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Metabolic related traits in Mexicans: an approach to the study of complex diseases

By

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Por medio del presente nos permitimos hacer de su conocimiento que la tesis de doctorado del **M. en C. Armando Totomoch Serra** intitulada: **"Variables metabólicas en Mexicanos: una aproximación al estudio de las enfermedades complejas"**, ha sido aprobada por su Comité Tutorial por lo que corresponde a la versión final de la misma.

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ABSTRACT

The type 2 diabetes (T2D) is increasing worldwide, and Mexico is one of the countries with more people affected. In 2016, 9.4% of the population had a previous diagnosis of T2D, and 54.4% of them had diminished vision. These diseases are accompanied by comorbidity increasing the risk to develop macrovascular and microvascular complications. This study aims to identify single nucleotide variants (SNV) associated with T2D and related traits in a convenience sample from Mexico City. This study is an epidemiologic, observational, descriptive, and cross-sectional study, designed in two steps. In step one was selected 415 patients with recent T2D diagnosis, according to de American Diabetes Association criteria and phenotypic data for age, body mass index (BMI), weight, height, hip and waist perimeter, waist to hip ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose, cholesterol (CHOL), triglycerides (TRG), High Density Lipoprotein-Cholesterol (HDL-C), insulin, glycosilated hemoglobin (HbA1C), and Homeostasis Model Assesment-Insulin Resistance(HOMA-IR), and genotyped the variants *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *SIRT1* rs7896005 and *UCP2* rs659366. In step two were selected 415 T2D patients and 415 healthy controls with complete phenotype data for sex, age, DBP, SBP, glucose, CHOL, TRG, HDL-C, insulin, BMI, TRG/HDL index, and HOMA-IR and genotyped the variants *PPARG* rs1801282, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *ADRA2A* rs553668, *KCNQ1* rs2237892, *SIRT1* rs7896005, *IGF2BP2* rs4402960 and *UCP3* rs3781907. In the group of 415 T2D patients analyzing the genotypes was found an association for *VEGFA* rs2010963 with HDL-C ($p=7x10^{-4}$). In a linear regression *VEGFA* rs2010963 was significantly associated with HDL-C (p=0.007) in the additive model, and with nominal significance with HbA1C (p=0.020) in the dominant model, and DBP (p=0.032) in the recessive model. In another hand in the case-control analysis, *ADRA2A* rs553668 was associated with T2D ($p<1x10^{-4}$) under the additive and recessive model. In the analysis included T2D patients recently diagnosed was found an association of *VEGFA* and *SIRT1* with low levels of HDL-C and high levels of DBP respectively. Additionally, in the case-control analysis was confirmed a high association of *ADRA2A* with T2D, under top statistical criteria. These results highlight the need to study the Mexican population from Mexico City in diverse genetic approaches to elucidate the genetic participation of the variants associated in other people with T2D and propose new variants and genes that can be of involvement for Mexicans with origin in Mexico City.

RESUMEN

La diabetes tipo 2 (DMT2) ha incrementado sustancialmente en el mundo y México es uno de los países con más personas afectadas. Para el año 2016, 9.4% de la población había recibido el diagnóstico de DMT2, 54.4% de ellos informan disminución de la visión. Esta enfermedad se acompaña de comorbilidad incrementando el riesgo de desarrollar complicaciones macro o microvasculares. El presente estudio tiene como objetivo identificar variantes de nucleótido único asociadas con DMT2 y sus variables complejas relacionadas en un muestreo de conveniencia tomado en la ciudad de México. Se trata de un estudio epidemiológico, observacional, descriptivo, y transversal, diseñado en dos pasos. En el paso uno se seleccionaron 415 pacientes con diagnostico reciente de DMT2 de acuerdo a los criterios de la American Diabetes Asociación y que contaran con datos fenotípicos para edad, índice de masa corporal (IMC), peso, talla, perímetro de cintura y cadera, índice cintura-cadera, presión sistólica (PS), presión diastólica (PD), glucosa, CHOL, TRG, HDL-C, insulina, HbA1C y el HOMA-IR, así como genotipificación de las variantes *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *SIRT1* rs7896005 y *UCP2* rs659366. En el segundo paso se seleccionaron 451 pacientes con DMT2 y 416 controles sanos con datos completos para las variables sexo, edad, PD, PS, glucosa, CHOL, TRG, HDL-C, insulina, IMC, ínidice TRG/HDL-C y HOMA-IR, así como genotipificación para *PPARG* rs1801282, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *ADRA2A* rs553668, *KCNQ1* rs2237892, *SIRT1* rs7896005, *IGF2BP2* rs4402960 y *UCP3* rs3781907. En el grupo de 415 pacientes con DMT2 cuando fueron analizados por genotipo se encontró asociación para *VEGFA* rs2010963 con HDL-C ($p=7x10^{-4}$). Al realizar una regresión lineal *VEGFA* rs2010963 fue asociado de manera significativa con HDL-C (p=0.007), en un modelo aditivo y con significancia nominal para HbA1C (p=0.020) en modelo dominante, y PD (p=0.032) en modelo recesivo. Por otra parte en el análisis de casos y controles *ADRA2A* rs553668 fue asociado con DMT2 ($p<1x10^{-4}$) bajo el modelo aditivo y recesivo. Estos resultados resaltan la necesidad de estudiar a la población mexicana de la ciudad de México en distintas aproximaciones genéticas, con el objetivo de dilucidar la participación de variantes asociadas en otras poblaciones y propone variantes y genes que podrían ser de importancia para la población de la ciudad de México.

