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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ COACTIVATOR 1-α (*PPARGC1A*) GENETIC ASSOCIATIONS WITH 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

Amanpreet K. Cheema

2014

To: Dean Michele Ciccazzo
R. Stempel College of Public Health and Social Work

This dissertation, written by Amanpreet K. Cheema, and entitled Peroxisome Proliferator-Activated Receptor-γ Coactivator 1-α (*PPARGC1A*) Genetic Associations with 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.

Vijaya Narayanan

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Tan Li

Fatma G. Huffman, Major Professor

Date of Defense: October 28, 2014

The dissertation of Amanpreet K. Cheema is approved.

Dean Michele Ciccazzo
R. Stempel College of Public Health and Social Work

Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2014

DEDICATION

I dedicate this dissertation to my family, especially my parents Amrik and Bhupinder, my sister Kiran and brother-in law Anjum, my parents-in-law Felix and Maria, and my late grandparents for their unfaltering support, unconditional love and belief in me. To my husband, Juan who has been my rock during the tough times and who always brings out the best in me. Last but certainly not the least; I give my thanks to the Almighty for giving me the opportunity and surrounding me with the people whom I value the most.

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ABSTRACT OF THE DISSERTATION

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ COACTIVATOR 1-α (*PPARGC1A*) GENETIC ASSOCIATIONS WITH TYPE 2 DIABETES IN THREE ETHNICITIES

by

Amanpreet K. Cheema

Florida International University, 2014

Miami, Florida

Professor Fatma G. Huffman, Major Professor

Genetic heterogeneity, lifestyle factors, gene-gene or gene-environment interactions are the determinants of T2D which puts Hispanics and populations with African ancestry at higher risk of developing T2D. In this dissertation, the genetic associations of *PPARGC1A* polymorphisms with T2D and its related phenotypes (metabolic markers) in Haitian Americans (cases=110, controls=116), African Americans (cases=120, controls=124) and Cuban Americans (cases=160, controls=181) of South Florida were explored. Five single nucleotide polymorphisms of gene *PPARGC1A* were evaluated in each ethnicity for their disease association. In Haitian Americans, rs7656250 (OR= 0.22, $p<0.01$) and rs4235308 (OR=0.42, $p=0.03$) had significant protective association with T2D but had risk association in African Americans for rs7656250 (OR=1.02, $p=0.96$) and rs4235308 (OR=2.53, $p=0.03$). We found that in Haitian American females, both rs7656250 (OR=0.23, $p<0.01$) and rs4235308 (OR=0.38, $p=0.03$) had protective association with T2D. In African American females, rs7656250 (OR=1.14, $p=0.78$) had risk association whereas in males, it had

significant protective effect (OR=0.37, $p=0.04$). However, the risk association exhibited by rs4235308 was stronger in African American females (OR=2.69, $p=0.03$) than males (OR=1.16, $p=0.72$). In Cuban Americans, only rs7656250 showed significant risk association with T2D (OR=6.87, $p=0.02$) which was stronger in females alone (OR=7.67, $p=0.01$). We also observed significant differences among correlations of *PPARGC1A* SNPs and T2D phenotypes. Positive correlation was observed for log Hs-CRP with rs3774907 ($p<0.05$) in African Americans and rs4235308 ($p=0.03$) in Cuban Americans respectively. Correlation of log A1C with rs7656250 ($p=0.02$) was positive in Cuban Americans while it was negative for rs3774907 in Haitian Americans ($p<0.01$). Haitian Americans also had negative correlations between rs3774907 and log FPG ($p<0.01$), rs1172438 and log insulin (p=0.02). Results showed that (i) there are significant differences with regards to *PPARGC1A* correlations with T2D and its phenotypes among the three ethnicities studied (ii) the associations of *PPARGC1A* SNPs showed significant effect modification by sex. The findings suggest that variations in effects of *PPARGC1A* gene polymorphisms among three ethnicities and between sexes may have biomedical implications for the development of T2D as well as the phenotypes related to T2D.

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ABBREVIATIONS AND ACRONYMS

Ancestry-informative marker	AIM
Confidence interval	CI
Deoxyribonucleic acid	DNA
Estrogen receptor-related receptor	ESRR
Glucose transporter	GLUT-4
Genome wide studies	GWAS
Hispanic Health and Nutrition Examination Survey	HHANES
Linkage disequilibrium	LD
Minor allele frequency	MAF
Nuclear receptor factors	NRFs
Odds ratio	OR
Polymerase chain reaction	PCR
Peroxisome proliferative activated receptor gamma coactivator1- alpha	PPARGC1A/PGC1-α
Reactive oxygen species	ROS
Single nucleotide polymorphism	SNP
Ubiquitous protein 1	UCP1
Type 2 diabetes	T2D

CHAPTER I

INTRODUCTION

Type 2 Diabetes (T2D) is a major public health issue and its incidence is increasing worldwide (CDC, 2014). About 9.3% of the United States population is afflicted with diabetes (CDC, 2014). The prevalence of T2D in 65 years and older is 25.9%, 16.2% in 45-64 year olds whereas 4.1% in 20-44 year olds (CDC, 2014). The rates of new cases are much high in individuals with Hispanic and African lineages as compared to Non-Hispanic Whites (CDC, 2014). Among all ethnicities, T2D is diagnosed in 15.9 % of American Indians and Alaska Natives, 12.8 % in Hispanics and 13.2 % in non-Hispanic blacks (CDC, 2014). Diabetes brings with itself many associated chronic diseases such as high blood pressure, cardiovascular diseases, blindness, kidney disease and even amputation and death. The risk of developing T2D is reported to be increased by certain modifiable factors like obesity, physical inactivity, cigarette smoking and diet with low fiber and high glycemic index (Bouchard, 1995; Yki-Ja¨rvinen, 1995; Boden, 1997). Many factors such as age, gender, diet, ethnicity and distribution of fat, that impact insulin secretion and sensitivity, are controlled by genetics, lifestyle or both (Qi et al., 2010; Yamauchi et al., 2010). Additionally, the fundamental clinical characteristic of T2D is high blood glucose level, which may be caused by anomalies in one or more of the different molecular pathways regulated by certain genes (Narayan, Boyle, Thompson, Sorensen & Williamson 2003).

Peroxisome proliferator-activated receptor-γ coactivator-1 α (*PPARGC1A*) has been identified as a transcriptional co-activator of a series of nuclear receptors, which regulate processes that impact cellular energy metabolism, thermogenesis regulation, glucose metabolism, adipogenesis, and oxidative metabolism (Stefan et al., 2003; Gerhart-Hines et al., 2007; Puigserver, Wu, Park, Graves, Wright & Spiegelman, 1998; Puigserver et al., 2003; Rhee et al., 2003). *PPARGC1A* gene encodes a protein called PPARGC1A or PGC1-α. PGC1-α is a versatile master co-activator of various metabolic processes and therefore is involved in a variety of human diseases such as type 2 diabetes. PGC1-α interacts with a wide array of nuclear receptor factors (NRFs) that further regulate several mitochondrial genes responsible for maintaining energy metabolism and mitochondrial function and biogenesis (Fernandez-Marcos, & Auwerx 2011; Scarpulla, Vega & Kelly, 2012). PGC1-α also regulates gluconeogenesis, through transcription factors including nuclear receptor subfamily 3, group C, member 1(NR3C1) and forkhead box protein O1 (FoxO1). PGC1-α, expressed by *PPARGC1A*, regulates the expression of Fndc5 which is further proteolysed to irisin (Puigserver, Wu, Park, Graves, Wright & Spiegelman, 1998). Irisin upon activation stimulates 'browning' of adipose tissue and increases expression of ubiquitous protein 1 (UCP1) (Puigserver, Wu, Park, Graves, Wright & Spiegelman, 1998). The up-regulation of UCP1 is linked to increased mitochondrial function and increased energy utilization, therefore confers resistance to obesity induced insulin resistance.

PGC1-α also regulates fatty acid oxidation as well as oxidative phosphorylation by interaction with peroxisome proliferative activated receptor alpha (*PPARA*) and estrogen receptor-related receptor (*ESRR*) (Fernandez-Marcos & Auwerx, 2011;

Scarpulla, Vega & Kelly 2012). Oxidative phosphorylation in patients with type 2 diabetes (T2D) is impaired, and as *PPARGC1A* regulates the mitochondrial genes, impaired oxidative phosphorylation is most likely to be linked to variations in *PPARGC1A* gene possibly resulting in diseases such as T2D, and metabolic syndrome (Vimalananda, Rosenweig, Cabral, David, & Lasser, 2011). PGC1-α dysregulation is often associated with insulin resistance and Type 2 diabetes (T2D) (Vimalananda, Rosenweig, Cabral, David, & Lasser, 2011). In both pre-diabetes, and T2D, PGC1-α expression is low along with reduction in NRF expression (Patti et al., 2003). Reduced expression of PPARGC1A has not only been reported in individuals with T2D, but also in individuals who are unaffected, but have a family history of T2D (Gillberg et al., 2013). Recently a study established association between *PPARGC1A* haplotype and 30 min, 60 min post load glucose levels and beta cell function indices. This study also demonstrated a borderline significant association of *PPARGC1A* with T2D (Oberkofler et al., 2004). In aged mice, up-regulated *PPARGC1A* caused increase in insulin sensitivity, and insulin signaling (Wenz, Rossi, Rotundo, Spiegelman, & Moraes, 2009). PGC1-α also increases glucose uptake by up-regulating glucose transporter (GLUT-4) in skeletal muscle cells and increases gene phosphoenolpyruvate carboxy-kinase and glucose-6-phosphatase activities (Yoon et al., 2001). PGC1-α protein expression is found to be lower in adipose tissue of obese, as compared to lean individuals, independent of ethnicity (Chen, Yan, Huang, Yang & Gu, 2004).

PPARGC1A variants related to insulin resistance, and impaired insulin secretions are therefore important in the pathogenesis of T2D. *PPARGC1A* coactivates peroxisome

proliferator-activated receptor gamma (*PPARG*), implicated in several regulative pathways such as lipid and glucose homeostasis, adipocyte proliferation and adaptive thermogenesis by interaction and regulation of various other genes (Huss, Koop & Kelly, 2002; Puigserver, Wu, Park, Graves, Wright & Spiegelman, 1998; Rosen & Spiegelman, 2001). Due to its extensive role in regulation of various biochemical processes, the human *PPARGC1A*, located in chromosome 4p15.1 has been identified to be linked to T2D and related phenotypes. PGC1-α is therefore involved in regulation of mitochondrial metabolism, adaptive thermogenesis, as well as many other biochemical processes (Handschin & Spiegelman, 2006).

Mitochondrial metabolisms produces reactive oxygen species (ROS) such as super-oxides but then are neutralized by various detoxifying enzymes such as superoxide dismutase or glutathione peroxidase, to maintain integrity of the system. PGC1-α protein controls the detoxification of these ROS by increasing expression of these detoxifying enzymes especially in muscles and mitochondria, thus a major role player in lowering oxidative stress (St-Pierre et al., 2006; St-Pierre et al., 2003; Valle, Alvarez-Barrientos, Arza, Lamas, & Monsalve, 2005). The ROS build up in cells perhaps due to low detoxifying enzyme expression may create havoc on the cell machinery. ROS can damage DNA causing mutations, and cell membranes by lipid peroxidation and eventually lead to cell death. Additionally, nuclear respiratory factors (NRFs) expression is also stimulated by PGC1-α (Wu et al., 1999). NRFs are regulators of various genes involved in mitochondrial function (Wu et al., 1999). Thus, the *PPARGC1A* variants may result in production of non-functional protein increasing oxidative stress and thus leading to many chronic diseases.

Animal studies

Master regulator role of *PPARGC1A* has been established through numerous animal studies. In a mice study, knocking off *PPARGC1A* gene not only resulted in reduced expression of mitochondrial genes involved in electron transport chain but also mitochondrial respiration (Li, Monks, Ge, & Birnbaum, 2004; Leone et al., 2005). This *PPARGC1A* knock out mice also demonstrated inability to increase UCP1 expression, therefore dys-regulation of adaptive thermogenesis (Li, Monks, Ge, & Birnbaum, 2004; Leone et al., 2005). Lehman et al. (2000) reproduced the study but using transgenic mice instead of knocking off *PPARGC1A* gene and found increase in gene products involved with mitochondrial biogenesis which was later supported by another study (Wende et al., 2007). Resistance to age related obesity and T2D has also been observed in transgenic mice with up-regulated muscle PGC1-α (Wenz, Rossi, Rotundo, Spiegelman, & Moraes, 2009).

Ethnicity and Type 2 Diabetes

Complex interactions between ethnicities such as genetic variations and environmental factors (food, lifestyle and physical inactivity) have been identified to be associated with T2D (Chapp-Jumbo, Edeoga, Wan, & Dagogo-Jack, 2011; Schulze & Hu, 2005; Hansen & Pedersen, 2005; Kobberling & Tillil, 1990). Compared to non-Hispanic whites, the risk of T2D is 66% higher among Hispanics and 77% among Non-Hispanic Blacks (Colditz, Willett, Stampfer, Manson, Hennekens, Arky, & Speizer, 1990).Various population studies suggested a higher risk for T2D in Japanese and Danish populations, and the associated complications like hypertension (Hara, 2002). Early insulin secretions in Austrian population, Pima Indians respectively have also

been linked with higher risks for the carriers of *PPARGC1A* variants (Hara et al., 2002; Ek et al., 2001; Muller, Bogardus, Pedersen, & Baier, 2003; Oberkofler et al., 2003). The absence of association with diabetes- related traits have also been reported in German, Dutch, French Caucasians and the Chinese (Ambye et al., 2005; Chen, Yan, Huang, Yang & Gu, 2004; Lacquemant, Chikri, Boutin, Samson, & Froguel, 2002). Specifically, one variant, rs8192678 (Gly482Ser), of *PPARGC1A* gene has been established to be associated with T2D in Danish (Ek et al., 2001), Japanese (Hara et al., 2002), Southern Chinese (Zhang et al., 2007) and North Indians (Bhat et al., 2007), but no such association was reported in Pima Indians (Muller, Bogardus, Pedersen, & Baier, 2003) or in French Caucasians (Lacquemant, Chikri, Boutin, Samson, & Froguel, 2002). Another variant, rs2970847 (Thr394Thr), of *PPARGC1A* has also been linked with insulin resistance and T2D in Chinese (Song et al., 2010), with T2D in two North Indian (Kashmiri and Punjabi) populations (Bhat et al., 2007). These discrepancies in genetic associations in different populations could merely be due to different genetic admixture or due to errors in sampling, low statistical power, population not being homogenous, stringency for genome wide studies (GWAS). According to Centre for Disease Control and Prevention (CDC), adult Cuban Americans and individuals with African origins have higher incidences of T2D and associated complications like hypertension, obesity, cardiovascular diseases and mortality (CDC, 2014). The current Cuban population resulted from the complex process of overlapping and racial mixture between groups from Asia, Africa, Native America and Spain which could be a major influence for the higher prevalence of T2D. In this population, lifestyle also is a huge player in the development of T2D.

Hispanics are rather a large group of individuals that includes Mexicans, Puerto Ricans, Cubans, and Peruvians among many others (Garcia, 2000). The differences among Puerto Ricans and Peruvians or Mexicans and Cubans are substantial. These differences trace back to the ancestry of individuals that differed widely in very important factors such as language, food, religion and even genetic makeup (Lentz, 1995). Most of the Cubans that have migrated to United States are white and of higher socioeconomic status. The access to more food and other factors may amplify already prevalent unhealthy lifestyle like smoking, physical inactivity, and diet after moving to United States. While the data on dietary patterns in Cubans are unavailable, however 1982–84 HHANES survey suggest higher consumption of junk foods (Taylor et al., 2008). On average Cuban Americans have fewer servings of fruits and vegetables, and grains in their daily diet (Huffman et al., 2012; Helmrich, Ragland, Leung, & Paffenbarger, 1991; Ling et al., 2008). All of these factors make Cuban Americans an interesting population to study. Despite the fact that Cuban Americans have the high risk to develop T2D; detailed information on Cuban Americans is missing. Moreover, no study so far has looked at genetic associations of Cuban Americans with chronic diseases such as T2D. Specifically, there has been no study that investigated the association of *PPARGC1A* variants, T2D and its related complications in Cuban Americans.

Higher cardiovascular risk, diabetes, and obesity have been demonstrated in African Americans than in their White counterparts could be due to many factors such as lower socio economic status, physical inactivity, genetic and dietary factors (Boden, 1997). The prevalence of T2D is highest among African Americans (CDC, 2014).

Obesity, one of the major risk factors for T2D is also highly prevalent in African American women and adults as compared to Whites (CDC, 2014). The African American diet is poor in fruits and vegetables, but high in fat, sugar, and salt that increases the risk of developing high blood pressure, obesity and consequently T2D. Although the adipogenic diet puts African Americans at high risk for T2D, the role of genetics cannot be ruled out. African Americans received 'thrifty gene' from their African ancestors that helped them survive in case of unavailability of food (Neel, 1987). The 'thrifty gene' along with diet with poor nutrition has made African Americans the high risk population for T2D (Neel, 1987). Moreover, according to CDC statistics, more than 19.4% of African American adults smoked cigarettes in 2011 and smoking among men is found to be higher than women (CDC, 2012). Cigarette smoking is an established risk factor for heart disease and stroke and may be instrumental in development of T2D and associated oxidative stress (CDC, 2012). Haitian Americans are grouped together with other populations of African origins. Apart from African descent, populations in Haiti also have lineage from France, and Spain making them unique (Zephir, 2004). The distinctness of Haitian Americans is not only accounted for by different, genetics but also lifestyle and dietary factors. Haitian Americans generally eat two meals a day and their meals usually are predominantly starch based which could be the reason why Haitian Americans have worst glycemic control than African and Non-Hispanic Whites with diabetes (NDEP, 2012). The differences between Haitian Americans and African Americans are more evident from the healthy eating index (HEI) scores, and level of physical activity (Huffman et al. 2012). There exists an assumption that ethnic groups are homogenous

and obvious differences among different members of the ethnic group and many times subgroups with the ethnicity are overlooked. The *PPARGC1A* association studies are virtually absent in Haitians. To the best of our knowledge there is only a single study that determined the association of *PPARGC1A* in obese African Americans (Edwards et al., 2012). Moreover, the ethnic differences that exist within ‘Black’ population are not yet explored. The opportunity to investigate the association between *PPARGC1A* genetic variations and T2D in Cuban American, African American and Haitian American population of United States of America is present in Miami. Therefore, the study was undertaken to examine the relationship between *PPARGC1A* and T2D.

