetic association studies. This is particularly true for African Americans due to diverse heterogeneous subgroups within Blacks and further complicated by environmental factors [9, 10]. From our study, we were able to identify ethno-specific associations of *TCF7L2* SNPs with the risk of T2D and metabolic traits in African Americans and Haitian Americans and suggested that the two ethnic groups may have different genetic risk factors under the complex interactions between genetic and environmental factors. Some recent investigation also suggested TCF7L2 involved in adipogenesis and demonstrated risk associations with Metabolic Syndrome [11-14]. Consistent with these studies, we identified one SNP (rs12255372) in *TCF7L2* gene was correlated with BMI and some important metabolic traits in African American population. Additionally, gender effect on the genetic association that reported in previous genetic investigations was also found in our study. The gender differences in risk association of T2D could be ascribed to genetic effects and epigenetic mechanisms that further complicated by non-genetic factors such as environmental exposure, anatomical differences, and sex hormones [15].

Minority populations that possess lineage from two or more genetic sources are usually at high risks for metabolic diseases such as T2D and cardiovascular diseases [16]. Understanding the similarities as well as differences among these ethnicities provided important public and clinical implications in T2D prevention and treatment. For the present research, we demonstrated entho-specific associations of *TCF7L2* polymorphisms with the risk of T2D and T2D related quantitative traits in three underrepresented minority populations. Additionally, environmental modifications on such association provided new insights into the understanding of etiology of diabetes and have implications for nutrition intervention in individuals carrying *TCF7L2* risk allele(s).

Future study

Due to the limited sample size, the present study may not have enough statistical power to detect the significant associations that were true to these three populations. Therefore, larger sample sizes will be optimal to observe credible and substantial effects of such genetic variants. The present study focused on association of polymorphisms of only one gene with T2D and did not look into adjoining genetic effects on the risk of T2D. Investigation with combined SNPs from multiple validated risk T2D candidate genes might provide a better evaluation and estimation for the overall risk of T2D. Recently, epigenetic study that investigated macro-/micro-nutrients and bioactive food elements constituting the nutritional environment that alters the genome, the epigenome, the posttranslational regulation and modification is emerging as an interesting field in Nutrition Science [17]. Results from our study as well as some previous investigations suggested modification effect of gene-diet interaction on the association of *TCF7L2*

polymorphisms and T2D. In vivo or in vitro functional follow up studies at molecular level are warranted to confirm these observed associations from cross-sectional studies. Once confirmed, further human clinical trials would be conducted for pursing individualized nutrition intervention.

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Publications

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