DEDICATION

To god, and all the members who are part of the nuclear and extended family to which belong, and for those who are part of it without blood ties.

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CHAPTER 1. INTRODUCTION TO THE STUDY OF COMPLEX DISEASES

In health sciences, there is a group of diseases with a high genetic component including the monogenic, the non-classical Mendelian, the chromosomal, and the genomic disorders. These diseases are considered as rare disorders and affect fewer than 200,000 people in the United States or fewer than 1 in 2,000 people in Europe (Boycott et al. 2013). They are near to 6000-8000 diseases, and 80% have a genetic basis suspected or identified (EURORDIS 2010; Tambuyzer 2010). In the present, these diseases are intensively analyzed with a substantial improvement in the phenotypic determination and with the development of technology that allows simultaneous sequencing genes like Next Generation Sequencing (NGS)(Boycott et al. 2013). Furthermore, the diffusion of the knowledge worldwide through specialized databases, increased the odds to find a correlation between genotypes and phenotypes one example is The Online Mendelian Inheritance in Man, (OMIM) (Amberger and Hamosh 2017). All of these resources help to identify subtle phenotypes hard to determine in the past, and it envisions a scenario where this rare diseases will be prevented, reverse o curated more efficiently than in the past.

However, there is another group of diseases that are more common, has a high prevalence worldwide and are more concerned by the general population. This group of diseases are named as complex disorders, and are polygenic (many genes affected) - multifactorial disorders (affectation occurred in combination with environmental factors). These diseases diagnosed in a high proportion of the population in the adult life; the sporadic cases are typical. Familial aggregation can be present, the genetic component is variable, and each gene has a small effect (Mitchell 2012). Between the group of complex diseases are type 2 diabetes (T2D), obesity, cancer, acute coronary syndrome, arthritis rheumatoid, schizophrenia, and so forth. Recently, the improvement in genotyping and statistical techniques have made approachable to study genes with small effect in complex diseases, a task hard to perform in the past. In this context, the Genetic Epidemiology emerges as a discipline that explores the influence of genes and environment on measures of health and disease susceptibility in populations (Teare and Koref 2011). This discipline has continuously studied the significant effect of selected genes in Mendelian diseases, and in the last decades made an incursion to the study of

complex conditions (Teare and Koref 2011). In this incursion improved the results in the detection of genetic susceptibility factors with low penetrance and a significant part of the results obtained from developed countries. Unfortunately, this discipline has limited research groups working in developing countries just as Mexico, getting only a few genetic variants with enough statistical power and significance.

The genetic background implicated in complex diseases and related traits has been identified in first instance in population studies using pedigrees mainly, through studies of heritability in a broad sense (Almasy and Blangero 2010) where is represented the relationship between the additive genetic variance (V_a) and the total phenotypic variance (V_p), and is expressed as $\mathbf{h^2 = V_a/V_p}$

In studies of heritability when a disease or trait has an essential percentage of heritability identified (in a particular population), family studies or case (proband) - control studies (**Table 1**) can be used to identify genes or metabolic pathways influencing the disease or related trait under study (DiPietro 2010; Sahebi et al. 2013). At present, GWAS (Genome Wide Association Studies) are implemented in a hypothesis-free methodology with the aim to identify new genes and variants associated with T2D in the diverse populations worldwide (Visscher et al. 2012). In the last years through the sequencing of the whole genome (Carlson et al. 2004) or exome, new variants and susceptibility genes can be found using complex statistics tools and intensive computational resources (Kiezun et al. 2013). In both cases despite the findings of positive results identifying genes and variants in the European population, is conceptually necessary, the replication of the results obtained with positive association among and between the diverse populations worldwide. The present study is part of the effort to confirm replication results obtained in Europeans, Asians, Mexican natives and Mexican mestizo, also propose new variants and candidate genes to be studied in association with T2D and/or related traits, contributing to the multiple efforts to clarify part of the missing heritability (Van Den Oord 2008) for this disease (Li and Meyre 2013).