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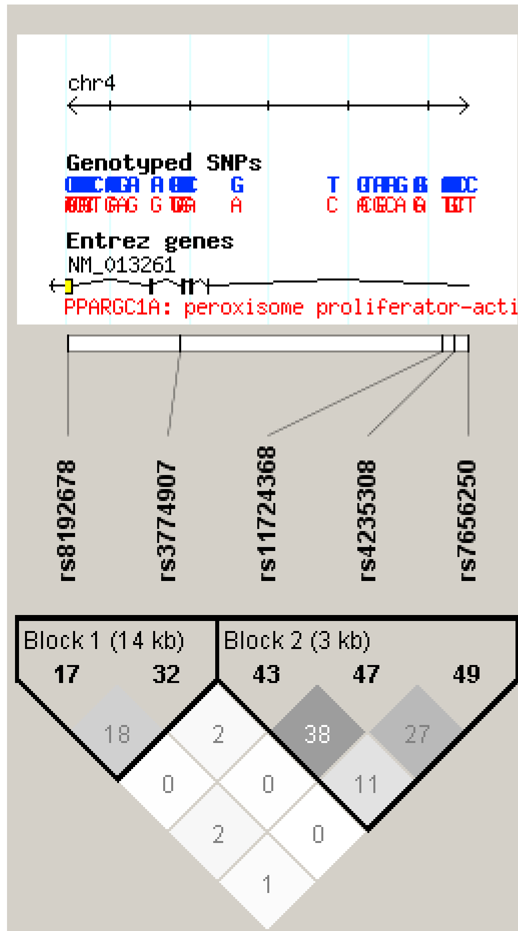
B. Table 1: Risk factors for development of type 2 diabetes

Established	**Proposed**
Age	Persistent Organic Pollutants
Parent or sibling with diabetes	Exposure to agricultural pesticides during first trimester of pregnancy
Ethnicity	Vitamin γ-tocopherol
Prediabetes	Exposure to arsenic
History of cardiovascular disease	
History of gestational diabetes	
History of polycystic ovary syndrome (PCOS)	
Genetics	
High blood pressure	
Physical inactivity	
Abdominal obesity	
Being overweight	
High cholesterol	

C. Figure 1: Haploview plot showing Linkage disequilibrium (LD) with r^2 values for five selected SNPs of *PPARGC1A* gene.



***Note**: Black coloring display strong LD, dark grey display less strong LD, light grey displays intermediate LD and white displays weak LD.*

D. Table 2: Characteristics of most studied SNPs in the *PPARGC1A* gene

NCBI ref SNP number*	Chromosome nucleotide position ǂ	Disease risk associations	Location	Minor allele	F-Score	Minor allele frequency†		
						Global	CEU	ASW
rs8192678	23815662	T2D, CVD, Obesity	intron	G	0.50	0.29	0.35	0.07
rs3774907	23829862	Not enough information	intron	C	0.18	0.18	0.27	0.13
rs11724368	99418507	CVD	intron	G	0.25	0.11	0.21	0.08
rs4235308	23864412	CVD	intron	C	0.28	0.39	0.41	0.31
rs7656250	23866016	T2D, CVD	intron	C	0.27	0.26	0.26	0.12

*Note: * National Center for Biotechnology Information (NCBI) reference single nucleotide polymorphism (SNP) number (http://www.ncbi.nlm.nih.gov/)*
ǂGenome Reference Consortium Human Build 37 patch release 13 (GRCh37.p13) used for nucleotide position (http://www.ncbi.nlm.nih.gov/SNP)
†Minor allele frequencies are from a reference population genotyped in HapMap Project. Population descriptors: CEU (C): Utah residents with Northern and Western European ancestry from the CEPH collection; ASW (A): African ancestry in Southwest USA. CVD=Cardio Vascular Disease; T2D=type 2 diabetes; F-score=functionality score.

CHAPTER II

SPECIFIC AIM AND HYPOTHESIS

Multiple lines of studies have identified that the *PPARGC1A* plays a crucial role in glucose metabolism, insulin signaling, adipogenesis and oxidative metabolism. The involvement of *PPARGC1A* in all these metabolic pathways puts this gene in central role suggesting strong relationship of polymorphisms (variants) of *PPARGC1A* with T2D. However, inconsistent replication of results is often present when studying genetic associations, due to ethnic differences present in these complex interactions between genetic and lifestyle factors. Therefore, due to this gap in knowledge, this dissertation research assessed *PPARGC1A* polymorphisms: rs8192678, rs3774907, rs11724368, rs4235308, rs7656250 as genetic determinants of T2D and related phenotypes in three ethnicities at high risk for T2D; Haitian Americans, African Americans and Cuban Americans. It is well known that T2D does not affect all ethnicities and both sexes equally. We aim to establish some understanding regarding the differences in T2D prevalence observed between three ethnicities, with specific emphasis on the correlations of *PPARGC1A* polymorphisms with T2D. As *PPARGC1A* is a genetic determinant of T2D, the relationship between intermediate phenotypes was also investigated with *PPARGC1A* polymorphisms. The correlations of *PPARGC1A* polymorphisms were further studied against physical activity and lifestyle factors such as use of smoking, and alcohol.

The following hypotheses were tested:

Hypothesis 1: *PPARGC1A* variants mediate genetic predisposition to T2D and its related phenotypes such as obesity.

- The mediation by gene *PPARGC1A* polymorphisms in predisposition of T2D was tested for African Americans and Haitian Americans in chapter III (for SNPs rs8192678, rs7656250, rs4235308 and rs11724368) and V (for SNP rs3774907). The association of *PPARGC1A* polymorphisms with related phenotypes of T2D was tested for Haitian Americans in chapter IV, V and VII. It was tested for African Americans in chapter VII. Hypothesis 1 was tested in Cuban Americans in chapter VI and VII.

Hypothesis 2: If *PPARGC1A* mediates genetic predisposition to T2D, its association will be stronger in cases with young-onset; who are non-obese; and who have family history of T2D.

- Hypothesis 2 was tested in chapter VII for Haitian Americans, African Americans and Cuban Americans.

The proposed study is important because complex interactions between *PPARGC1A* polymorphisms and lifestyle factors in these three study populations are unexplored. The differences between ethnicities and both sexes, hypothesized to be observed may provide explanations as to why different trends are observed for T2D prevalence in among ethnicities and males-females. The research findings provide evidence for the influence of genotypes on the phenotypes (metabolic markers) characteristic to T2D development. The findings provide pivotal

information which can be applied towards ethnic specific T2D management, treatment and community health policies.

CHAPTER III

GENETIC ASSOCIATIONS OF *PPARGC1A* WITH TYPE 2 DIABETES; DIFFERENCES AMONG POPULATIONS WITH AFRICAN ORIGINS

A. Abstract

Aim: To investigate the differences in genetic association of *PPARGC1A* polymorphism with phenotype such as T2D between Haitian American and African American adults.

Methods: The case-control study consisted of >30 years old, self-identified Haitian Americans (n=110 cases, n=116 controls) and African Americans (n=124 cases and n=122 controls) living in South Florida with and without T2D. Information was collected on socio-demographics; anthropometrics; medication use; smoking history; and family history of the participants. TaqMan allelic discrimination assays (LifeTech, Foster City, CA) used to genotype whole genome DNA using real-time PCR amplification on BioRad CFX96 real time PCR instrument (Hercules, CA).

Results: Adjusted logistic regression indicated that the SNP rs7656250 showed protective association with T2D (OR=0.22, p<0.01) in Haitian Americans but risk association with T2D (OR=1.02, p=0.96) though it did not reach statistical significance. The rs4235308 also showed protective association with T2D in Haitian Americans (OR=0.42, p=0.03) but significant risk association with T2D in African Americans (OR=2.53, p=0.03). After stratification with sex, in Haitian Americans, both rs4235308 (OR=0.38, p=0.03) and rs7656250 (OR=0.23, p<0.01) showed protective association with T2D in females but could not reach statistical significance

in males. In African American females, rs7656250 showed risk association though statistically non-significant (OR=1.14, *p*=0.78), whereas in males, it had statistically significant protective effect on T2D (OR=0.37, *p*=0.04). In African American females, rs4235308 had stronger risk association with T2D (OR=2.69, *p*=0.03) than both sexes combined.

Conclusions: The trends observed for genetic association of *PPARGC1A* SNPs; rs4235308 and rs7656250 between Haitian Americans and African Americans point out differences among Black race. The lack of genetic association studies in these two ethnicities warrants replicative study with larger sample size.

B. Introduction

Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha (*PPARGC1A*) gene encodes a well-known protein, PGC1-α (Lehman, Barger, Kovacs, Saffitz, Medeiros, & Kelly, 2002; Huss, Kopp, & Kelly, 2002; Handschin & Spiegelman, 2006; Gerhart-Hines et al., 2007; Fernandez-Marcos & Auwerx, 2011). PGC1-α interacts with a wide array of nuclear receptor factors (NRFs) that further regulate several mitochondrial genes responsible for maintaining energy metabolism, mitochondrial function and biogenesis (Lehman, Barger, Kovacs, Saffitz, Medeiros, & Kelly, 2002; Huss, Kopp, & Kelly, 2002; Handschin & Spiegelman, 2006; Gerhart-Hines et al., 2007; Fernandez-Marcos & Auwerx, 2011). PGC1-α regulates gluconeogenesis through transcription factors including NR3C1 and FoxO1 (Puigserver et al., 2003). In addition, PGC1-α regulates fatty acid oxidation as well as oxidative phosphorylation by interaction with peroxisome proliferative activated receptor alpha (PPARA) and estrogen receptor-related receptor (ESRR) (Wu et al., 2000; Lehman, Barger, Kovacs, Saffitz, Medeiros, & Kelly, 2002; Huss, Kopp, & Kelly, 2002; Handschin & Spiegelman, 2006; Gerhart-Hines et al., 2007; Fernandez-Marcos & Auwerx, 2011). Upregulation of glucose transporter-4 (GLUT-4) by PGC1-α increases glucose uptake in skeletal muscle cells and increases phosphoenolpyruvate carboxy-kinase and glucose-6-phosphatase activities (Michael et al., 2001; Miura, Kai, Ono, & Ezaki, 2003). This versatility of PGC1-α as a master co-activator of various metabolic processes has put it on a center stage for variety of human metabolic diseases such as type 2 diabetes (T2D) (Oberkofler et al., 2004) .

Reduced expression of PGC1-α has been reported not only in individuals with T2D, but also in individuals who are unaffected, but also in those who have a family history of T2D (Gillberg et al., 2013). Ethnic heterogeneity observed in genetic associations of *PPARGC1A* polymorphisms with T2D could be due to the presence of causal or other polymorphisms in strong linkage disequilibrium (LD) with the polymorphism in question. Differences in LD or gene to gene interactions among ethnicities could also be a possible explanation for such observed differences. Moreover, the environment in which populations live varies around the world. This variation in the interaction of environment with gene of interest could also be instrumental in different associations of *PPARGC1A* polymorphisms with T2D across ethnicities.

Differences in genetic variations and environmental factors (diet, lifestyle and physical inactivity) between ethnicities have in fact been identified to be associated with T2D (Chapp-Jumbo, Edeoga, Wan, & Dagogo-Jack, 2011; Schulze & Hu, 2005; Hansen & Pedersen, 2005; Kobberling & Tillil, 1990). Compared to non-Hispanic Whites, the risk of T2D is 77% higher among Non-Hispanic Blacks (Narayanan, Boyle, Thompson, Sorensen, & Williamson, 2003). Although the adipogenic diet puts African Americans at high risk for T2D, the role of genetics cannot be ruled out. African Americans received 'thrifty gene' from their African ancestors that helped them survive in case of unavailability of food (Neel, 1989). The 'thrifty gene' along with diet with poor nutrition has made African Americans the high risk population for T2D (Neel, 1989). Quite often the lines that separate various sub populations within the 'Black' community are blurred in research studies, which make association studies

difficult, due to presence of genetic heterogeneity within the sample. The latest US Census Bureau data (2008) indicates the presence of 546,000 Haitian immigrants in the United States, 46% of total Haiti- born population resides in Florida and more specifically 34.2 % reside in the Miami-Dade and Broward Counties, FL (Rosen, Sharpe, Rosen, Doddard, & Abad, 2007). Haitian Americans are generally grouped together with other populations of African origins. Apart from African descent, populations in Haiti also have lineage from France, and Spain making them unique (Zephir, 2004). In 2010, the International Diabetes Federation estimated the T2D prevalence in Haiti to be 7.2% for 20 to 79 year olds (International Diabetes Federation, 2000) yet the official data for Haitian-Americans are not available. Therefore genetic association studies are important for *PPARGC1A* gene, which is implicated in energy metabolism and T2D in populations with African origins. However, there is lack of data on the relationship between *PPARGC1A* polymorphisms and T2D outcomes in Haitian Americans. Therefore, the principle focus of this study was to investigate the differences in genetic association of *PPARGC1A* polymorphism with phenotype such as T2D between Haitian American and African American adults residing in south Florida.

C. Materials and methods

1. Study population

Self-identified Haitian Americans and African Americans living in South Florida, ages >30 years, were recruited at the Human Nutrition Laboratory, Department of Dietetics and Nutrition, Robert Stempel College of Public Health and Social Work,

Florida International University for a case control cross sectional study. Recruitment of participants was done using invitational flyers, community-based sources and advertisements in English and Creole. The presence of T2D was self-reported by the participants and was confirmed with laboratory tests using American Diabetes Association criteria (fasting plasma glucose concentration ≥126 mg/dl or use of insulin or diabetes medication). Individuals with any other chronic condition, pregnancy or lactation, were excluded from the study. The research purpose and protocol was explained in English as well as Creole to the participants and voluntary informed consent was procured. Institutional Review Board (IRB) approval was received from Florida International University prior to study initiation.

2. Socio-demographics, anthropometrics and medical assessment

The information on demographics such as age, gender, T2D medication use and smoking history was collected using questionnaire to match cases and controls for both ethnicities by trained research staff. Height as well as weight were measured using SECA balance scale (Seca Corp, USA). Body mass index (BMI) was then calculated in kg/height in m2. A non-stretchable measuring tape measured waist circumference (WC) to the nearest 0.1 cm by placing it midway between the 12th rib and iliac crest at minimal respiration. After 15 minute rest, sphygmomanometer (Tycos 5090-02 Welch Allyn Pocket Aneroid Sphygmomanometer, Arden, NC, USA) and a stethoscope (Littmann Cardiology, 3M, St Paul, MN, USA) were used to measure blood pressure (BP).

3. Blood collection and DNA isolation

Twenty ml of venous blood was collected from each individual after an overnight fast (at least 8 hours) by a certified phlebotomist using standard laboratory techniques. Genomic DNA was then 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 tested using 2000c nanodrop spectrophotometer (Thermo Scientific, USA).

4. Single nucleotide selection and genotyping

The *PPARGC1A* gene is located in 4p15.1 region spanning ~110 kb. The rationale behind SNP selection was to give equal emphasis to functionality, already known disease associations, statistical power and cost. The four SNPs were selected for genotyping (Table 1) using HapMap (http://www.hapmap.org) genotype data from Africans, and taking into account their relationships with each other. These SNPs were tested for inter-relationships using linkage disequilibrium (LD) plots. TAGGER on Haploview was used for selection of haplotype tagging SNPs. The independence of each SNP from others is evident in the LD plot (Fig. 1). The values shown in this plot are r2 values showing the correlation between any pair of SNPs. The highest r2 value for any pairwise comparison for the four selected SNPs is 0.38 as shown in Fig 1. An integrated selection on the basis of genetic associations and human genome epidemiology was done using HuGE Navigator and dbSNP. Functionality of SNPs was assessed bio-informatically on F-SNP website (http://compbio.cs.queensu.ca/F-SNP/). Thus, seventy five SNPs were narrowed down using mathematical, biological

and bioinformatics approach to four that have high minor allele frequencies (MAF), robust disease associations, high functionality and no correlation with one another. The main characteristics for the selected *PPARGC1A* gene Single Nucleotide Polymorphisms (SNPs) genotyped are shown in Table 1. Genotyping for all four 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). PCR amplification (20 μL) was performed in 96 well plates using Bio-Rad's SsoFast™ Probes Supermix as the reaction buffer with the TaqMan Assay. To ensure reproducibility and reliability of genotyping method, 10% of the DNA samples were duplicated during genotyping. Bio-Rad CFX Manager software (version 3.0) was utilized for both data acquisition and assignment of genotypes for each SNP.

5. Statistical Analysis

The statistical analysis were done using SPSS version 20 (SPSS Inc., Chicago, IL, USA). All statistical tests were two-tailed, and the threshold for statistical significance was set at $P \leq 0.05$. Sample size calculation was performed prior to the initiation of the study. Sample size of n=62 was calculated for significance threshold of 0.05 and odds ratio of 1.5 for equal case and control, to have statistical power of 80%. Genotype counts in each SNP were checked for Hardy-Weinberg equilibrium (HWE) in controls using the Chi-squared goodness-fit test. 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 recessive genetic model to detect recessive effects, often

overlooked by other genetic models. Logistic regression methods were used to calculate unadjusted and adjusted 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) before and after adjusting for potential confounding factors such as age, sex, smoking status, and BMI. The analysis also included interaction term for SNPs and sex. Due to heterogeneity among two ethnicities, these two groups were analyzed separately. Stratified analysis by ethnicity and sex was performed, to assess their effect modification on the relationship of polymorphisms with the phenotype i.e. T2D. The analysis was then repeated adjusting for age, BMI and smoking status.

D. Results

A total of 226 Haitian Americans (n=110 cases, n=116 controls) and 246 African Americans (n=124 cases and n=122 controls) comprised the study population for his study.

General characteristics

Table 2 shows the general characteristics of the individuals in the study. In brief, individuals with T2D (cases) were older than those without T2D (controls) in both Haitian Americans (p=0.001) and African Americans (p=0.022). Cases in Haitian American (p=0.019), as well as African American group (p=0.000) had higher waist circumference than controls. However, BMI was significantly higher for cases as compared to controls in African Americans only (p=0.000). There was no significant difference between cases and controls in Haitian American group for either SBP or

DBP, whereas, SBP was significantly higher in cases as compared to controls in African American group (p=0.006).

The cases in Haitian American group included 48 males (44%) and 62 females (56%) and the controls included 54 males (47%) and 62 females (53%). The cases in African American group constituted of 59 males (48%) and 65 were females (52%). The controls in African American group included 61 males and females each (50%).

Frequency of *PPARGC1A* polymorphisms

All cases and controls were genotyped for the four candidate SNPs. Genotype call rates were higher than 95% for cases and controls in both ethnicities. None of the four *PPARGC1A* SNPs showed any deviation from Hardy-Weinberg equilibrium in controls. Table 3 shows genotype distribution of all four *PPARGC1A* SNPs in the case-control sample for both ethnicities. The minor allele frequency (MAF) for rs8192678, rs7656250, rs4235308 and rs11724368 SNP was 0.145 and 0.060; 0.118 and 0.090; 0.414 and 0.327; 0.072 and 0.069 for cases and controls of Haitian Americans respectively. In African American group, the MAF for rs8192678, rs7656250, rs4235308 and rs11724368 for cases and controls was 0.093 and 0.074; 0.165 and 0.110; 0.343 and 0.336; 0.093 and 0.069 respectively (Table 3). The MAF seen in the study were very close to NCBI's genotyped data validating our study (http://www.ncbi.nlm.nih.gov/SNP/).