To find genes or candidate regions that contribute to T2D, association studies are useful, the aim is to test correlation between disease status and genetic variation (Lewis and Knight 2012), in these studies the most common type of DNA variation analyzed is the single nucleotide variant (SNV), with a frequency of 1 modification

each ~1000 pair bases in the human genome. SNVs are the preferred type of variation studied in complex diseases (Seal et al. 2014) and are used for the indirect identification of functional variants mainly through linkage disequilibrium, fine mapping and haplotype analyses (Pittman et al. 2005). Despite being less informative than another type of variation in the genome as the variable number tandem repeat (VNTR) or short tandem repeats (STR), the SNVs are preferred because the high density and facility in genotyping making them the better option for most genetic epidemiology studies (Elfatih 2011).

Table 1. Main genetic epidemiology studies (Sahebi et al. 2013).

Research question/problem	Type of study	Unit of study	Type of measurement	Aim
Is there evidence of phenotype of phenotype aggregation within families?	Familial aggregation	Case-parent, Case-parent-grandparent	Recurrence risk ratio, correlation, Odds ratio, conditional regression logistic	Identifying new disease genes
Is the evidence of aggregation reliable with an effect of genes?	Heritability	Twin study	Variance component heritability	Identifying new disease genes
Is there evidence of genes with substantial enough effect to justify expensive studies	Segregation	Familial pedigree	Maximum likelihood estimation, Binomial distribution	Identifying new disease genes
Where in the genome is a causative gene most likely to lie?	Linkage	Case, disease of interest and control group, disease-free	Parametric; maximum likelihood, Nonparametric: mean, proportion, chi-square, likelihood test	Identifying new disease genes

Is there a causative variant?	Association, linkage disequilibrium, cohort, case only study	Related-case control, Unrelated-case control, exposure and non-exposure group, case groups	Transmission disequilibrium test, Chi-square, Independence Odds ratio, conditional regression logistic, Lewontin's D prime, relative risk, attributable risk, attributable risk, exposure odds ratio	Characterizing know disease genes
Estimate allele frequency	Cross-sectional		Prevalence correlation	Characterizing know disease genes
Estimate penetrance	Association Cohort	Related-case control, Familial cohort	Odds ratio, conditional regression logistic, risk ratio, attributable risk	Characterizing know disease genes
Evaluating strategies for prevention of genetic diseases	Experimental, retrospective cohort	Clinical trial (intervention and control group), exposure and non-exposure groups	Risk ratio, attributable risk	Characterizing know disease genes

T2D is an example of complex disease studied worldwide, where at least 80 robust variants are identified in association with it (Fuchsberger et al. 2016). Unfortunately, a considerable proportion of the studies have been performed in Europeans populations (Popejoy and Fullerton 2016) and recently with the bump in Asian economies the number and quality of studies with positive association have been increasing. However, the risk attributed to genetic factor described at present is 10% (Lyssenko and Laakso 2013). Multiple methodology alternatives are conducted to clarify the remaining risk, one of them is to explore the influence of rare and infrequent variants for T2D, using consortiums data to increase the sample size, effect size identified and power. Nonetheless cannot be found essential participation for rare variants in T2D (Willemsen et al. 2015; Fuchsberger et al. 2016). These findings have led to reconsider the study of extreme phenotypes, the occurrence of complex diseases in low-risk groups, and the study of private variants in diverse populations, such as the Mexican mestizo (Martorell 2005; Gamboa-Meléndez et al. 2012; Williams et al. 2014). Furthermore, the study of T2D and related traits in Mexicans could help to identify sources of covariance in metabolic characteristics and private variants no identified previously in other populations, because a better understanding of Mexican genetic composition and susceptibility to diseases could impact positively in the health of the Mexicans through better preventive decisions, diagnostic and management.

This thesis is also an approach to the study of complex diseases in genetic epidemiology. Analyzing T2D and related traits, this work divided into two principal parts: 1) The analysis of SNVs and T2D traits in one population with this disease, 2) An association case-control study of SNVs with T2D in Mexican Mestizo from Mexico City.

CHAPTER 2. LITERATURE REVIEW

1) T2D epidemiology

Mexico is surprised in the second stage of an epidemiological transition. In this transition, there is a notable increase in the incidence of chronic-degenerative diseases and a decrease in the rate of communicable diseases. Leading to an overload of the health system and increase in the consumption of supplies (medicines, laboratory tests for monitoring, lancets, glucose test strips, syringes, needles, measuring devices, insulin pumps, etc.), and also of the requirements of human resources and long-term care in patients with chronic-degenerative diseases. Because there is a modification of the population pyramid, the number of individuals susceptible to suffer at least one of the chronic degenerative diseases will be increasing in the next years. According to the projections by the National Population Council of Mexico (CONAPO) for the year 2030, the population over 60 years old will be more than a quarter of the people in Mexico (CONAPO 2012).

In 2013, Mexico had 8.7 million of people with T2D, this disease is the second cause of death in the country, and Mexico is the sixth place in the world ranking with more T2D affected patients, in the rest of this rank were China, India, the United States, Brazil, and Russia. According to the National Health Survey (ENSANUT) performed in 2012, Mexico had identified 6.4 million adults with this condition, representing 9.17% of all the adults in the country (Gutiérrez et al. 2012), the range between 60 to 64 years was the highest in prevalence. T2D as a highly prevalent disease traced in the lasts years through national programs. The National Institute of Geography and Statistics (INEGI), issued a document in 2013 highlighting the next data:

a) Nine of 100 persons without access to health care services who had a diabetes test were positive in screen tests.
b) By every 100 patients with hospitalizations due to complications derived from T2D, 24 had renal complications.

c) T2D incidence will increase with population aging and the age rank between 60-64 years was the highest in 2013.
d) Deaths derived from T2D complications are 61% for men and 62% for women.

The costs derived of attending T2D patients according to recent studies (Arredondo et al., 2013), were calculated for the case of Mexico, in an analysis of time series for the period 1990-2008 and determined epidemiological changes and trends of cases expected for the period 2009-2011, to more than 778 million dollars. Being mostly paid by the patient and their families with more than 429 million dollars (**Table 2**) (Barquera et al., 2013).

The states with the highest T2D prevalence in 2012 were Mexico City, Nuevo León, State of Mexico, Veracruz, and Tamaulipas; therefore, these entities require an additional level of attention in the training of human resources and an increase in the culture of prevention (Gutiérrez JP, 2012).

T2D diabetes is a chronic and multifactorial disease, characterized by an alteration in the metabolism with hyperglycemia, because of change in the insulin secretion, synthesis, and action. T2D is classified into four groups; these are Type 1 Diabetes (T1D), T2D, gestational diabetes and diabetes as consequence of other causes (American Diabetes Association 2014).

Table 2. Direct and indirect costs derived from T2D attention.

ITEM	SSA (1)	IMSS (2)	ISSSTE (3)	Users (4)	Private insurance	Total
Direct costs (DC)	n (351,172)	n (443,279)	n (175,607)	n (249,619)	NV	
Medical Attention/Diagnosis	7,101,113	16,029,089	3,750,300	31,061,914	1,792,032	59,734,448
Medicaments	15,813,331	35,749,875	8,351,475	69,234,743	3,994,310	133,143,734
Hospitalization	4,747,670	10,716,748	2,507,381	20,767,414	1,198,118	39,937,331
Complications	13,125,433	29,627,572	6,931,914	57,413,683	3,312,326	110,410,928
Subtotal DC	40,787547	92,123384	21,541070	178,477754	10,296786	343,226541
Indirect costs(IC)						
Premature mortality costs	2,267,624	5,326,703	1,217,070	10,811632	NV	19623029
Permanent disability costs	47,188,661	110,847,272	25,326,919	225,842994	NV	409205846
Temporal disability Costs	712,395	1,673,432	382,353	3,603879	NV	6372059
Subtotal	50,168,680	117,847,407	26,926,342	240,258505	NV	43520093
Total cost	90,956,227	209,970,791	48,467,412	429,033045	NV	778427475