Correlations between *PPARGC1A* polymorphisms and type 2 diabetes

In total, four *PPARGC1A* SNPs were examined for genetic associations with T2D using logistic regression analysis. Results including unadjusted odds ratios and odds ratios adjusted for covariates (age, sex, BMI, smoking status) and interaction terms

between SNPs and sex are shown in table 4(a & b). Two out of four SNPs showed significant association with T2D in Haitian Americans. However, only one SNP was significantly associated with T2D in African Americans (Table 4b). The SNP rs7656250 showed protective association with T2D with adjusted OR of 0.22 (*p*=0.005) in Haitian Americans (Table 4a). This association was not significant for African American group but when adjusted for confounders, rs7656250 showed risk association with T2D with OR of 1.02 (*p*=0.940) though it did not reach statistical significance (Table 4b). The interaction between sex and rs7656250 was found to be significant only in Haitian Americans (*p*=0.008). In Haitian Americans, rs4235308 had an unadjusted odds ratio (OR) of 0.53 (*p*=0.033) as shown in table 4a. The adjustment for age, BMI, sex, smoking and interaction terms for SNPs and sex lowered the effect (OR=0.42, *p*=0.026).This SNP showed significant risk association with T2D in African Americans (OR=2.53, *p*=0.028) (Table 4b).

Effect modification of sex on *PPARGC1A* SNPs association on T2D was also explored by stratification by sex adjusted for age, BMI and smoking status, as shown in table 5a & b. In Haitian Americans, rs4235308 showed protective association with T2D both in females (OR=0.38, *p*=0.026) as well as in males (OR=0.62, *p*=0.326), though not statistically significant. In Haitian Americans, rs7656250 also had a protective effect on T2D in females (OR=0.23, *p*=0.006) and but risk association in males (OR=1.62, *p*=0.409). The association in males was statistically insignificant. In African American females, rs7656250 showed risk association though statistically non-significant (OR=1.14, *p*=0.788), whereas in males, it had statistically significant protective effect on T2D (OR=0.37, *p*=0.043). In African American females,

rs4235308 had stronger risk association with T2D (OR=2.69, p=0.029) but not in males (OR=1.16, p=0.723).

E. Discussion

High prevalence of T2D in populations with African origins is well established (Carter, Pugh, & Monterrosa, 1996; EL-Kebbi, Cook& Ziemer, 2003; Wolfe, 2000). Recently, only few studies have documented existing metabolic differences in the sub populations of African ancestry (Agyemang, Bhopal, & Bruijnzeels, 2005; Cooper & David, 1986; Cheema et al., 2014). Despite being well established, the ethnic disparity is not always addressed in genetic association studies. There exists an assumption that ethnic groups within a race are homogenous and obvious differences among different members of the ethnic group and many times subgroups with the ethnicity are overlooked. This study revealed such differences among Haitian Americans and African Americans, often grouped together with other populations of African origins. This study also provides some confirmation of minor allele frequencies of previously discovered genetic markers associated with T2D, furthermore validating our case-control study for investigation.

Of the four *PPARGC1A* SNPs, rs4235308 showed significant overall association with T2D, while rs8192678, rs7656250 and rs11724368 did not show any associations in African American group. However, in Haitian American group, both rs7656250 and rs4235308 showed overall association. Ling et al (2008) reported association of reduced *PPARGC1A* mRNA expression with rs8192678 SNP, making some to speculate it as a functional SNP. The association of rs8192678 SNP with T2D has also

been reported in Danish (Ek et al., 2001), Japanese (Hara et al., 2002), Southern Chinese (Song et al., 2010; Zhang et al., 2007), and North Indians (Bhat et al., 2007), but no such association was reported in Pima Indians (Muller, Bogardus, Pedersen, & Baier, 2003) or in French Caucasians (Lacquemant, Chikri, Boutin, Samson, & Froguel, 2002). These discrepancies in genetic associations in different populations could merely be due to different genetic admixture or due to errors in sampling, low statistical power, population not being homogenous, confounding by gene-environment interactions and stringency for genome wide studies (GWAS).These conflicting results suggest ethnic differences in distribution of the SNPs in different populations and thus differences in susceptibility for T2D in various ethnicities. It is often seen that a genetic association is rather with a nearby SNP than the SNP being tested due to confounding by locus. We made sure that the SNPs selected for the study were independent and the associations were not due to linkage disequilibrium between these gene variants.

One interesting finding in the study was the protective association of rs7656250 as well as rs4235308 with T2D in Haitian Americans whereas risk association in African Americans. Both rs7656250 as well as rs4235308 exhibited protective effect in females of Haitian American group. However, a risk association was observed for both SNPs in females of African Americans in the study. Haitian Americans have poor diabetes control but lower prevalence than African Americans (Vimalananda, Rosenweig, Cabral, David, & Lasser, 2011). The collective protective effect of *PPARGC1A* polymorphisms rs7656250 and rs4235308 in this study in Haitian Americans could be just a glimpse of why such a difference exists. One study pointed

out the differences between both ethnicities of South Florida in diet quality (Huffman, 2012). Although both ethnicities were found to have lower than optimal diet quality, Haitian Americans had better diet quality scores in general but not in women (Huffman, 2012). The prevalence of T2D has been reported to be higher in Haitian females than males in one study (Jean-Baptiste, Larco, Charles-Larco, Vilgrain, Simon, & Charles, 2006) although the study population comprised of only the members of the households present at the time of the visit. This selection bias could have resulted in overestimation of diabetes prevalence in females. Additionally, the gender differences in prevalence of T2D in Haitian Americans are not well known due to lack of literature. The poor access to health care, educational status, exposure to gestational diabetes and diet quality often seen in ethnicities of African origins may increase the lifestyle burden on physiological functioning increasing prevalence of T2D in females (Shai et al., 2006). According to a recent study published in Journal of American Medical Association, African American females had 2.4-fold greater diabetes incidence per 1000 person as compared to 1.5-fold greater in men than their White counterparts (Brancati, Kao, Folsom, Watson & Szklo, 2000). The strong risk association for rs4235308 in African American females observed in this study supports the same trend. However, the risk association of rs7656250 in African Americans could not reach statistical significance, probably due to insufficient sample size. In African males, the association of rs7656250 was marginally protective for T2D; probably it can explain why African American males have lower T2D prevalence than African American females. As there is lack of genetic association

studies in African American population and virtually nonexistent in Haitian American population, further research is warranted.

There are few limitations of this study. Although, the sample size of the study had sufficient statistical power (>80%) to detect odds ratio of 1.5 or more, for equal case and control at significance threshold of 0.05, it may have been inadequate to detect association of SNPs with a modest effect. As with any case-control approach, bias exists for genetic association studies, due to unacceptable designation of cases and controls. In this study, participants were classified as cases or controls (T2D=Yes/NO) with the use of medical history and the standard criteria described by American Diabetes Association. Self-reported ethnicity is a common method with population based association studies and due to population stratification it may increase the false positive results. 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, for each respective ethnicity. The heterogeneity however within the African American and Haitian American population and thus residual confounding is still a concern.

Despite the low *p*-values, the likelihood of true disease associations mostly depends on the biological plausibility. Polymorphisms located within the *PPARGC1A* gene with strong associations with T2D have been reported in multiple genetic association studies (Villegas et al., 2014; Zhu et al., 2009; Lai et al., 2008; Su, Peng, Li, & Huang, 2008; Jing, Xueyao & Linong, 2012). The *PPARGC1A* gene has been identified as a transcriptional co-activator of a series of nuclear receptors, which regulate processes that impact cellular energy metabolism, thermogenesis regulation,

glucose metabolism, adipogenesis, and oxidative metabolism via protein PGC1-α (Gerhart-Hines et al., 2007; Puigserver, Wu, Park, Graves, Wright, & Spiegelman, 1998; Rhee et al., 2003). Acetylation of PGC1-α is in fact essential for its transcriptional co-activator functions (Rodgers et al., 2005) and any hindrance in acetylation-deacetylation process may adversely affect its functioning. PGC1-α dysregulation is often associated with insulin resistance and T2D (Finck & Kelly, 2007), which suggests that variations within the *PPARGC1A* gene may influence transcriptional homeostasis of the genes involved.

In summary, this is the only study that successfully examined differences in genetic associations of *PPARGC1A* with T2D between Haitian American and African Americans. As T2D is a complex disease with strong environmental influence, the contribution of differences in ancestry may be behind the ethnic disparities observed in risk of type 2 diabetes development in this and other populations.

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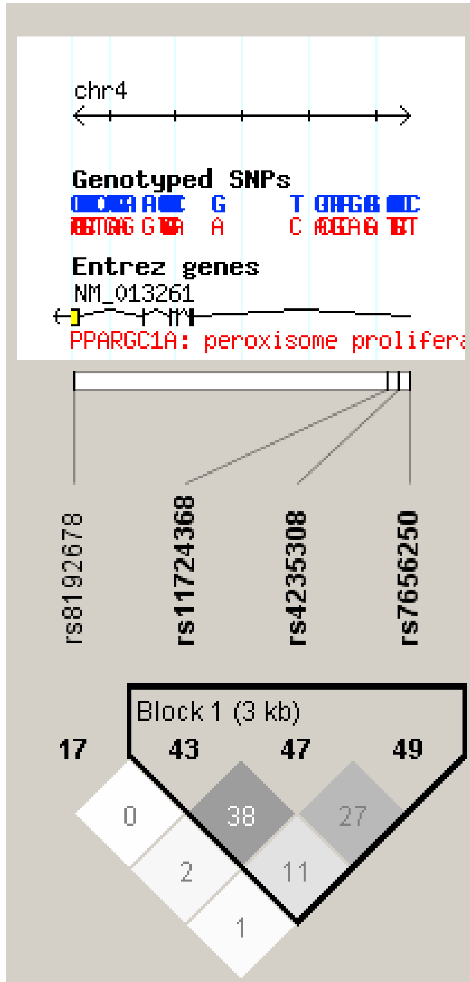
G. Table 1. Characteristics of *PPARGC1A* SNPs

NCBI ref SNP number*	Chromosome nucleotide position‡	MAF†	Disease risk associations	F-Score
rs8192678	23815662	0.291	T2D, CVD, Obesity	0.50
rs7656250	23866016	0.265	T2D, CVD	0.27
rs4235308	23864412	0.396	CVD	0.28
rs11724368	99418507	0.106	CVD	0.25

*Note: * National Center for Biotechnology Information (NCBI) reference single nucleotide polymorphism (SNP) number (http://www.ncbi.nlm.nih.gov/)*
‡Genome Reference Consortium Human Build 37 patch release 13 (GRCh37.p13) used for nucleotide position (http://www.ncbi.nlm.nih.gov/SNP/)
†Minor allele frequencies are from a global population genotyped in HapMap project
MAF=minor allele frequency; T2D=type 2 diabetes; CVD=cardiovascular disease; F-Score= Functionality score.

H. Figure 1. Haploview plot showing Linkage disequilibrium (LD) with r^2 values for four selected SNPs of *PPARGC1A* gene.



Note: Black coloring display strong LD, dark grey display less strong LD, light grey displays intermediate LD and white displays weak LD.

I. Table 2. Descriptive characteristics of individuals by ethnicity and T2D status

Variables		**Haitian Americans**			**African Americans**		
		Cases (n=110)	**Controls (n=116)**	***p*-value**	**Cases (n=124)**	**Controls (n=122)**	***p*-value**
Age, yr.		58.55±10.15	54.03±11.05	<0.01	54.31±10.07	51.20±8.65	0.01
Sex (Male)		48 (44)	54 (46)	0.66	59 (48)	61 (50)	0.70
Waist Circumference (cm)		100.25±12.16	95.97±12.72	0.01	114.15±18.12	102.02±14.98	<0.01
BMI (kg/m^2)		29.50±5.45	28.96±5.157	0.44	35.86±8.28	31.21±6.77	<0.01
Smoke (Yes)		7 (6)	5 (4)	0.49	44 (35)	49 (40)	0.45
Blood pressure	**SBP**	148.24±25.76	144.63±26.206	0.29	140.85±20.11	133.15±18.41	<0.01
(mm of Hg)	**DBP**	90.82±13.22	90.44±13.55	0.83	89.76±11.59	88.37±12.97	0.38
Diabetes Meds (Yes)		98 (89)	0 (0)	NA	96 (77)	0 (0)	NA

Note: Values are unadjusted mean ± SD for continuous variables or N (%) for categorical variables. Diabetes medication is only for cases. So statistical test is not necessary and the p-value is not available (NA). Cases= with T2D; Controls= without T2D; BMI= body mass index; Diabetes Meds= Diabetes medications; SBP= systolic blood pressure; DBP= diastolic blood pressure.

J. Table 3. Genotype distribution of *PPARGC1A* SNPs by ethnicity and T2D

	SNPs		Minor Allele	Haitian Americans (n=226)					African Americans (n=246)				
				Cases (n=110)	Controls (n=116)	*p*-value	MAF (%) Cases	MAF (%) Controls	Cases (n=124)	Controls (n=122)	*p*-value	MAF (%) Cases	MAF (%) Controls
Genotype frequencies (n, %)	**rs8192678**	**CC**	T	92 (84)	102 (88)	<0.01	0.145	0.060	103 (84)	104 (85)	0.36	0.093	0.074
		CT		4 (4)	14 (12)				19 (15)	18 (15)			
		TT		14 (13)	0 (0)				2 (2)	0 (0)			
	rs7656250	**TT**	C	85 (73)	96 (83)	0.58	0.118	0.090	90 (72)	98 (80)	0.26	0.165	0.110
		CT		24 (22)	19 (16)				27 (22)	21 (17)			
		CC		1 (0.9)	1 (0.8)				7 (6)	3 (2)			
	rs4235308	**TT**	C	35 (32)	52 (45)	0.12	0.441	0.327	56 (45)	51 (42)	0.33	0.343	0.336
		CT		59 (54)	52 (45)				51 (41)	60 (49)			
		CC		16 (14)	12 (10)				17 (14)	11 (9)			
	rs11724368	**CC**	G	93 (84)	100 (86)	0.72	0.072	0.069	103 (83)	105 (86)	0.35	0.093	0.069
		CG		17 (15)	16 (14)				19 (15)	17 (14)			
		GG		0 (0)	0 (0)				2 (2)	0 (0)			

Note: Genotype frequencies are depicted as n (%). Cases= with T2D; Controls= without T2D; SNP= single nucleotide polymorphism; MAF= Minor allele frequency.

K. Table 4a. *PPARGC1A* SNP association with T2D in Haitian Americans

Variables		Haitian American							
		Unadjusted OR	**95% C.I.**		***p*-value**	**Adjusted OR**	**95% C.I.**		***p*-value**
rs8192678	**TT +CT vs CC**	0.66	0.30	1.42	0.28	0.49	0.15	1.60	0.24
rs7656250	**CC+CT vs TT**	0.66	0.34	1.30	0.23	**0.22**	**0.07**	**0.64**	**<0.01**
rs4235308	**CC+CT vs TT**	**0.53**	**0.30**	**0.95**	**0.03**	**0.42**	**0.17**	**0.93**	**0.03**
rs11724367	**CC+CG vs GG**	1.14	0.52	2.52	0.74	1.73	0.55	5.49	0.35
rs8192678*sex	**-**	-	-	-	-	1.77	0.34	9.27	0.50
rs7656250*sex	**-**	-	-	-	-	**7.53**	**1.66**	**34.15**	**<0.01**
rs4235308*sex	**-**	-	-	-	-	1.56	0.46	5.34	0.48
rs11724367*sex	-	-	-	-		0.54	0.09	2.95	0.48

*Note**:** The statistically significant results are in bold. Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis for all the SNP. p is considered significant at 0.05. OR= Odds ratio; CI= Confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.*

L. Table 4b. *PPARGC1A* SNP association with T2D in African Americans

Variables		African American								
		Unadjusted OR	**95% C.I.**		***p*-value**	**Adjusted OR**	**95% C.I.**		***p*-value**
rs8192678	**TT +CT vs CC**	0.90	0.45	1.87	0.78	0.55	0.19	1.56	0.26
rs7656250	**CC+CT vs TT**	0.62	0.34	1.13	0.12	1.02	0.43	2.43	0.96
rs4235308	**CC+CT vs TT**	1.29	0.75	2.21	0.36	**2.53**	**1.08**	**5.92**	**0.03**
rs11724367	**CC+CG vs GG**	0.69	0.33	1.25	0.33	0.29	0.08	1.14	0.08
rs8192678*sex	**-**	-	-	-	-	1.46	0.38	5.60	0.58
rs7656250*sex	**-**	-	-	-	-	0.36	0.11	1.13	0.08
rs4235308*sex	**-**	-	-	-	-	0.48	0.15	1.59	0.23
rs11724367*sex	-	-	-	-	-	3.78	0.82	17.31	0.09

*Note**:** The statistically significant results are in bold. Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis for all the SNP. p is considered significant at 0.05. OR= Odds ratio; CI= Confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.*

M. Table 5 a. Associations of the single nucleotide polymorphisms of *PPARGC1A* with type 2 diabetes by ethnicities in males

Variables		**Male**							
		Haitian American				**African Americans**			
		OR	**95% CI**		***p*-value**	**OR**	**95% CI**		***p*-value**
rs8192678	**TT +CT vs CC**	0.89	0.25	3.10	0.85	0.86	0.29	2.53	0.79
rs7656250	**CC+CT vs TT**	1.62	0.51	5.09	0.41	**0.37**	**0.14**	**0.97**	**0.04**
rs4235308	**CC+CT vs TT**	0.62	0.24	1.61	0.33	1.16	0.50	2.68	0.72
rs11724368	**CC+CG vs GG**	0.84	0.23	3.08	0.79	1.11	0.42	2.94	0.83

Note: The statistically significant results are in bold. Controlled variables included in the logistic regression analysis for OR (adjusted) were age, sex, BMI, and smoking status. OR= Odds ratio; CI= Confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.

N. Table 5 b. Associations of the single nucleotide polymorphisms of *PPARGC1A* with type 2 diabetes by ethnicity in females

Variables		**Female**							
		Haitian American				**African Americans**			
		OR	**95% CI**		***p*-value**	**OR**	**95% CI**		***p*-value**
rs8192678	**TT +CT vs CC**	0.51	0.15	0.16	0.26	0.48	0.15	1.49	0.20
rs7656250	**CC+CT vs TT**	**0.23**	**0.08**	**0.65**	**<0.01**	1.14	0.43	3.07	0.79
rs4235308	**CC+CT vs TT**	**0.38**	**1.59**	**0.89**	**0.03**	**2.69**	**1.11**	**6.52**	**0.03**
rs11724368	**CC+CG vs GG**	1.41	0.45	4.40	0.55	0.32	0.07	1.54	0.15

Note: The statistically significant results are in bold. Controlled variables included in the logistic regression analysis for OR (adjusted) were age, sex, BMI, and smoking status. OR= Odds ratio; CI= Confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.

CHAPTER IV

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA COACTIVATOR-1 ALPHA *(PPARGC1A)* POLYMORPHISM ASSOCIATED WITH MICROALBUMINURIA IN HYPERTENSIVE HAITIAN AMERICANS WITH TYPE 2 DIABETES?

A. Abstract

Aim: To explore the relation of *PPARGC1A* polymorphism with microalbuminuria in hypertensive Haitian American adults with type 2 diabetes (T2D).

Methods: Haitian Americans, ages >30 years, with and without T2D were recruited for a cross-sectional, case-control study using community based sources, and advertisements. Sociodemographic data, and medical history, was collected using questionnaires. Anthropometrics and medical assessment performed using standard procedures. Measurements of serum glucose using hexokinase method, A1C using Roche Tina Quant method (Laboratory Corporation of America, LabCorp, FL), lipid panel using automatic chemical analyzer and albumin using ImmunoDip method (Diagnostic Chemicals Limited, Oxford, CT, USA) was performed. Urinary albumin concentrations of 0.18 mg/L as a cut off for microalbuminuria (Yes) were considered corresponding to albumin: creatinine ratio 0.30 ug/mg values. Real-time PCR amplification was performed using TaqMan allelic discrimination assay (Life Technologies Inc, Carlbad, CA) for genotyping rs3774907 polymorphism of *PPARGC1A* from whole genome DNA isolated using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany).

Results: The risk for hypertensive Haitian Americans with T2D to have microalbuminuria was much lower with 'C' allele of *PPARGC1A* polymorphism (OR=0.35, *p*=0.031) than common allele 'T' suggesting protective effect of minor allele for rs3774907 SNP (OR=2.88, p=0.272).

Conclusions: The risk for hypertensive Haitian Americans with T2D to have microalbuminuria was much lower with 'C' allele of *PPARGC1A* polymorphism than common allele 'T' suggesting protective effect of minor allele for rs3774907 SNP. In future, larger replicative studies in several ethnicities should examine the relationship observed in this study to validate the role of *PPARGC1A* in microalbuminuria.

B. Introduction

Microalbuminuria (MA) defined as urinary albumin excretion greater than normal but lower than 300 mg/day, is associated with cardiovascular risk, irrespective of the diabetes status (Mattock et al., 1992; Beilin, Stanton, McCann, Knuiman, & Divitini, 1996; Hillege et al., 2001). The prevalence of microalbuminuria varies in different ethnicities, populations of African origin being at highest risk amongst Caucasians and Polynesians (Goldschmid, Domin, Ziemer, Gallina, & Phillips, 1995). Additionally, the prevalence of type 2 diabetes (T2D) is also the highest (13.2%) among populations of African origin but no data is available on Haitian Americans exclusively (CDC, 2014). Several studies have reported microalbuminuria to be a strong predictor of cardiovascular complications and kidney diseases, particularly in individuals with T2D (Miettinen et al., 1996; Mattock et al., 1998; Borch-Johnsen, Feldt-Rasmussen, Strandgaard, Schroll, & Jensen, 1999). The high odds of microalbuminuria in Haitian Americans with T2D and poor glycemic control have been reported (Cuervo, Zarini, Exebio, McLean & Huffman, 2012). The risk for developing T2D and poor glycemic control increases with elevated urinary albumin excretion. Microalbuminuria is not the causative agent of cardiovascular events but is rather a marker for increased risk (Garg & Bakris, 2002). Several environmental factors have been implicated with the high rates of microalbuminuria, but hypertension and systolic blood pressure (BP) association has been seen across the ethnicities (Konen, Summerson, Bell, & Curtis, 1999; Goldschmid, Domin, Ziemer, Gallina, & Phillips, 1995). Hypertension increases glomerular hydrostatic pressure, accelerating urinary albumin excretion and accelerating microalbuminuria (Palatini, 2003). Elevated glomerular hydrostatic pressure could very well be an indicator of endothelial

dysfunction, making leakage of albumin and other macromolecules of blood into the vascular wall, thereby initiating atherosclerosis (Palatini, 2003). Common genetic markers predisposing one to both high BP and microalbuminuria could also be at play.

Predictive markers for microalbuminuria, including genetic ones, are now being under the close scrutiny of scientific community. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (*PPARGC1A*) is located on chromosome 4. The protein expressed by this gene, PGC1- α is involved in mitochondrial biogenesis via transcriptional regulation of nuclear respiratory factor (NRF) (Fernandez-Marcos & Auwerx, 2011; Scarpulla, Vega, & Kelly, 2012). Vascular endothelial growth factor-1 (VEGF-1) expression is also regulated by PGC1-α (Thom, Rowe, Jang, Safdar, & Arany, 2014). In individuals with hypertension, mitochondrial content of endothelial cells is reported to be lower (Tang, Luo, Chen, & Liu, 2014). Its expression is also lower in diabetes (Pangare & Makino. 2012). Several epidemiological studies have linked *PPARGC1A* polymorphisms with hypertension, carotid atherosclerosis, and coronary artery disease, proposing its involvement in development of vascular disease (Kluge, Fetterman, & Vita, 2013). The region in which the *PPARGC1A* gene is located, chromosome 4p15.1, have been reported to be linked with high BMI (Stone et al., 2002), microalbuminuria (Prior et al., 2012), hypertension (Rojek et al., 2014), elevated systolic blood pressure (Vimaleswaran et al., 2008) in other studies. Haitians have similar diabetes care and outcomes as African Americans but fewer microvascular or macrovascular complications (Vimalananda, Rosenweig, Cabral, David, & Lasser, 2011). This study was therefore designed to explore the relation of *PPARGC1A* polymorphism with microalbuminuria and hypertension in Haitian American population, with T2D.

C. Materials and methods

1. Study population

Participants, ages >30 years, were recruited for a cross-sectional study conducted with Haitian Americans with and without type 2 diabetes. Recruitment of participants was done using invitational flyers, community-based sources and advertisements. The participants self-reported the presence of type 2 diabetes which was further confirmed with laboratory tests using American Diabetes Association criteria. Individuals were classified as having T2D if fasting plasma glucose concentration was ≥126 mg/dl or use of insulin or diabetes medication was reported. Participants were instructed to refrain from smoking, consuming any food or beverages except water, and engaging in any heavy physical activity for at least eight hours prior to their blood collection. Participants were explained protocols of the study and an informed voluntary consent in English or Creole was obtained prior to the commencement of the study. This study was approved by the Institutional Review Board at Florida International University. Individuals with any other chronic condition, pregnancy or lactation, were not eligible for participation.

2. Socio-demographics

Validated questionnaire was used to collect information on demographics such as age, gender, and smoking history. Data on T2D status (yes/no), duration of T2D, medication use (for diabetes, Nonsteroidal Anti-inflammatory Drugs (NSAIDs), and family history of T2D was collected using validated questionnaire by trained staff.

3. 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 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. The BP was measured twice and then averaged. Presence of hypertension was established if participant had either systolic BP ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg or they were using antihypertensive agents (AHA).

4. Blood collection

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, LabCorp, 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) values.

5. Urinary albumin and Microalbuminuria

Albumin levels in fresh, single-voided, first morning urine samples were quantitated by a semiquantitative assay (ImmunoDip, Diagnostic Chemicals Limited, Oxford, CT, USA) according to validated methods published by Davidson et al [24] to assess urinary albumin and microalbuminuria status. The ImmunoDip dipstick fulfilled the

requirements from the National Academy of Clinical Biochemistry (NACB) as a screening tool to detect microalbuminuria. In this study, urinary albumin concentrations of 0.18 mg/L were considered as a cut off for microalbuminuria (Yes) according to vendor (ImmunoDip, Diagnostic Chemicals Ltd) which corresponded to albumin: creatinine ratio 0.30 ug/mg values.

6. DNA isolation and Real-time TaqMan-based genotyping

Whole blood genomic DNA was isolated and tested for quality using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) and 2000c nanodrop spectrophotometer (Thermo Scientific, USA), respectively. Real-time PCR amplification on BioRad CFX96 real time PCR instrument using commercially available TaqMan allelic discrimination assay (Life Technologies Inc, Carlbad, CA) was performed for Single Nucleotide Polymorphisms (SNPs).

7. Quality Control and Internal validity

All biochemical parameters measured in the study had <10% of both intra- and inter-assay coefficient of variations (CVs). Both cases and controls were derived from same study base. To ensure reproducibility and reliability of genotyping method, 10% of the DNA samples were duplicated during genotyping.

8. Statistical analysis

All statistical analyses were performed using SPSS 20 (SPSS, Inc., Chicago, IL, USA). A p-value of <0.05 (two-tailed) was considered statistically significant. The genotype frequencies for rs3774907 SNP was tested for Hardy-Weinberg's equilibrium (HWE). Genetic associations for rs3774907 were assessed using both the recessive (CC+CT vs TT) as well as dominant (TT+CT vs CC) genetic model. These models were employed to

detect recessive effects of the rare allele and dominant effects of common allele respectively. Sample size analyses for case-control ratio of 1:1 were conducted at significance threshold of 0.05 to detect odds ratio of 1.5 or more and minimum sample calculated was n=62. Student's t-test and Chi-squared test were used to compare demographic and clinical information between individuals with and without type 2 diabetes, for continuous and categorical variables respectively. Logistic regression was employed to assess the relationship of SNP and hypertension with binary outcome for case-control status (Microalbuminuria= Yes / No) before and after adjusting for potential confounding factors such as age, sex, BMI, and smoking status among Haitian Americans with T2D.

D. Results

The general characteristics by microalbuminurea status are shown in Table 1. In the present study, genotype call rates for rs3774907 SNP were greater than 95%. The minor allele frequency of C allele was found to be 0.410 in individuals with microalbuminuria as compared to 0.246 in individuals without microalbuminuria (Table 1). The genotype frequencies for TT/CT/CC in individuals with microalbuminurea were 10/26/3 and in without microalbuminuria the frequencies were 39/29/3. Individuals with microalbuminurea had higher proportion of C allele than the individuals without microalbuminurea who had higher proportion of T allele (p=0.013).

No statistical difference in BMI (p=0.482), sex (p=0.231), smoking status (p=0.215), waist circumference (p=0.374) and proportion of individuals with hypertension (p=0.226) was found between individuals with microalbuminurea (cases)

and those without microalbuminurea (controls). The levels of triglycerides (*p*=0.143), total cholesterol (*p*=0.268), FPG (*p*=0.262) or LDL-C (*p*=0.070) were not statistically different between individuals with and without microalbuminurea. However, the microalbuminurea group had higher SBP (*p*=0.001) and DBP (*p*=0.026), A1C (*p*=0.013) and HDL-C (p=0.039).

As shown in Table 2, the logistic regression analysis for rs3774907, shows unadjusted odds ratios indicating that the individuals with T2D and CC and CT genotype were 0.28 times as likely as those with TT genotype for rs3774907 to have microalbuminurea (*p*=0.004). After controlling for the effect of age, sex, BMI, smoking status, the Haitian Americans with T2D and CC and CT genotype remained steady at 0.29 times as likely as individuals with T2D and genotype TT to develop microalbuminuria (*p*=0.006). On the contrary, the individuals with T2D were 1.84 times as likely to have microalbuminuria if they had TT or CT genotype of rs3774907 compared with those with CC genotype (*p*=0.47). The risk decreased to OR= 1.71 when adjusted for confounding variables; age, sex, smoking status, BMI (*p*=0.53). However, the results were not statistically significant for TT+CT model. Hypertension was not significantly associated with microalbuminuria (Table 2).

In Table 3, likelihood of microalbuminuria in hypertensive Haitian Americans with T2D is shown by presence of rs3774907 alleles. The hypertensive individuals with T2D and CC and CT genotype of rs3774907 were 0.351 times as likely as TT genotype to have microalbuminuria (*p*=0.031) after adjusting for age, sex, BMI and smoking status. On the other hand, the adjusted risk for microalbuminuria increased to 2.882 times in hypertensive Haitian Americans with T2D and TT +CT genotype (*p*=0.272).

E. Discussion

This study investigated the relationship of rs3774907 SNP of the *PPARGC1A* gene with microalbuminuria in hypertensive Haitian Americans with T2D. As anticipated, individuals with microalbuminuria had higher blood pressure and A1C. Surprisingly no difference was seen between with and without microalbuminurea groups for BMI, waist circumference, cholesterol, or triglycerides. Despite higher blood pressure the reason why no difference in lipid profile was seen in microalbuminuria group except HDL-C being higher in individuals with microalbuminuria, is not clear. Interestingly, the minor allele frequency for rs3774907 (C) was higher in individuals with microalbuminuria than without microalbuminuria, suggesting some interaction of this allele with microalbuminuria.

Microalbuminuria is frequently observed in individuals with hypertension (Redon & Pascual, 2006). We tested the effect of this association in either of the two alleles for the SNP rs3774907. Upon testing, the risk for hypertensive individuals to have microalbuminuria was much lower with minor allele ‘C’ of rs3774907 SNP than allele ‘T’ in this study sample of Haitian Americans with T2D. The interaction however was never before examined in Haitian American population, despite African origin populations being at high risk for development of microalbuminuria. Microalbuminuria patients usually show elevated blood pressure, compared to patients without microalbuminuria along with atherogenic lipid profile (Bigazzi & Bianchi, 1995). Same relationship was observed between microalbuminuria and some of the atherogenic factors; blood pressure and A1C but not cholesterol, or triglycerides, in this study consisting of Haitian Americans. This correlation is well documented to be the

manifestation of endothelial dysfunction, reported in hypertension due to the involvement of endothelial cells in permeability and blood pressure control (Pedrinelli et al., 1994). In this study, interaction between hypertension and rs3774907 was also significant suggesting association of hypertension with this SNP. Recent studies have recognized mitochondrial influence in endothelial function due to its involvement in multiple cellular processes. The complex process of mitochondrial content is based on the delicate equilibrium between selective mitochondrial degradation and mitochondrial biogenesis. Mitochondrial biogenesis is regulated primarily by *PPARGC1A* expressed PGC1-α, through activation of nuclear respiratory factor (NRF) (Nisoli et al., 2003; Dominy, Lee, Gerhart-Hines, & Puigserver, 2010). In addition, PGC1-α regulates glucose and lipid metabolism as well as vascular endothelial growth factor-1 (VEGF-1) expression and thus it stimulates angiogenesis (Patten & Arany, 2012).The importance of PGC1-α in angiotensin induced hypertension was established by a recent study (Kröller-Schön et al., 2013). The influence of PGC1-α on elevated blood pressure and thus hypertension could be a major determinant of microalbuminuria.

There are several limitations to the study. Only one variant for the gene *PPARGC1A* was chosen to test the association with microalbuminuria in this study population. Haplotype based analysis should be therefore performed in further studies by assessing other variants within the regulatory region of *PPARGC1A* gene. As type 2 diabetes is a multifactorial disease, environmental factors must be included in such studies to get comprehensive analysis. Ethnic specific case-control studies generally have intrinsic bias due to possible genetic heterogeneity among cases and controls. This study however recruited both cases and controls from the same geographical region for Haitian

Americans. The small sample size with cross sectional design could have been a factor in inability to see few statistically significant interactions.

In conclusion, this is the first study that examined rs3774907 relation to microalbuminuria in hypertensive Haitian Americans with T2D. As it is an exploratory study, additional larger studies however are warranted to confirm the contribution of *PPARGC1A* gene polymorphisms in susceptibility of microalbuminuria in hypertensive Haitian Americans with T2D.

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G. Table 1. Characteristics of study population by Microalbuminurea status

Variables		**With Microalbuminurea (n=39)**	**Without Microalbuminurea (n=71)**	***p*-value**
Age		59.49±9.62	58.04±10.46	0.478
Sex, Female		20 (51)	28 (39)	0.231
BMI (kg/m2)		29.01±4.79	29. 77±5.80	0.482
Waist circumference (cm)		98.85±12.75	101.01±11.85	0.374
SBP (mm of Hg)		159.13±26.89	142.25±23.20	0.001
DBP (mm of Hg)		94.58±13.28	88.75±12.82	0.026
Smoke, Y		4 (10)	3 (4)	0.215
Total cholesterol (mg/dl)		195.62±43.04	186.92±36.98	0.268
Triglycerides (mg/dl)		113.33±60.83	99.00±40.63	0.143
Log TG		1.99±0.17	1.96±0.18	0.335
HDL-C		50.46±14.09	56.90±16.15	0.039
LDL-C		122.44±37.49	110.24±30.97	0.070
FPG (mmol/L)		176.87±75.40	156.66±98.89	0.262
A1C (%)		9.28±2.77	7.96±2.56	0.013
Log A1C		0.94±0.12	0.88±0.12	0.008
Hypertension, Y		33 (84)	53 (72)	0.226
	TT (%)	10 (25)	39 (55)	0.013
rs3774907	**CT (%)**	26 (67)	29 (41)	
	CC (%)	3 (8)	3 (4)	
MAF	**C**	0.410	0.246	

Note: Data were expressed as mean ± SD for continuous variables or N (%) for categorical variables. BMI= body mass index; WC= waist circumference; Log TG= log transformed triglyceride; FPG= fasting plasma glucose; A1C= hemoglobin A1C; HDL-C=high-density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAF=minor allele frequency.

H. Table 2. Logistic regression analysis for rs3774907 and hypertension with microalbuminuria in Haitian Americans with type 2 diabetes.

Parameter	Unadjusted OR	95% CI		*p*-value	Adjusted OR	95% CI		*p*-value
CC+CT vs TT	0.28	0.12	0.68	0.004	0.29	0.12	0.67	0.006
Hypertension	1.79	0.62	5.17	0.274	1.99	0.63	6.25	0.238
Hypertension *rs3774907	-	-	-	-	3.21	0.23	43.59	0.381
TT+CT vs CC	1.84	0.35	9.71	0.47	1.71	0.32	9.19	0.533
Hypertension	1.85	0.66	5.15	0.237	2.08	0.68	6.33	0.197
Hypertension *rs3774907	-	-	-	-	0.35	0.13	0.93	0.035

Note: Controlled variables included in the logistic regression analysis were age, sex, BMI, smoking status. CI= confidence interval; OR= odds ratio.

I. Table 3. Likelihood of microalbuminuria by rs3774907 among hypertensive Haitian Americans with type 2 diabetes.

Parameter		Unadjusted OR	95% CI		p-value	Adjusted OR	95% CI		p-value
rs3774907	**CC+CT vs TT**	0.33	0.13	0.85	0.022	0.35	0.13	0.91	0.031
	TT+CT vs CC	2.55	0.40	16.13	0.32	2.88	0.43	19.0	0.27

Note: Controlled variables included in the logistic regression analysis were age, sex, BMI, smoking status. CI= confidence interval; OR= odds ratio.