(1)=Secretaria de Salud; (2)=Instituto Mexicano del Seguro Social; (3)=Instituto de Servicios y Seguridad Social para los Trabajadores del Estado; (4)= Personal expenses for users in private health institutions NV=No value. The DC were calculated based on the cases estimated in 2010 by sub-sector. Exchange rate: January 2010, 1US$11.35 Mexicans Pesos BANXICO 2010, for January 2018, 1US$18.51(Barquera et al. 2013)

In the health care system in Mexico the diagnostic criteria are followed according to the American Diabetes Association (American Diabetes Association 2014), and also has been created clinical guidelines to be followed by medical staff in public institutions. One of the more critical health institutions is the Instituto Mexicano del Seguro Social (IMSS)(IMSS 2009) with the aim to help timely in the diagnosis and treatment, this institution encourages these guides containing diagnostic algorithms that assist in the learning and understanding of the disease and directed to the medical staff in training and first contact personnel.

Diagnosis of T2D establish when occurs polydipsia, polyuria, polyphagia, and weight loss, with a glycemia at any time of the day >200 mg/dL without relation to the time elapsed since the last meal. The biochemical criteria used with or without symptoms to confirm the diagnosis of diabetes are:

- Glycosylated hemoglobin is higher than 6.5 % (using standardized method).
- Fasting glucose greater than 126 mg/dL (fasting for at least 8 hours).
- Plasma glucose at two hours greater than or equal to 200 mg/dL after an oral glucose tolerance test (load 75 g of anhydrous glucose dissolved in water).

Additionally, there are intermediate stages concerning T2D, their treatment and optimum diagnosis can reduce the presence of sequelae, comorbidity in the short and medium-term and the progress to T2D, these stages are prediabetes, abnormal fasting glucose, postprandial glucose, and glucose intolerance.

It is known that T2D is associated with physiopathological factors derived from conditions such as obesity, chronic inflammation, sedentary lifestyle and eating habits with a high content of fat and carbohydrates, (IMSS 2009). An example of the importance of interaction between environment and genetic factors influencing T2D development is the Pima ethnic group. This native group has been divided throughout the history in various occasions, since the arrival of the Spaniards, to the loss of half of the territory of Mexico because of the war with the United States of North America (USA). A portion of this population is in the southern United States (Arizona) and the second portion in northern Mexico (Sonora and Chihuahua). It has been found that the prevalence of diabetes in North American Pimas is 38% is five times higher than in the Pimas of Northern Mexico (6.9%), which is attributed to differences in lifestyle and environmental factors (Ravussin et al. 1994; Pratley 1998).

Mexico has more than 60 Native groups this makes a significant difference in eating habits and genetic background for the diverse sites of the national territory. However, the Mexican mestizo is a group of people product of recent miscegenation between Europeans and Native Americans and in lesser extent Africans and Asian populations. The Mexican mestizo from Mexico city is the product of constant miscegenation between millions of people through the years (López-Beltrán and Deister; Silva-Zolezzi et al. 2009), and are the most homogeneous populations who assist to the public health systems.

2) Genetic association studies

In genetic epidemiology exist the association studies where the aim is to associate one allele or allele groups (genome region) with the susceptibility to

develop a disease. Under the theory of different distribution for the risk alleles in the groups elected, classically there are two ways to study a disease through association studies those are the family bases studies and the independent population-based (http://www.dorak.info/epi/genetepi.html). The study of candidate genes in genetic association studies uses SNVs because of its high frequency in the human genome (1 each 1000 pairs bases). Also can be used different markers such as microsatellites, variable-number tandem repeat, copy-number variants, and so forth, some of them even more informative than SNV, but with high requirements of DNA for genotypification. The most common application in association studies is the population-based as a case-control study where the specific phenotype election for the cases may define the hypothesis to test (**Figure 1 and Figure 2**). In general terms, the goodness-of-fit tests, likelihood-based or regression methods can be used to analyze association (Lewis and Knight 2012). Also, the quantitative measure can be tested for association in a linear regression framework, assessing whether the genotypes (as an explanatory variable) predict trait value (Lewis and Knight 2012).