CHAPTER V

PGC1-α POLYMORPHISM ASSOCIATION WITH TYPE 2 DIABETES; DUE TO ROLE IN HIGHER FASTING PLASMA GLUCOSE, AND INFLAMMATION IN HAITIAN AMERICANS.

A. Abstract

Aim: The primary aim of this study was to examine the correlation of *PPARGC1A* polymorphism, rs3774907 with type 2 diabetes (T2D) in Haitian Americans with and without T2D. Secondary aim was to explore if this relationship is due to the involvement of PGC1-α in FPG and Hs-CRP levels in Haitian Americans with obesity and T2D status.

Methods: Whole genome DNA was extracted from n=228 Haitian Americans with (n=118) and without (n=110) T2D for this IRB approved case control study using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany). Socio-demographic and anthropometric data comprising of age, sex, smoking history and medication intake was collected using questionnaires. Body mass index (BMI) was calculated as weight/height, both measured using SECA balance scale (Seca Corp, MD, US). If Waist circumference, measured by a measuring tape, was ≥102cm in males and WC ≥88cm in females, the individual was classified as abdominally obese. Measurement of high sensitivity C-reactive protein (Hs-CRP) by immulite method and serum glucose was measured with hexokinase method. Genotyping for rs3774907 polymorphism was performed with whole genome DNA using TaqMan allelic discrimination assay (Life Technologies Inc, Carlbad, CA) on a BioRad CFX96 real time PCR instrument. Multiple linear regression was used to test the relation between rs3774907 genotypes and log transformed FPG or

Hs-CRP with diabetes status (Yes/No) adjusted for age, sex, smoking status and BMI on SPSS version 18 (SPSS Inc., Chicago, IL, USA).

Results: PGC1-α polymorphism was significantly associated with decreased risk for T2D in Haitian Americans with CC or CT genotype than TT genotype (OR=0.33, p=0.005). The individuals with CC+CT genotype for rs3774907 had 0.04 log mmol/L lower FPG (p=0.025) and 0.24 log ng/ml lower CRP (p=0.02) values than TT genotype. Conversely, the individuals with TT+CT genotype had 0.19log mmol/L lower log FPG levels (p=0.653) but 0.67log ng/ml higher log CRP values (p=0.01).

Conclusions: Lower levels of log FPG and Hs-CRP among Haitian Americans who are rs3774907 homozygous for rare allele suggests implication of this *PPARGC1A* polymorphism in metabolic disorders often followed by T2D. The interactions observed with *PPARGC1A* polymorphism may contribute to the explanation of metabolic outcomes such as hyperglycemia or chronic inflammation among certain Black ethnicities which are at high risk for T2D.

B. Introduction

Plasma glucose levels are tightly regulated under normal physiological conditions (Jiang & Zhang, 2003). This normal physiological range of glucose levels in plasma varies from 70 to 100 milligrams per deciliter (mg/dL), depending on feeding or fasting state (Desvergne, Michalik, & Wahli, 2006). Circulating fasting plasma glucose (FPG) is determined by interplay between gastric emptying time and hepatic processes, such as glycogenolysis and gluconeogenesis (Jiang & Zhang, 2003). The plasma levels of fasting glucose of 100 to 125 mg/dL are classified as with impaired fasting glucose (IFG), whereas, FPG levels ≥126 mg/dL, is considered as type 2 diabetes (T2D). The elevation in FPG levels is linked with risk of T2D and cardiovascular diseases (CVD) (De Vegt et al., 1999; De Vegt et al., 2001; Snieder, Boomsma, Van Doornen & Neale, 1999). High blood glucose level is the fundamental clinical characteristic of T2D, which may be caused by anomalies in one or more of the different molecular pathways regulated by certain genes (Narayan, Boyle, Thompson, Sorensen, & Williamson, 2003; Nelson, Vogler, Pedersen, Hong & Miles, 2000). T2D in conjunction with hyperglycemia contributes to vascular dysfunction by instigating various biochemical mechanisms that brings changes in the intricate metabolic pathways.

Hyperglycemia has been reported to stimulate the activation of protein kinase C isoforms, elevating Glycated end product levels, increasing oxidative stress in the vascular endothelium (Lin et al., 2005). The presence of hyperglycemia is also known to trigger the inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) release from various cells, leading to chronic low grade inflammation (Tsigos, Papanicolaou, Kyrou, Defensor, Mitsiadis & Chrousos, 1996). This chronic inflammation

can also be stimulated with the presence of adiposity although inflammation is also observed in non-obese, lean individuals (Peraldi & Spiegelman, 1998; Munoz, Abate & Chandalia, 2013).

Peroxisome proliferator-activated receptor-γ coactivator-1 α (*PPARGC1A*) encoded protein PGC1-α coordinates a tissue-specific gene regulation, affecting the expression of genes involved in hepatic glucose metabolism and mitochondrial biogenesis (Lin, Handschin & Spiegelman, 2005; Yoon et al., 2001). PGC1-α increases glucose uptake by up-regulating GLUT-4 in skeletal muscle cells and it regulates enzymes phosphoenolpyruvate carboxy-kinase and glucose-6-phosphatase levels involved at critical points in gluconeogenesis (Yoon et al., 2001; Mootha et al., 2004). PGC1-α also regulates glycolysis by controlling the levels of NAD+ regenerated from NADPH (Wende et al., 2007). Elevated levels of PGC1-α in muscle have been reported to show decreased rates of glycolysis (Wende et al., 2007). Recently a study established association between *PPARGC1A* haplotype and 30 min, 60 min post load glucose levels and beta cell function indices (Oberkofler et al., 2004). By regulating the expression of enzymes of gluconeogenesis, PGC1-α could in fact be pivotal in regulation of glucose uptake or production.

PGC1-α also protects against inflammatory response caused by high oxidative stress prevalent in several metabolically deregulation linked cases such as T2D (Dillon, Rebelo & Moraes, 2012). PGC1-α reduces both the synthesis and release of pro-inflammatory cytokines (Dillon, Rebelo & Moraes, 2012). Interestingly, high levels of inflammatory cytokines have been reported in the tissues where PGC1-α protein was lost or down-regulated (Handschin et al., 2007). Additionally, a study reported reduced

expression of PGC1-α in mice with heterozygous *PPARGC1A* gene, comparable to human with T2D (Mootha et al., 2004), suggesting a correlation between inflammatory cytokines and PGC1-α. It is therefore imperative to study variations in this gene to enhance knowledge on T2D prognosis.

Ethnic disparity exists in the development of T2D and its comorbidities. Individuals with African origin are at higher risk for T2D than Caucasians (Colditz et al., 1990). Haitian Americans have high blood pressure, poor control of LDL cholesterol as well as poor glycemic control but lower insulin resistance and vascular complications compared to African Americans or Caucasians (Narayan, Boyle, Thompson, Sorensen& Williamson, 2003; Cheema et al., 2014). Therefore, the relationship of *PPARGC1A* polymorphism, rs3774907 on FPG and Hs-CRP levels in Haitian Americans with obesity and T2D status was assessed.

C. Materials and methods

1. Study population

The case-control study sample consisted of n= 228 Haitian Americans with n=118 and n=110 without T2D. The participants were recruited from Miami-Dade and Broward counties using multiple community sources, for a previous parent study with cross-sectional design conducted at Human Nutrition Laboratory at FIU. Local diabetes educators and community health practitioners in both counties contributed in recruitment. Invitational flyers with information on the research protocol of the parent study were distributed to Florida International University (FIU) faculty, staff and students. Additionally, Advertisements were placed in local Haitian American newspapers, Creole

radio station, churches, restaurants and supermarkets to recruit the participants. The intent of the research study as well as protocol was explained to the individuals who agreed to participate and anthropometric information such as age, sex was collected. The age of diagnosis and treatment modalities were also noted for the participants who self-reported to have T2D. The study was approved by the Institutional Review Board at FIU. Voluntary written consent was collected from the eligible participants in Creole or English.

Sociodemographic data, smoking history and status, and medications intake were collected using standard questionnaires. SECA balance scale (Seca Corp, MD, US) was used to measure both height and weight and body mass index (BMI) was calculated as weight/ height (kg/m^2). Those who had BMI≥30 kg/m^2 were considered obese (NIH, 1998). Waist circumference (WC) was measured by a non-stretchable measuring tape between 12th rib and iliac crest. Abdominal obesity was determined with WC in males to be ≥102cm and in females with WC ≥88cm (NIH, 1998).

2. Biological measurements

Whole blood was collected using standard procedures by a phlebotomist from participants with instructions not to smoke, or consume food or beverages except water overnight (at least for 8 hours). The hexokinase enzymatic methods were employed to measure glucose levels in serum stored in a Vaccutainer Serum Separator tube. Blood collected in EDTA collection tube was used to measure glycosylated hemoglobin (A1C) percentages with Roche Tina Quant method by a certified laboratory (Laboratory Corporation of America, LabCorp, FL, USA). Immulite method was employed to

measure values of high-sensitivity C-reactive protein (Hs-CRP) in serum (Roberts, Sedrick, Moulton, Spencer& Rifai, 2000).

3. Genotype detection

After isolating genomic DNA from the peripheral blood using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), single nucleotide polymorphism (SNP) genotyping was performed by real-time PCR amplification on BioRad CFX96 real time PCR instrument. Commercially available TaqMan allelic discrimination assay specific for rs3774907 SNP (Life Technologies Inc, Carlbad, CA) was used for assessment of genotypes along with SsoFast™ Probes Supermix reaction buffer. Each 96 well plate with singleplex reactions contained two no template controls (NTC), random samples for replication and a positive control. The cycling program for PCR for rs3774907 SNP was enzyme activation for 2 minutes at 95°C, 5 seconds of denaturation at 95°C (42 cycles), and then 5 seconds of annealing and extension at 61°C. Data acquisition and assignment of genotype was conducted using Bio-Rad CFX Manager software (version 3.0).

4. Statistical Analysis

All statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, US) with p values <0.05 considered as statistically significant. Log transformations of continuous variables (FPG and Hs-CRP) were made if the variable was not normally distributed. Differences between the general continuous characteristics between case and control subjects were determined using Student's t-test and analysis of variance (ANOVA). Chi-squared test was used to analyze differences between categorical variables such as gender, alleles, and genotypes of two groups and to determine if the SNP conforms to the Hardy Weinberg Equilibrium (HWE). Logistic regression analysis

tested the correlation of rs3774907 with binary outcome diabetes status (Yes/No) adjusted for age, sex, BMI and smoking status. Recessive model was used to test the recessive effect of rare allele on the outcome and dominant model was used to observed effects of common allele on the outcome. The relationship of log FPG or log Hs-CRP with diabetes status (Yes/No), Obesity (Yes/No) and rs3774907 SNP (CC+CT vs TT) and their interactions was examined by multiple linear regression adjusted for age, sex, smoking status and BMI.

D. Results

Table 1 provides general characteristics of the study population by diabetes status. The mean age of individuals without T2D was 54.15 ±11.13 years whereas it was 58.55±10.15 years for individuals with T2D (p=0.002). There was no statistically significant difference between two groups for gender (p=0.56), smoking status (p=0.67), BMI (p=0.56) or obesity (p=0.40). The individuals with T2D had larger waist circumference (p=0.02), higher A1C (p<0.001), and higher FPG (p<0.001) than the participants without T2D. There was significant difference observed for rs3774907 SNP genotype frequencies between two groups (p<0.001). In individuals without T2D, the frequencies (%) for TT/CT/CC genotypes of rs3774907 SNP were 80/20/6 whereas in individuals with T2D, the genotype frequencies were 49/55/6. The minor allele frequency (MAF) in individuals with T2D was 0.304, and in those without T2D, MAF was 0.181.

The descriptive data of the study population by rs3774907 SNP genotype is shown in Table 2. No significant difference was observed for sex (p=0.85), smoking status (p=0.91), obesity (p=0.50), age (p=0.25) or BMI (p=0.73) between the three

genotypes. Individuals with TT genotype had lower proportion of individuals with T2D (p<0.001), lower FPG (p=0.003), lower A1C (p=0.005) than CC and CT genotypes. The heterozygous CT genotype was seen to have higher FPG values than any of CC or TT homozygotes (p=0.003) but when the FPG values were log transformed, there was no significant difference in values among the three genotypes. A1C was also highest in CT genotype followed by CC (p=0.005). The Hs-CRP was lower for CT (p=0.012) genotype than TT or CC (Table 2). Haitian Americans with CC or CT genotype were at lower risk (OR=0.33, 95% CI=0.15-0.71, p=0.005) of T2D than those with TT genotype.

Table 3 shows findings of multiple linear regression analyses of relationship between rs3774907 and log FPG values, with non-significant interaction terms removed. The individuals with CC+CT genotype had significantly lower FPG by 0.04 log mmol/L than TT genotype (p=0.02). The results for TT+CT vs CC genotype were not statistically significant. Results from multiple linear regression analyses for relation of rs3774907 with log CRP values are shown in Table 4. The log CRP values were significantly lower by 0.24 log ng/ml in those with CC+CT genotype than TT genotype (p=0.02). On the other hand, individuals with TT+CT genotypes had 0.67 log ng/ml higher CRP values than those with CC genotype for rs3774907 (p=0.01).

E. Discussion

The effect of *PPARGC1A* polymorphism on FPG and Hs-CRP was explored in Haitian Americans with and without T2D. As expected, individuals with T2D had higher FPG than individuals without T2D however, the values for Hs-CRP was lower among individuals with T2D. High Hs-CRP levels are a sub-clinical indicator for chronic

inflammation, often seen in T2D (Helmersson, Vessby, Larsson & Basu, 2004). It is well known that genetics play a partial role in the levels of FPG (Snieder, Boomsma, Van Doornen & Neale, 1999). The presence of higher MAF in individuals with T2D as compared to without T2D and association of rs3774907 with T2D in this study suggests an association of the minor allele C of rs3774907 SNP with the T2D and possibly its modalities.

When general characteristics of participants were inspected by genotype, carriers of heterozygous (CT) genotype had the highest FPG and, A1C values but surprisingly the lowest Hs-CRP values among the three genotypes. However when log transformed values for FPG and Hs-CRP was compared, the difference was not significant. Homozygous (TT) genotype carriers however had the lowest number of individuals with T2D, lower FPG, and A1C values. The results suggest a protective effect of T allele against metabolic parameters such as Hs-CRP associated with T2D and association of heterozygosity with elevated metabolic values.

Furthermore, results from multiple linear regression analysis show that the individuals with CC+CT genotypes had lower Log FPG as well as Log CRP values than TT genotype. The individuals with T allele had much lower Log CRP values than those with C allele supporting the above projected idea of T allele of rs3774907 being protective for CRP values in Haitian Americans. Despite of poor diabetes control, Haitian Americans have lower type 2 diabetes prevalence than African Americans (Vimalananda, Rosenweig, Cabral, David, & Lasser, 2011). The findings of this study may add to the understanding of why Haitian Americans are protective towards T2D.

Elevated FPG (hyperglycemia) and Hs-CRP are often associated with metabolic diseases such as T2D. Presence of hyperglycemia enhances the oxidative stress by activating Tumor necrosis factor (TNF-α), Interleukin 6 (IL-6), and producing reactive oxygen species (ROS), which further impairs the Nitric oxide (NO) mediated vasodilation (Dandona, Aljada & Bandyopadhyay, 2003). Under normal physiology of vascular endothelium, a balance exists between vasodilators and vasoconstrictors to maintain a selective barrier for various molecules in blood. When NO availability is reduced, endothelial dysfunction results initiating inflammatory response releasing CRP. Inflammation sets up an atherogenic cycle that increases the risk of the individual for cardiovascular diseases and T2D (Laakso, 2010). The role of low levels of NO as antioxidant is well established (Mohanakumar et al., 2002). The antioxidant effect of NO is elucidated via PGC1-α expression and therefore the other stress protection genes (Mohanakumar et al., 2002). However, PGC1-α in turn regulates endothelial Nitric Oxide Synthase (eNOS) expression establishing a feedback regulation of ROS detoxification mechanism (Borniquel, Valle, Cadenas, Lamas& Monsalve, 2006). PGC1-α control the expression of various enzymes directly involved in producing inflammatory response (Dillon, Rebelo, & Moraes, 2012). It is also reported that PGC1-αmodulates circulating glucose levels by controlling the expression of genes involved in hepatic glucose production via gluconeogenesis and glucose metabolism by glycolysis, making PGC1-α critical in T2D prognosis (Yoon et al., 2001; Mootha et al., 2003; Wende et al., 2007). If polymorphism in *PPARGC1A* causes PGC1-α level to vary significantly or structural changes occur that can alter the function of this protein, it could influence the many processes it regulates.

The study has several limitations. Small sample size and case-control study design being some of them. We could not see statistical significance in the T allele carriers Log CRP, probably due to the small sample size. The population though was homogenous, recruited from the same geographical area and base population. Only one SNP was used in the *PPARGC1A* gene in this study. However, this SNP is not in linkage disequilibrium with any other causal SNP (data not shown). Therefore the association demonstrated is not due to correlation to any nearby loci but rather an independent one.

This study explored the possibility that certain ethnicities within Black race may have *PPARGC1A* gene induced metabolic consequences that put them at high risk for T2D. To the best of our knowledge, no other study is available that explored the effect of rs3774907 SNP on FPG or Hs-CRP levels as well as its association with T2D in Haitian Americans. This study should be replicated in a larger study sample as well as in other ethnicities to validate the results.

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G. Table 1. General characteristics of study population by diabetes status

Variables		With T2D (n=110)	Without T2D (n=116)	*p*-value
Age		58.55±10.15	54.15±11.13	0.002
Sex (male)		48 (44)	56 (47)	0.56
Smoke (yes)		7 (6)	6 (5)	0.67
T2D_ meds (yes)		98 (89)	0 (0)	-
Obese (yes)		47 (43)	44 (37)	0.40
BMI (kg/m2)		29.50±5.45	29.10±5.25	0.56
WC (cm)		100.25±12.17	96.29±12.86	0.02
A1C (%)		8.43±2.70	6.05±0.66	<0.001
Log A1C		0.91±0.13	0.78±0.04	<0.001
FPG (mmol/L)		163.83±90.03	101.22±19.79	<0.001
Log FPG		2.16±0.187	1.99±0.08	<0.001
Hs-CRP (ng/mL)		2.92±3.30	3.69±7.29	0.30
Log Hs-CRP		0.38±0.34	0.40±0.39	0.71
MAF		0.304	0.178	-
	TT	49 (69)	80 (69)	0.001
rs3774907	**CT**	55 (25)	30 (26)	
	CC	6 (5)	6 (5)	

Note: Values are unadjusted mean ± SD for continuous variables or N (%) for categorical variables. Diabetes medication is only for with T2D. T2D= type 2 diabetes; BMI= body mass index; T2D_Meds= type 2 diabetes medications; WC= waist circumference; FPG= fasting plasma glucose; A1C= hemoglobin A1C; Hs-CRP= high sensitivity C- reactive protein. Obese= Yes (BMI≥30 kg/m^2).

H. Table 2. General characteristics of Haitian Americans by rs3774907 genotype

Variables	TT (n=129)	CT (n=85)	CC (n=12)	*p*- value
T2D (yes)	49 (38)	55 (65)	6 (50)	0.001
Sex (male)	58 (44)	41 (48)	5 (42)	0.85
Smoke (yes)	7 (5)	5 (6)	1 (8)	0.91
Obese (yes)	49 (37)	38 (5)	4 (33)	0.50
Age	54.80±10.77	58.09±10.52	59.90±9.65	0.25
BMI (kg/m2)	29.73±5.64	29.96±5.05	28.81±4.42	0.73
FPG (mmol/L)	119.14±60.12	150.89±85.72	125.20±68.57	<0.01
Log FPG	2.04±0.14	2.13±0.17	2.04±0.22	<0.01
A1C (%)	6.88±2.18	7.77±2.31	7.00±1.88	<0.01
Log A1C	0.82±0.10	0.87±0.11	0.83±0.10	<0.01
Hs-CRP (ng/mL)	3.52±3.82	3.42±4.53	9.70±18.36	0.01
Log Hs-CRP	0.38±0.35	0.36±0.34	0.60±0.53	0.17

Note: Values are unadjusted mean ± SD for continuous variables or N (%) for categorical variables. Diabetes medication is only for with T2D. T2D= type 2 diabetes; BMI= body mass index; T2D_Meds= type 2 diabetes medications; WC= waist circumference; FPG= fasting plasma glucose; A1C= hemoglobin A1C; Hs-CRP= high sensitivity C- reactive protein. Obese= Yes (BMI≥30 kg/m^2).

I. Table 3. Multiple linear regression analysis for relationship of rs3774907 with log FPG

Variables		Coefficient	SE	p- value	95% CI	
					Lower bound	Upper bound
rs3774907	**CC+CT vs TT**	-0.04	0.01	0.025	-0.08	-0.06
	TT+CT vs CC	-0.19	0.04	0.653	-0.10	0.06

Note: Model adjusted for age, sex, smoking status, T2D status, obesity, two way as well as three way interaction terms between rs3774907, T2D status and obesity.

J. Table 4. Multiple linear regression analysis for relationship of rs3774907 with log CRP

Variables		Coefficient	SE	p- value	95% CI	
					Lower bound	Upper bound
rs3774907	**CC+CT vs TT**	-0.24	0.10	0.020	-0.43	-0.03
	TT+CT vs CC	0.67	0.20	0.010	0.27	1.08

Note: Model adjusted for age, sex, smoking status, T2D status, obesity. Non-significant interactions removed.

CHAPTER VI

TYPE 2 DIABETES IN CUBAN AMERICANS; INFLUENCE OF *PPARGC1A*

A. Abstract

Aim: To explore the influence of *PPARGC1A* gene variants on type 2 diabetes (T2D), and its related phenotypes in Cuban Americans.

Methods: Cuban Americans aged ≥ 30 years with and without T2D were included in the study. Data on socio-demographics, and anthropometrics as well as medical history and alcohol intake was collected. Measurement of A1C by Roche Tina Quant (Laboratory Corporation of America, LabCorp, FL), lipid panel by automated analyzer, glucose by hexokinase assay and hs-CRP by immulite method were conducted. Whole genome DNA was isolated by QIAmp DNA blood mini kit (Qiagen, Hilden, Germany) from whole blood. TaqMan allelic discrimination assay (LifeTech, Foster City, CA) was used for *PPARGC1A* genotyping on BioRad CFX96 real time PCR (Hercules, CA).

Results: In Cuban Americans with CC+CT genotype of *PPARGC1A* SNP rs7656250, the likelihood of T2D was 6.87 times than those with TT genotype (*p*=0.02), after adjusting for age, sex, BMI and smoking status. Significantly higher adjusted odds ratio were found in females (OR=7.67; p=0.01) for CC+CT genotype vs TT genotypes. Additionally, the odds of cases with C allele (CC and CT genotypes) for rs4235308 SNP increased for higher log CRP by 0.33 times (p=0.002) as compared to TT genotype. Further, the likelihood of pulse pressure being higher was 7.39 times in C allele carriers than the ones who had TT genotype (*p*=0.001).

Conclusions: The findings suggest implication of *PPARGC1A* polymorphisms in higher FPG levels, blood pressure that are typical indicators of metabolic abnormalities resulting

in T2D. Cuban Americans being one of the ethnicities in which T2D prevalence is high, the results contribute to the understanding of why some ethnicities are at higher risk of T2D than others. As with any other genetic association study, replicative studies with large samples are needed for this study in order to generalize the findings to Cuban American population.

B. Introduction

Type 2 diabetes (T2D) is a complex cluster of essentially different metabolic disorders. Genetic heterogeneity, lifestyle factors, gene-gene or gene-environment interactions are the determinants of T2D development. Hispanics have higher prevalence of metabolic disorders, hypertension, cardiovascular diseases and T2D than non-Hispanic Whites (Najjar & Kuczmarski, 1989; Smith & Barnett, 2005; Huffman, Gomez & Zarini, 2009). The prevalence of obesity and hypertension among Cuban Americans is comparable to Mexican Americans, Puerto Rican Americans and other Latin American ethnicities, according to HHANES study done in 1982-84 (Najjar & Kuczmarski, 1989). However, Cuban Americans aged 45-74 years have lower prevalence of T2D and diabetes related mortality than Mexican Americans, Puerto Rican Americans (Najjar & Kuczmarski, 1989; Smith & Barnett, 2005). Interestingly, lifestyle factors or acculturation has not been associated with obesity in Cuban Americans (Khan, Sobal & Martorell, 1997). Therefore, it is possible that such metabolic differences within these ethnicities exist due to the genetic admixture.

Recently, peroxisome proliferator activated receptor gamma coactivator-1 alpha (*PPARGC1A*) has emerged as a candidate gene associated with T2D. *PPARGC1A* is pivotal in regulation of several genes, including the ones involved in mitochondrial function, energy homeostasis, lipid oxidation, thermogenesis regulation and oxidative phosphorylation (Gerhart-Hines et al., 2007; Puigserver et al., 1998; Puigserver et al., 2003; Rhee et al., 2003). The protein expressed by *PPARGC1A* called PGC1-α is reduced in T2D and its associated phenotypes. The reduction in PGC1-α expression as a result of Single Nucleotide Polymorphisms (SNPs) in the gene could vary among different

ethnicities. One variant of *PPARGC1A*, rs8192678 (Gly482Ser), is reported to be correlated with T2D in Danish (Ek et al., 2001) but not in Pima Indians (Muller, Bogardus, Pedersen& Baier, 2003) and French Caucasians (Lacquemant, Chikri, Boutin, Samson & Froguel, 2002). The PGC1-α gene variants have also been implicated with obesity in African Americans (Edwards, et al. 2012). As current Cuban population is a result of complex overlap of genes from African, European (Spain) and Native Indian populations, the exploration of a genetic component in metabolic functioning becomes imperative. Hence, the influence of variants of *PPARGC1A* gene and *PPARGC1A*-environment interactions on T2D and its related phenotypes was explored in this study in a Cuban American population.

C. Materials and methods

1. Study population

The individuals for this case-controlled study were recruited for an age matched cross-sectional parent study conducted at Human Nutrition Laboratory, Florida International University (FIU). Cuban Americans with and without T2D were recruited in an alternate manner for duration of one year by letters of invitation. Approximately ten thousand letters, in both English and Spanish, with information about the purpose of the study were mailed to randomly selected individuals with age≥30 years. The lists for Cuban Americans with T2D and without T2D, living in South Florida, were purchased from KnowledgeBase Marketing, Inc., Richardson, TX, USA. The interested participants (4% of total delivered mails) were telephonically interviewed for basic screening for age, gender and T2D status (methods of treatment and age of diagnosis). These individuals

were then explained the purpose, protocol of the parent study in detail. Out of 388 individuals who responded, 18 were ineligible for the study due to ethnicity; age (<30 years); treatment with insulin; pregnant or lactating women; and other chronic conditions. Participants were also excluded if their A1C values could not be measured or caloric intake >5000 kcals for analyses purposes. All eligible candidates were requested for participation in the study and informed voluntary consent obtained from the participants prior to the outset of the study. For the parent study, Cuban Americans, aged ≥30 years with and without T2D were included in the study. Data on age, gender, smoking status, and diabetes medications was collected using a sociodemographic questionnaire. Seca balance scale was used to measure both height and weight (Seca Corp, Columbia, MD, USA) and body mass index (BMI) was calculated in kg/m^2. Blood pressure was measured by a sphygmanometer (Tycos 5090-02 Welch Allyn Pocket Aneroid Sphygmomanometer, Arden, NC, USA) and a stethoscope (Littmann Cardiology, 3M, St Paul, MN, USA) using standard laboratory technique. If values of systolic blood pressure (SBP) ≥140mm Hg and diastolic blood pressure (DBP) ≥90 mm Hg or if the individual was under antihypertensive treatment, the individual was determined hypertensive. Pulse pressure was determined by subtracting DBP from SBP. Food frequency questionnaire (FFQ) developed was utilized to collect information on alcohol consumption (Willet et al., 1985). A certified phlebotomist collected twenty ml of venous blood using standard laboratory techniques from each participant after an overnight fast. Quantification of A1C percentages by Roche Tina Quant method, lipid panel by enzymatic methods, immulite method for serum high sensitivity C-reactive protein (Hs-CRP), and glucose by hexokinase method was performed. Total serum adiponectin was measured using an

enzyme-linked immunosorbent assay (ELISA). Institutional review board at Florida International University approved the study.

2. DNA isolation and genotyping

QIAmp DNA blood mini kit was utilized to isolate genomic DNA from stored whole blood (-80ºC) (Qiagen, Hilden, Germany). Isolated DNA was then tested for both quality and quantity using 2000c nanodrop spectrophotometer (Thermo Scientific, USA). All five SNPs of *PPARGC1A* gene (rs8192678, rs765250, rs3774907, rs4235308 and rs11723468) were genotyped using TaqMan allelic discrimination assays (LifeTech, Foster City, CA) after real-time PCR amplification on BioRad CFX96 real time PCR instrument (Hercules, CA). Data was acquired and assigned using Bio-Rad CFX Manager software (version 3.0).

3. Statistical analysis

The statistical analysis employed SPSS version 18 (SPSS Inc., Chicago, IL, US). All statistical tests were two-tailed, with the statistical significance threshold set at $P \leq 0.05$. The sample size was calculated prior to the data acquisition with statistical threshold of 0.05 and 80% statistical power. Sample size of n=62 was calculated for case-control ratio=1:1 to detect odds ratio of 1.5 or greater. Genotype counts in each of the five SNPs were tested for conformity to Hardy-Weinberg equilibrium (HWE) in controls by the Chi-squared goodness-fit test. All continuous variables were tested for normality using the Kolmogorov-Smirnov test. The variables FPG, A1C, Hs-CRP, insulin, adiponectin, triglyceride (TG) and total cholesterol were log transformed. Student t-test for continuous variables and Chi-squared test for categorical variables were used to compare demographic and clinical data between cases (with T2D) and controls (without T2D).

The combined effect of all five SNPs on outcome case-control status (T2D Yes/T2D No) was assessed using logistic regression. Covariates adjusted for the logistic regression analyses were age, gender, and smoking, BMI and interaction terms for each of the five SNPs with sex. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate if the observed probability was equal to the expected probability based on the fitted model; a lack of fit for the fitted logistic regression model was reflected by a p-value <0.05. The sensitivity and accuracy of our method in prioritizing SNPs associated with T2D was tested using Receiver Operating Characteristic (ROC) curves. The association between T2D and SNPs was then further analyzed by logistic regression after stratifying by sex. The relationship of T2D phenotypes: Log A1C; Log FPG; Log CRP; log adiponectin; log insulin; log triglyceride and total cholesterol with all five SNPs adjusted for age, sex, BMI and smoking status was evaluated by linear regression.

D. Results

Cuban Americans with (cases) and without T2D (controls) were compared for the general and metabolic characteristics in Table 1. The mean age was 64.90±12.29 years for cases and 62.24±11.17 years for controls (p=0.04). There was no significant difference observed between cases and controls for sex (p=0.49), smoking status (p=0.56), SBP (p=0.11), DBP (p=0.30), or Hs-CRP (p=0.44). Both waist circumference (p<0.001) and BMI (p=0.03) were significantly higher in cases as compared to controls. Additionally, the levels for FPG (p<0.001), A1C (p<0.001), Insulin (p=0.002), and TG (p=0.01) were significantly higher in cases than controls. However, adiponectin (p<0.001), TC (p=0.02), HDL (p<0.001), LDL (p=0.006) levels and Kcal (p=0.01) were significantly lower in

cases than in controls. Table 2 shows genotype frequencies and MAF of all five SNPs were compared between cases and controls. The MAF for rs8192678, rs7656250, rs4235308, rs11724368, and rs3774907 were calculated as 0.327, 0.223, 0.377, 0.238 and 0.222 in controls whereas they were 0.335, 0.264, 0.378, 0.198 and 0.182 in cases respectively. There was no statistical significance observed in genotypes of rs8192678 (p=0.78), rs7656250 (p=0.06), rs4235308 (p=0.99), rs11724368 (p=0.43) or rs3774907 (p=0.25) between cases and controls (Table 2).

Results from logistic regression analysis to test combined association of all five SNPs with T2D status are shown in Table 3. Out of five SNPs, only one SNP (rs7656250) showed significant unadjusted disease risk association with T2D with OR=2.98 (p=0.02). After adjusting for age, sex, BMI, smoking status and individual interactions of all five SNPs with sex, the OR increased to 6.87 (p=0.02) for rs7656250 (CC+CT vs TT). The interactions between SNPs and sex were not significant. The receiver operating characteristic (ROC) curve is shown in figure 1. The area under the curve (AUC) was 65.4%, which indicates a good fit of the model. When the T2D association was explored in the population for rs7656250, separated by sex, the odds (unadjusted) in female were significant (OR=6.50; p=0.02) (Table 4). This association was not statistically significant in males. After adjusting for confounders for T2D, such as age, BMI and smoking status, the association remained statistically significant and odds were greater (OR=7.67; p=0.01) for females than both sexes combined (Table 4 vs Table 3).

Multivariable linear regression was performed on T2D intermediate phenotypes with five SNPs adjusted by age, sex, BMI, smoking status in cases. Out of five SNPs, rs4235308 showed association with log Hs-CRP. In cases, the presence of C allele in rs4235308

SNP increased the likelihood of higher log CRP by 0.33 times (p=0.002) as compared to TT genotype. Out of four remaining SNPs, none showed any significant association with the CRP levels. Pulse pressure was also found to be significantly associated with SNP rs3774907 in cases. In individuals with T2D, the likelihood of higher pulse pressure was 7.39 times in C allele carriers than the ones who had TT genotype (p=0.001). Of A1C, FPG, TG, TC, insulin or adiponectin levels, no phenotype was found to be associated with any SNP in Cuban American cases.

E. Discussion

In recent decades, obesity-linked T2D epidemic in the United States has been on the rise (CDC, 2012). The diabetes prevalence rate of >6% which once was seen only in two states: Puerto Rico and Washington, D.C., recently increased to all 50 states by 2012 (CDC, 2012). In 2010, 25.8 million Americans had type 2 diabetes, but the number has jumped to 29.1 million in 2012, according to '*National Diabetes Statistics Report, 2014'* (CDC, 2014). The burden of the disease, however, is not shared equally by all ethnicities. American Indians/Alaskan Natives (15.9%) have highest rates of diagnosed diabetes followed by non-Hispanic blacks (13.2%), Hispanics (12.8%), Asian Americans (9.0%) and non-Hispanic whites (7.6%) (CDC, 2014). Out of all the ethnic minorities in the Unites States, Hispanics, with 52 million people, form the largest group. Hispanic population comprises of diverse Spanish speaking inhabitants of Caribbean, Central and South American countries (Garcia, 2000). Some states in US have more concentrated Hispanic populations (Lopez, Gonzalez-Barrera & Cuddington, 2003). South Florida has seen the influx of ethnicities from Caribbean and Latin countries. Hispanics comprise

65.6% of Miami-Dade population, and 54 % are Cuban Americans (Lopez, Gonzalez-Barrera & Cuddington, 2003).

Hispanic population is a mixture of European, African and Native American lineage, the proportion of each of the three varying in different Hispanic groups (Bryc et al., 2010). The difference in genetic lineage makes each Hispanic subgroup unique. Hispanics are at higher risk for T2D development and related phenotypes as compared to their European counterparts (CDC, 2012; CDC, 2014). One possibility could be due to lineage from Native American and African ancestors. It is well documented that metabolic diseases are more prevalent in Native American and populations with African origins (CDC, 2014). These two populations have proposed to contain a 'thrifty gene' that enabled them to survive scarcity of food (Neel, 1989). In the 21st century, the abundance of poor quality food had put these populations at greater risk for metabolic diseases (Champagne et al., 2007; Edwards & Patchell, 2009).

In this study, we observed higher waist circumference, BMI, FPG, A1C, TG and insulin but low adiponectin, HDL, LDL, TC and Kcal intake values for cases than controls. The T2D was significantly associated with rs7656250 SNP. The C allele carriers for rs7656250 SNP were at greater risk for T2D than those with TT genotype. When separated by sex, the odds were significantly higher in females. The results in males however were not statistically significant. A recent study in Cubans ≥60 years has reported the prevalence of T2D to be 19.9% in females and 7.3% in males (Rodrigues Barbosa, Balduino Munaretti, Da Silva Coqueiro & Ferreti Borgatto, 2011). The higher prevalence of T2D in females could be due to exposure to gestational diabetes or presence of obesity that affects Cuban women more than men (Herrera-Valdés et al.,

2008). However, several studies have found lower obesity among both urban and rural elderly Cubans than their Latin America and the Caribbean counterparts (Valencia, Alemán-Mateo, Salazar & Hernández Triana, 2003; Rodrigues Barbosa, Balduino Munaretti, Da Silva Coqueiro & Ferreti Borgatto, 2011). The HHANES in 1982-84 also reported higher rates of T2D in Cuban American women (34%) than Cuban American men (30%) (Najjar & Kuczmarski, 1989). The obesity in Cuban American women (15%) was also reported to be higher than men (9%) (Pawson, Martorell & Mendoza, 1991). The absence of acculturation and socioeconomic status with higher BMI (Khan, Sobal & Martorell, 1997) indicates possible genetic influences. The genetic variations are now known to modify an individual's susceptibility to disease (Shriver et al., 2005).

Reduced expression of *PPARGC1A* has not only been reported in individuals with T2D, but also in individuals who are unaffected, but have a family history of T2D (Mootha et al., 2004; Gillberg et al., 2013). Defects in regulation by PGC1-α have been associated with T2D and insulin resistance (Finck & Kelly, 2006). The 4p15 chromosomal location where *PPARGC1A* is located has been associated with obesity in multigenerational Utah residents (Stone et al., 2002). Fasting plasma insulin in Pima Indians has also been mapped to this location (Pratley et al., 1998). In Pima Indians, variants of *PPARGC1A* gene were associated with early insulin secretion and modifications in lipid oxidation (Muller, Bogardus, Pedersen & Baier, 2003). The *PPARGC1A* variants have also been associated with BMI in African Americans (Edwards et al., 2012). In the present study, we found the association of rs4235308 SNP with higher log CRP values whereas SNP rs3774907 was found to be associated with pulse pressure. High CRP and pulse pressure are predictors of cardiovascular health and

thus prognosis of T2D. The presence of the association in both Native Indian and African origin populations suggests some involvement of this gene with T2D prognosis in Cuban Americans.

This study is unique and most likely the only one that investigated the influence of *PPARGC1A* polymorphisms on T2D and its intermediate phenotypes in Cuban Americans. The five genotyped SNPs were selected after testing for correlations using LD plot and TAGGER on Haploview to ensure independence in association with T2D. There are however some limitations of the study. Due to small sample size we may have not been able to detect modest effects of the association. Second, the sampled population was restricted in geographical location; therefore, the results may lack generalizability, in order to extrapolate to general Cuban Americans. The genetic homogeneity is insured by Ancestry Informative Markers (AIM), which was not employed in this study making population stratification a concern. However, the sample population was recruited from Cuban American dense Miami-Dade County and participants were self-identified white Cuban Americans. As with any candidate gene study, the possibility of false positive results is a concern for this study. In future, independent replicative studies should investigate this association in more representative Cuban American population to corroborate the findings.

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G. Table 1. General characteristics of study population

Variables		Cuban Americans With T2D (n=161)	Cuban Americans Without T2D (n=180)	*p*-value
Age, yr.		64.90±12.29	62.24±11.17	0.04
Sex (Male)		61(37.88)	61(33.88)	0.49
Waist Circumference (cm)		105.41±14.53	100.24±12.45	<0.001
BMI (kg/m^2)		30.90±6.24	29.58±5.24	0.03
Smoke (Yes)		25(15.52)	33(18.33)	0.56
Blood pressure	**SBP**	129.48±21.15	129.48±21.15	0.11
(mm of Hg)	**DBP**	80.86±11.42	80.86±11.42	0.30
FPG (mmol/L)		145.38±60.85	97.69±17.77	<0.001
Log FPG		2.13±0.16	1.98±0.06	<0.001
A1C (%)		7.65±1.62	6.01±0.64	<0.001
Log A1C		0.87±0.08	0.78±0.04	<0.001
Hs-CRP (ng/mL)		5.37±6.24	4.83±6.30	0.44
Log CRP		0.58±0.41	0.51±0.40	0.13
Insulin (µIU/mL)		18.91±13.04	14.77±10.08	0.002
Log insulin		1.18±0.30	1.08±0.27	0.004
Adiponectin (ng/mL)		4.90±3.67	12.61±7.95	<0.001
Log adiponectin		0.55±0.38	0.97±0.41	<0.001
TC (mg/dL)		193.15±40.54	204.15±43.40	0.02
TG (mg/dL)		173.90±88.99	149.68±84.16	0.01
Log TG		2.19±0.19	2.12±0.21	0.001
HDL-C (mg/dL)		50.36±11.08	55.44±14.40	<0.001
LDL-C (mg/dL)		107.05±33.30	117.81±36.51	0.006
Alcohol		2.42±5.61	3.95±8.20	0.05
Kcal		2151.01±798.32	2363.60±792.65	0.01

Note: Values are unadjusted mean ± SD for continuous variables or N (%) for categorical variables. Diabetes medication is only for cases. BMI= body mass index; DM_Meds= Diabetes Mellitus medications; SBP= systolic blood pressure; DBP= diastolic blood pressure; A1C=Glycated hemoglobin; FPG= fasting plasma glucose; CRP=C-reactive protein; TC=total cholesterol; TG=triglycerides; HDL=high density lipoprotein; LDL= low density lipoprotein; Kcal=Kilo calories.

H. Table 2. Genotype frequencies of *PPARGC1A* SNPs by diabetes status

	SNPs		Minor Allele	With T2D (n=161)	Without T2D (n=180)	*p*-value
Genotype frequencies (n, %)	rs8192678	CC	T	70(43.47)	83 (46.11)	0.78
		CT		74(45.96)	76(42.22)	
		TT		17(10.55)	21(11.66)	
		MAF		0.335	0.327	
	rs7656250	TT	C	91(56.52)	102(56.66)	0.06
		CT		55(34.16)	72(40.00)	
		CC		15(9.31)	6(3.33)	
		MAF		0.264	0.233	
	rs4235308	TT	C	61(37.88)	69(38.33)	0.99
		CT		78(48.44)	86(47.77)	
		CC		22(13.66)	25(13.88)	
		MAF		0.378	0.377	
	rs11724368	CC	G	104(64.59)	104(57.77)	0.43
		CG		50(31.05)	66(36.66)	
		GG		7(4.34)	10(5.55)	
		MAF		0.198	0.238	
	rs3774907	TT	C	108(67.08)	106(58.88)	0.24
		CT		47(29.19)	68(37.77)	
		CC		6(3.72)	6(3.33)	
		MAF		0.182	0.222	

Note: Genotype frequencies are depicted as n (%); MAF= Minor Allele frequency.

I. Table 3. Logistic regression for *PPARGC1A* SNPs and T2D association

Variables		Cuban American Unadjusted OR	95% C.I.		*p*-value	Adjusted OR	95% C.I.		*p*-value
rs8192678	**TT +CT vs CC**	0.89	0.58	1.37	0.62	0.92	0.52	1.63	0.82
rs7656250	**CC+CT vs TT**	**2.98**	**1.12**	**7.87**	**0.03**	**6.87**	**1.41**	**33.30**	**0.02**
rs4235308	**CC+CT vs TT**	0.70	0.39	1.26	0.24	0.62	0.28	1.36	0.24
rs11724368	**CC+CG vs GG**	0.77	0.28	2.08	0.61	0.43	0.07	2.51	0.35
rs3774907	**CC+CT vs TT**	1.42	0.91	2.21	0.11	1.33	0.78	2.25	0.29
Age (Years)		**1.02**	**1.00**	**1.03**	**0.04**	**1.02**	**1.00**	**1.04**	**0.009**
Sex (Male/Female)		1.19	0.76	1.85	0.44	1.14	0.43	3.01	0.78
BMI (kg/m2)		**1.04**	**1.00**	**1.08**	**0.04**	**1.06**	**1.01**	**1.10**	**0.005**
Smoke		0.81	0.46	1.44	0.49	0.82	0.44	1.55	0.55
rs8192678*Sex		-	-	-	-	1.13	0.43	3.00	0.79
rs7656250*Sex		-	-	-	-	0.21	0.02	1.75	0.15
rs4235308*Sex		-	-	-	-	3.11	0.70	13.72	0.13
rs11724368*Sex		-	-	-	-	2.17	0.20	23.47	0.52
rs3774907*Sex		-	-	-	-	0.99	0.39	2.52	0.99

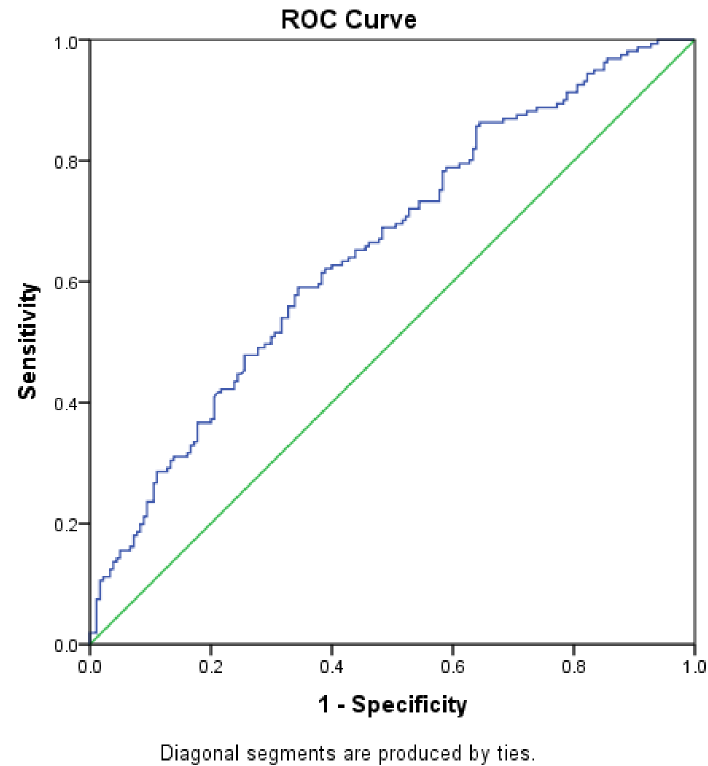
Note: The statistically significant results are in bold. Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis for all the SNP. p is considered significant at 0.05. Hosmer and Lameshaw test statistic p=0.907. OR= odds ratio; CI= confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.

J. Table 4. Logistic regression for *PPARGC1A* SNPs and T2D association separated by sex

Variables		Cuban American							
		Female				Male			
		OR	95% CI		*p*- Value	OR	95% CI		*p*- Value
rs8192678	**TT +CT vs CC**	0.91	0.51	1.61	0.75	1.01	0.47	2.16	0.97
rs7656250	**CC+CT vs TT**	**7.67**	**1.52**	**38.60**	**0.01**	1.44	0.35	5.87	0.60
rs4235308	**CC+CT vs TT**	0.62	0.28	1.35	0.23	1.89	0.53	6.80	0.32
rs11724368	**CC+CG vs GG**	0.42	0.07	2.48	0.34	0.85	0.17	4.25	0.84
rs3774907	**CC+CT vs TT**	1.27	0.70	2.31	0.42	1.39	0.63	3.06	0.41

Note: The statistically significant results are in bold. OR= odds ratio; CI= Confidence Interval; PPARGC1A= Peroxisome proliferator activated receptor, gamma, co-activator 1 alpha.

K. FIGURE 1: Receiver Operating Characteristic (ROC) curve.



Note: The ROC curve depicts the performance of our model used to identify combined genetic association of PPARGC1A SNPs with T2D. The area under the ROC curve is 65.4% which is significantly different from random chance (p<0.001). Maximum sensitivity and specificity are 59.6% and 71.1% respectively.

CHAPTER VII

ADDITIONAL SIGNIFICANT FINDINGS

A. Summary

After testing the main hypothesis of this dissertation research i.e. to explore *PPARGC1A* polymorphisms as genetic determinants of T2D in three ethnicities at high risk for T2D, additional statistical analysis were performed. The correlations of five *PPARGC1A* polymorphisms were studied against several phenotypes (bio-markers) associated with T2D: total cholesterol (TC), triglycerides (TG), high density cholesterol (HDL-C), low density cholesterol (LDL-C), high sensitivity C-reactive protein (Hs-CRP), insulin, glycated hemoglobin (A1C), fasting plasma glucose (FPG) and adiponectin levels. Additionally, the association between T2D and *PPARGC1A* polymorphisms was examined with age of onset; obesity; and family history of T2D. *PPARGC1A* gene regulates mitochondrial biogenesis, mitochondrial respiration, detoxification, energy and fat metabolism, and fast to slow skeletal muscle fiber transformation (Bishop, Granata & Eynon, 2014; Kang & Li, 2012; Olesen, Kiilerich & Pilegaard, 2010; Puigserver et al., 1998). Certain lifestyle factors affect metabolic outcomes by influencing molecular machinery. Recently various studies have reported the involvement of *PPARGC1A* with aerobic activity (Katzmarzyk, Church & Blair, 2004; Engeli et al., 2012). Moreover, the expression of NAD-dependent de-acetylase Sirtuis 1 (SIRT1), PGC1-α and FoxO1 is affected by chronic alcohol use (Lieber, Leo, Wang & Decarli, 2008). Therefore, interactions between *PPARGC1A* polymorphisms and lifestyle factors in these three study populations were also explored.

B. Materials and methods

The continuous variables were log transformed if variables were not normally distributed. Pearson correlations were analyzed between phenotypes (FPG, A1C etc.) and *PPARGC1A* polymorphisms for all three ethnicities. Logistic regression analyses were done to test the (i) association between T2D and *PPARGC1A* polymorphisms in African Americans and Haitian Americans (ii) effect modification by sex on correlation between *PPARGC1A* polymorphisms and T2D and (iii) interaction with age of onset; obesity; and family history of T2D, adjusting for age, BMI, smoking status as confounders. All statistical analyses were performed by SPSS (version 18 (SPSS Inc., Chicago, IL, US). Tests were considered statistically significant with $p<0.05$.

C. Results and discussion

Table 1a shows correlations of *PPARGC1A* SNPs with T2D intermediate phenotypes in African Americans. Only rs3774907 SNP was statistically correlated with log Hs-CRP ($p<0.05$). No statistically significant correlation was observed for SNPs rs8192678; rs7656250; rs4235308 or rs11724368 with T2D phenotypes (log TC, log TG, log HDL, log LDL, log insulin, log A1C, log FPG, log Hs-CRP or log adiponectin). In Haitian Americans, rs3774907 was significantly correlated with log A1C ($p<0.01$) and log FPG ($p<0.01$) values whereas rs11724368 was correlated with log insulin significantly ($p=0.02$) as shown in Table 1b. Another SNP; rs7656250 was also marginally correlated with log FPG in Haitian Americans ($p=0.09$). The gene *PPARGC1A* has been associated with T2D and related phenotypes in other ethnicities but never in the selected three ethnicities in this study. We observed positive correlation of rs3774907 with log Hs-CRP

in African Americans whereas in Haitian Americans, this SNP was negatively correlated with log Hs-CRP. Additionally, in Haitian Americans log FPG was negatively correlated with rs7656250 and rs3774907. Another SNP rs11724368 was negatively correlated with log insulin in Haitian Americans. The differences observed among African American and Haitian Americans for correlations of *PPARGC1A* SNPs with T2D phenotypes suggest the two ethnicities could not be pooled together for analyses. In Cuban Americans, *PPARGC1A* SNP rs7656250 was significantly correlated with log A1C (p=0.02) and rs4235308 was significantly correlated with log Hs-CRP (p=0.03) as shown in Table 1c. The positive correlation observed suggest high levels of log A1C and log Hs-CRP levels in CC+CT genotypes of both SNPs.

Table 2a shows protective association of SNP rs3774907 with T2D in both Haitian Americans as well African Americans after adjusting for confounding variables age, sex, BMI and smoking status. Haitian Americans Females and African Americans females with CC+CT genotype of the rs3774907 were 0.344 (p=0.006) and 0.369 (p=0.028) times less likely than those with TT genotype to have T2D. The results in males of either ethnicity were not statistically significant.

Table 3a shows that in African Americans, none of the *PPARGC1A* SNPs were significantly associated with T2D in individuals with family history of T2D (adjusting for age, BMI, sex and smoking status) when looking at recessive effects of the variant allele. The T2D association was also not significant in African Americans in non-obese after adjusting for age, sex, BMI and smoking status as shown in Table 3a. In Haitian Americans, no SNP (recessive model) had significant association with T2D after adjusting for confounding variable age, BMI, sex, and smoking status in those with

family history of T2D (Table 3b). However, rs3774907 was significantly associated with T2D in non-obese Haitian Americans with OR=0.27 (p=<0.01). Hence, non-obese Haitian Americans who have CC+CT genotype for rs3774907 were 0.27 times less likely to develop T2D than those with TT genotype. This suggests protective effect of rs3774907 SNP in Haitian Americans who are not obese. In Cuban Americans with family history of T2D, as shown in Table 3c, none of the SNPs was significantly associated with T2D after adjusting for confounders. Additionally, the association with T2D was also not significant in non-obese Cuban Americans (Table 3c).

Further, the association of *PPARGC1A* SNPs with T2D was explored in young onset of T2D; with physical activity (measured as met hours per week); alcohol consumption; or obesity in Haitian Americans, African Americans and Cuban Americans. In Haitian Americans, the association of T2D with interactions between age of onset; physical activity; family history; or alcohol intake and each respective SNP was not significant, as shown in Table 3b. Only the interaction term between rs4235308 and obesity had significant association with T2D after adjustment for age, sex, BMI and obesity (OR=0.24, p=0.03). This finding suggests that Haitian Americans with at least one rare allele have low OR for T2D despite being obese. The interactions of obesity with rest of *PPARGC1A* SNPs were not found to be significantly associated with T2D (Table 3b) suggesting no effect of obesity on the association. There was no statistical significance observed for the association of T2D with interactions between *PPARGC1A* SNPs and age of onset; obesity; physical activity; family history of T2D or alcohol intake in African Americans as shown in Table 3b. However, rs3774907 showed some interaction with alcohol intake (OR=1.04, p=0.08) which suggests slightly high risk for

T2D in African Americans who are carriers of rare allele for rs3774907 and are alcohol users.

In Cuban Americans, the effect of the interactions between age of onset; obesity; physical activity and any of the SNPs on the association with T2D was not statistically significant (Table 3c). No statistical significance observed for association of interaction between alcohol intake and each of *PPARGC1A* SNP, with T2D. The interaction effects of *PPARGC1A* SNPs and family history of T2D was also analyzed. The rs11724368 and family history (OR=5.32, p=0.08) had some interaction. The findings show high risk for T2D in rs11724368 rare allele carrier Cuban Americans who have family history of T2D. In Cuban Americans, none of the interaction was statistically significant for rest of the SNPs as shown in Table 3c.

The findings show some genetic mediation of *PPARGC1A* in T2D prognosis with interactions with age of onset; obesity; alcohol usage; family history of T2D. The absence of any statistical significance could merely be a result of small sample size within the sub categories such as with family history of T2D or absence of obesity, to allow the statistical model to reach significance.

D. References

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E. Table 1a. Pearson correlations of *PPARGC1A* SNPs with T2D phenotypes in African Americans

Variables	**rs8192678**		**rs7656250**		**rs3774907**		**rs4235308**		**rs11724368**	
	Pearson correlation	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**
Log TC	0.01	0.89	0.01	0.90	0.10	0.10	0.02	0.65	0.03	0.54
Log TG	0.09	0.14	0.07	0.23	0.04	0.49	0.07	0.23	0.10	0.09
Log HDL	-0.04	0.45	-0.05	0.38	0.06	0.28	-0.05	0.40	-0.05	0.38
Log LDL	-0.01	0.83	-0.02	0.73	0.10	0.10	0.02	0.79	0.02	0.66
Log insulin	-0.07	0.24	-0.04	0.47	0.02	0.70	-0.03	0.58	-0.04	0.46
LogA1C	0.02	0.70	0.02	0.75	-0.01	0.92	0.03	0.53	0.02	0.64
Log FPG	0.03	0.61	0.05	0.38	-0.02	0.73	0.07	0.27	0.04	0.53
Log Hs-CRP	-0.02	0.76	-0.04	0.50	**0.17**	**<0.05**	-0.01	0.87	-0.01	0.78
Log adiponectin	0.02	0.72	0.01	0.83	0.04	0.48	0.03	0.59	0.02	0.75

Note: The statistically significant results are in bold. TC= total cholesterol; TG= triglycerides; HDL= high density lipoprotein; LDL= low density lipoprotein; A1C= Glycated hemoglobin A1C; Hs-CRP= high sensitivity C reactive protein. PPARGC1A= peroxisome proliferator-activated receptor-γ coactivator-1 α; SNP= single nucleotide polymorphism; T2D= type 2 diabetes.

F. Table 1b. Pearson correlations of *PPARGC1A* SNPs with T2D phenotypes in Haitian Americans

Variables	rs8192678		rs7656250		rs3774907		rs4235308		rs11724368	
	Pearson correlation	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**
Log TC	0.01	0.95	0.05	0.38	-0.02	0.80	-0.11	0.08	-0.06	0.33
Log TG	0.01	0.92	0.08	0.2	-0.02	0.71	0.02	0.73	-0.00	0. 94
Log HDL	-0.09	0.16	0.03	0.56	-0.09	0.14	-0.12	0.06	-0.05	0.37
Log LDL	0.02	0.66	0.06	0.34	0.01	0.84	-0.07	0.26	-0.06	0.36
Log Insulin	-0.02	0.80	0.05	0.40	0.02	0.81	-0.02	0.73	**-0.02**	**0.02**
LogA1C	0.07	0.29	-0.01	0.77	**-0.23**	**<0.01**	0.07	0.28	0.07	0.25
Log FPG	0.01	0.91	**-0.11**	**0.09**	**-0.25**	**<0.01**	-0.03	0.64	0.07	0.27
Log Hs-CRP	0.01	0.84	0.09	0.17	-0.02	0.82	0.05	0.47	-0.04	0.52
Log adiponectin	-0.09	0.14	-0.04	0.52	<0.01	0.99	-0.07	0.26	-0.06	0.35

Note: The statistically significant results are in bold. TC= total cholesterol; TG= triglycerides; HDL= high density lipoprotein; LDL= low density lipoprotein; A1C= Glycated hemoglobin A1C; Hs-CRP= high sensitivity C reactive protein. PPARGC1A= peroxisome proliferator-activated receptor-γ coactivator-1 α; SNP= single nucleotide polymorphism; T2D= type 2 diabetes.

G. Table 1c. Pearson correlations of *PPARGC1A* SNPs with T2D phenotypes in Cuban Americans

Variables	rs8192678		rs7656250		rs3774907		rs4235308		rs11724368	
	Pearson correlation	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**	**Pearson correlation**	***p*-value**
Log TC	-0.02	0.67	-0.01	0.78	0.02	0.66	-0.02	0.73	0.07	0.17
Log TG	0.03	0.59	0.01	0.78	0.07	0.22	-0.09	0.10	-0.01	0.85
Log HDL	-0.03	0.64	-0.05	0.33	-0.04	0.48	0.05	0.39	-0.01	0.97
Log LDL	-0.01	0.83	-0.01	0.88	-0.01	0.81	-0.01	0.83	0.09	0.11
Log insulin	0.08	0.17	0.01	0.93	0.07	0.20	-0.01	0.83	0.05	0.37
LogA1C	0.01	0.85	**0.13**	**0.02**	0.08	0.12	0.03	0.59	-0.07	0.22
Log FPG	-0.01	0.93	0.04	0.44	0.07	0.16	-0.03	0.57	-0.03	0.96
Log Hs-CRP	-0.03	0.60	0.03	0.59	0.08	0.16	**0.12**	**0.03**	0.09	0.20
Log adiponectin	-0.02	0.97	-0.08	0.22	-0.02	0.75	-0.03	0.69		

Note: The statistically significant results are in bold. TC= total cholesterol; TG= triglycerides; HDL= high density lipoprotein; LDL= low density lipoprotein; A1C= Glycated hemoglobin A1C; Hs-CRP= high sensitivity C reactive protein. PPARGC1A= peroxisome proliferator-activated receptor-γ coactivator-1 α; SNP= single nucleotide polymorphism; T2D= type 2 diabetes.

H. Table 2a. Logistic regression of rs3774907 and type 2 diabetes by ethnicity

	Haitian American				African American			
Variables	**OR**	**95% C.I.**		***p*-value**	**OR**	**95% C.I.**		***p*-value**
rs3774907 CC+CT vs TT	**0.328**	**0.152**	**0.708**	**0.005**	**0.381**	**0.162**	**0.898**	**0.027**
rs3774907*Sex	1.391	0.451	4.291	0.566	2.187	0.687	6.957	0.185

Note: Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis. p is considered significant at 0.05. OR= odds ratio; CI= confidence Interval.

I. Table 2b. Logistic regression of rs3774907 in Haitian Americans by sex

Variables		**Haitian American**							
		Female				Male			
		OR	**95% C.I.**		***p*-value**	**OR**	**95% C.I.**		***p*-value**
rs3774907	**CC+CT vs TT**	**0.344**	**0.160**	**0.737**	**0.006**	0.500	0.209	1.195	0.119

Note: Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis. p is considered significant at 0.05. OR= odds ratio; CI= confidence Interval.

J. Table 2c. Logistic regression of rs3774907 in African Americans by sex

Variables		African American							
		Female				Male			
		OR	**95% C.I.**		***p*-value**	**OR**	**95% C.I.**		***p*-value**
rs3774907	**CC+CT vs TT**	**0.369**	**0.152**	**0.896**	**0.028**	0.842	0.387	1.830	0.664

Note: Controlled variables included in the logistic regression analysis for adjusted OR were age, sex, BMI, and smoking status. The interactions between sex and individual SNP were also included in logistic regression analysis. p is considered significant at 0.05. OR= odds ratio; CI= confidence Interval.

K. Table 3a. *PPARGC1A* SNP-age of onset/obesity/physical activity/alcohol/family history interaction's effect on association with T2D in African Americans.

Variable	**$OR_{interac}$**	***p*-value**
rs8192678*age of onset[a]	1.02	0.58
rs7656250*age of onset[a]	**0.93**	**0.07**
rs3774907*age of onset[a]	0.98	0.79
rs4235308*age of onset[a]	1.01	0.84
rs11724368*age of onset[a]	1.05	0.38
rs8192678*obesity[b]	0.25	0.10
rs7656250* obesity[b]	1.39	0.64
rs3774907* obesity[b]	0.79	0.71
rs4235308* obesity[b]	1.08	0.89
rs11724368* obesity[b]	0.89	0.90
rs8192678*met hours per week[c]	1.00	0.68
rs7656250*met hours per week[c]	1.01	0.05
rs3774907*met hours per week[c]	0.99	0.61
rs4235308*met hours per week[c]	1.00	0.78
rs11724368*met hours per week[c]	1.00	0.72
rs8192678*alcohol[d]	1.00	0.92
rs7656250*alcohol[d]	1.01	0.61
rs3774907*alcohol[d]	**1.04**	**0.08**
rs4235308*alcohol[d]	0.99	0.95
rs11724368*alcohol[d]	0.99	0.97
rs8192678*family history T2D[d]	3.65	0.19
rs7656250*family history T2D[d]	0.48	0.45
rs3774907*family history T2D[d]	2.28	0.27
rs4235308*family history T2D[d]	1.21	0.79
rs11724368*family history T2D[d]	0.17	0.10

Note: Variables adjusted for: a) sex, BMI; b, c, d) age, sex, BMI.

L. Table 3b. *PPARGC1A* SNP-age of onset/obesity/physical activity/alcohol intake/family history interaction's effect on association with T2D in Haitian Americans

Variable	$OR_{interac}$	*p*-value
rs8192678*age of onset[a]	0.94	0.19
rs7656250*age of onset[a]	1.02	0.78
rs3774907*age of onset[a]	1.07	0.13
rs4235308*age of onset[a]	0.95	0.29
rs11724368*age of onset[a]	1.03	0.59
rs8192678*obesity[b]	0.98	0.99
rs7656250* obesity[b]	0.87	0.86
rs3774907* obesity[b]	1.56	0.46
rs4235308* obesity[b]	**0.24**	**0.03**
rs11724368* obesity[b]	0.82	0.83
rs8192678*met hours per week[c]	0.99	0.34
rs7656250*met hours per week[c]	0.99	0.20
rs3774907*met hours per week[c]	0.98	0.70
rs4235308*met hours per week[c]	0.99	0.62
rs11724368*met hours per week[c]	0.98	0.15
rs8192678*alcohol[d]	0.94	0.58
rs7656250*alcohol[d]	0.97	0.82
rs3774907*alcohol[d]	0.92	0.52
rs4235308*alcohol[d]	1.07	0.48
rs11724368*alcohol[d]	1.23	0.41
rs8192678*family history T2D[d]	0.96	0.63
rs7656250*family history T2D[d]	1.06	0.40
rs3774907*family history T2D[d]	1.03	0.57
rs4235308*family history T2D[d]	0.96	0.57
rs11724368*family history T2D[d]	0.96	0.65

Note: Variables adjusted for: a) sex, BMI; b, c, d) age, sex, BMI.

M. Table 3c. *PPARGC1A* SNP -age of onset/obesity/physical activity/alcohol intake/family history interaction effect on association with T2D in Cuban Americans.

Variable	$OR_{interac}$	*p*-value
rs8192678*age of onset[a]	0.97	0.15
rs7656250*age of onset[a]	0.96	0.51
rs3774907*age of onset[a]	0.99	0.81
rs4235308*age of onset[a]	0.97	0.36
rs11724368*age of onset[a]	0.99	0.99
rs8192678*obesity[b]	0.64	0.33
rs7656250* obesity[b]	0.35	0.32
rs3774907* obesity[b]	1.57	0.36
rs4235308* obesity[b]	2.49	0.18
rs11724368* obesity[b]	0.45	0.48
rs8192678*met hours per week[c]	0.99	0.70
rs7656250*met hours per week[c]	1.01	0.41
rs3774907*met hours per week[c]	1.01	0.21
rs4235308*met hours per week[c]	1.01	0.39
rs11724368*met hours per week[c]	1.01	0.27
rs8192678*alcohol[d]	1.01	0.45
rs7656250*alcohol[d]	1.31	0.40
rs3774907*alcohol[d]	1.04	0.33
rs4235308*alcohol[d]	0.95	0.51
rs11724368*alcohol[d]	0.87	0.42
rs8192678*family history T2D[d]	1.00	0.50
rs7656250*family history T2D[d]	1.00	0.93
rs3774907*family history T2D[d]	1.01	0.50
rs4235308*family history T2D[d]	1.01	0.47
rs11724368*family history T2D[d]	**5.32**	**0.08**

Note: Variables adjusted for: a) sex, BMI; b, c, d) age, sex, BMI.

CHAPTER VIII

LIMITATIONS AND STRENGTHS

The research study has several strengths. This is the only study that explored the genetic associations of *PPARGC1A* polymorphisms with T2D in three high risk ethnicities; providing valuable information on the prognosis of T2D. Additionally, we attempted at studying the differences within the Black race with regards to genetic influences on T2D as well as its associated phenotypes. The effects of lifestyle factors, age of onset, and family history of T2D on the genetic determination of T2D with *PPARGC1A* polymorphisms were also investigated in all three ethnicities.

The case-control candidate gene study had the advantage of looking closely at polymorphisms of one single gene and its association with the disease. This study contributed in genotype determination of the candidate gene (*PPARGC1A*) polymorphisms with some biological importance to T2D. Relevance to pathogenesis of T2D and functionality was considered during the careful selection of the gene as well as polymorphisms utilizing established databases. The polymorphisms selected were also tested for linkage disequilibrium with adjoining polymorphisms to ensure independent genetic associations with T2D. Moreover, instead of using the default model (additive) in analyses; recessive model was specifically chosen to study the recessive effects of the variant allele of each polymorphism. The recruitment for unrelated controls in this study was done from the community sources matched by ethnic origin, age and sex which are an ideal practice for case-control studies. Moreover, the two Black ethnicities; African Americans and Haitian Americans were studies separately to avoid any population

stratification. Additionally, this study also explored effects of lifestyle factors on the genetic association with T2D that facilitated the estimation of the extent of the genetic risk factor.

As other candidate gene studies, this study has some limitations. As the study was focused on the genetic associations of only one gene (*PPARGC1A*), the influence of gene-gene interactions were not considered. The effects of polymorphisms on the expression of protein PGC1-α were not quantitated, so direct relationship with the gene product could not be established. In this study, sample size was calculated for detection of an association of the variant genotype with T2D, assuming odds ratio of 1.5 with threshold significance level set at 0.05 for 80% statistical power. However, for detection of significant odds ratio, the sample size may not have been sufficient if the frequency of minor allele is quite small especially with the case-control design. The participants in the study were self-reported ethnicities; resulting in possible genetic heterogeneity, a concern for candidate gene studies. Confounding due to population stratification can be controlled by ancestry-informative markers, AIMs, to avoid false associations were not used in this study. However, both controls and cases were selected from the same base population as well as same geographical location for each ethnicity. Additionally, the sample populations for Cuban Americans, Haitian Americans and African Americans being recruited from South Florida community may not be representative to general U.S. population.

CHAPTER IX

CONCLUSIONS AND FUTURE RESEARCH

Conclusions

The correlations among *PPARGC1A* polymorphisms, type 2 diabetes and its related phenotypes were examined in the Cuban American, Haitian American and African American populations of South Florida. Additionally the genetic associations of *PPARGC1A* polymorphism with T2D were also explored for the effects of age of onset, family history and lifestyle factors; obesity, physical activity and alcohol use.

The main objective of this study was to examine the correlations of *PPARGC1A* gene polymorphisms with T2D in three ethnicities which are at high risk of T2D. We were able to see associations between polymorphisms of this gene with T2D. The implication of *PPARGC1A* in T2D development reported earlier by other studies is validated by our findings. However, no other study has explored these polymorphisms in *PPARGC1A* gene in our unique ethnic populations for T2D disease association. We found significant differences among Haitian Americans and African Americans. *PPARGC1A* SNPs; rs4235308 and rs7656250 showed significant protective association with T2D in Haitian Americans but risk association in African Americans. One SNP, rs7656250 also had risk association with T2D in Cuban Americans. The different pattern of association of *PPARGC1A* polymorphisms among two Black ethnicities justifies our previously published hypothesis that these two ethnicities are distinct and may not be pooled together as Black race (Cheema, 2014).

Effect modification by sex, lifestyle factors and age of onset in T2D susceptibility was examined in all three ethnicities. Our findings further supported the trends of sex differences observed in T2D prognosis in these populations. The presence of stronger risk association in African American females seen in this study also identifies with the fact that African American females have higher T2D prevalence than African American males (CDC, 2012). Haitian Americans, despite their African origins and poor diabetes control have lower prevalence of T2D than their African American counterparts (Vimalananda, 2011). Our findings support and contribute to the complex explanation of this observed trend. We were unable to find significant effect modifications by family history of T2D, age of onset, alcohol intake or physical activity on T2D association with *PPARGC1A* polymorphisms in any ethnicity. However, some interactions were observed between *PPARGC1A* polymorphisms and family history; obesity; alcohol use in Cuban Americans, Haitian Americans and African Americans respectively. Moreover, the correlation between *PPARGC1A* polymorphism; rs3774907 and T2D still held true in non-obese Haitian Americans but not Cuban Americans or African Americans. The possibility of insufficient sample size could explain the inability to reach statistical significance for some of the analyses.

Our hypothesis that *PPARGC1A* polymorphisms will also be correlated with T2D phenotypes was based on other reports and the involvement of *PPARGC1A* in various genes in crucial metabolic pathways. We found positive correlations in African as well as Cuban Americans with these metabolic parameters whereas in Haitian Americans, negative correlations were found. The functional implication of *PPARGC1A* was seen in

Haitian Americans. Hypertensive Haitian Americans with T2D and CC and CT genotype were less likely than those with genotype TT to develop microalbuminuria.

The mixing of many races has given birth to ethnicities that possess lineage from two or more genetic sources. Such populations are at high risks for metabolic diseases such as T2D and cardiovascular diseases. Genetic polymorphisms that have direct effects on the gene product are the functional and often most useful polymorphisms, whereas others are genetic markers that may influence indirectly the metabolism of the gene product and thus the disease. To be able to study and understand the similarities as well as differences among these ethnicities is a big contribution to finding appropriate treatments and policies for the disease management and prevention.

Future research

Functional follow up studies are warranted as statistical correlations are the basis of the findings of this study. Carefully designed as well executed replication studies are necessary to validate the results in any case-control study. The associations of variants that occur with very low frequency might not have been statistically significant due to limited sample size. Therefore, larger sample sizes will be necessary to observe credible and substantial effects of such genetic variants. The study being focused on association of polymorphisms of only one gene with T2D did not look into adjoining genes that could have some interaction with *PPARGC1A*. The disregard of possible gene-gene interactions in a polygenic model in this study should be pursued by follow up studies. In near ideal scenario, a locus on a particular chromosome can be identified using whole genome scanning in the participant as well as family in a sample matched for age, sex and

ethnicity. This area can then be used to examine allelic associations of polymorphisms within and between candidate genes taking into account various environmental influences. Further, genomic studies assessing protein expression levels of functional proteins could be followed to explore the causative effect of such genetic polymorphisms.

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Table 3. Hypothesis testing

Hypothesis	**Conclusion**
1: *PPARGC1A* variants mediates genetic predisposition to T2D and related phenotypes	*PPARGC1A* polymorphisms were associated with T2D in three ethnicities which suggest the involvement of this gene in T2D prognosis. The polymorphisms were also correlated with T2D intermediate phenotypes such as FPG, A1C, Hs-CRP. Therefore, findings in this dissertation research indicate the genetic predisposition to T2D and related phenotypes being mediated by *PPARGC1A* polymorphisms.
2. If *PPARGC1A* mediates genetic predisposition to T2D, its association will be stronger in cases with young-onset, who are non-obese and who have family history of T2D	After adjusting for covariates (age, sex, BMI, smoking status, the interaction rs4235308 with obesity was significant in Haitian Americans. In Cuban Americans, high risk for T2D in rs11724368 rare allele carrier and with family history of T2D was seen. We did not see significant interaction of age of onset with T2D. The findings of this research indicate some genetic mediation of *PPARGC1A* in T2D prognosis with interactions with obesity; alcohol usage; and family history of T2D.

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