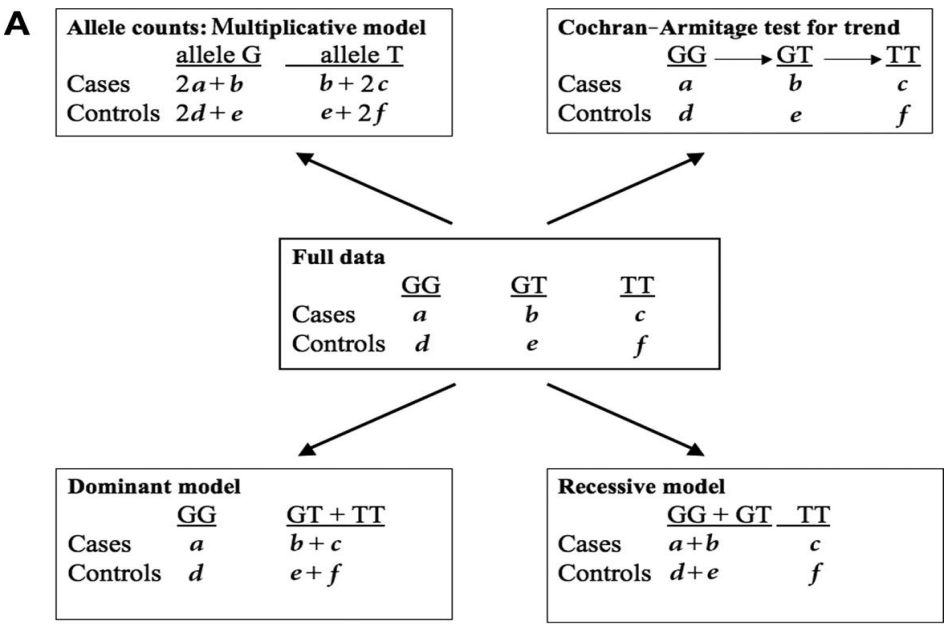

Figure 1. Association methods. Analysis methods for single SNV association studies, testing under the assumption of specific genetic models for arbitrary genotype counts (Lewis and Knight 2012)

However, false-positive findings, lack of power in replication studies, heterogeneity between and across studies, population stratification and lack of Hardy-Weinberg equilibrium or poor-quality control in genotyping can bias the results. In the last decade, GWAS and consortia conformation have improved the number of variants detected in complex diseases. One example of success is the inflammatory bowel disease where 163 risk loci have been identified in patients with IBD (Jostins et al. 2012), many of them involved in innate immune responses and mucosal homeostasis, but in T2D there are less than <80 robust variants associated and explaining only 10% of the T2D risk .

3) T2D genetic Association studies in Mexico

The implementation of genetic association studies has been performed in Mexico in the last years with results focused in candidate genes and replications studies and less extent in the implementation of GWAS one successful example is the Slim Initiative in Genomic Medicine for the Americas (SIGMA) (Williams et al. 2013) however, the number of variants associated with a P-value <0.005 (strict criteria for replication results), continue being scarce, until the last year the variants accomplishing with this significant value were *SLC16A11* rs13342232 ($p=5.5X10^{-12}$) *TCF7L2* rs7903146 ($p=2.5X10^{-7}$), *KCNQ1* rs2237897 ($p=4.9X10^{-16}$), *CDKN2A/2B* rs10811661 (p=0.003), *TNF-alpha* rs1800629 ($p=5x10^{-4}$), *ADRB3* rs4994 (p=0.002), *TCFL2* rs122553 (p=0.002), *IRS1* rs1801278 ($p=1x10^{-4}$) and *ABCA1* rs9282541 (p=0.001)(Sánchez-Pozos and Menjívar 2016).

In the year 2014, was performed by our group an extensive review in Pubmed of the variants and genes that could be included in replication studies in Mexican mestizo from Mexico City. In this review were included 3458 articles with the keyword SNP, polymorphism, variants, association, T2D, diabetes and type 2 diabetes, obtaining variants in 95 genes as candidates to be replicated in Mexicans mestizos, and there were at least 25 studies reporting association to T2D in Mexican Mestizo or Mexican-American, additionally there were 10 studies with positive association for Mexican-Natives. From this pool of genes and variants were elected the variants,

KCNQ1 rs2237892, *ADRA2A* rs553668, *VEGFA* rs2010963, *IGF2BP2* rs4402960, *PPARG* rs1801282, *PPARGC1A* rs8192678, *SIRT1* rs7896005, *UCP2* rs659366, *UCP3* rs3781907, *IGF2BP2* rs4402960 and *CDKN2A/2B* rs10811661 to be replicated in the samples obtained from volunteers included in this study.

4) ***KCNQ1 rs2237892***

The gene *KCNQ1* belongs to a family of genes responsible for the formation of potassium channels. The channel formed transports potassium ions out of the cell and participated in the generation and conduction of the action potential and is found in the inner ear and the heart muscle. The protein encodes by *KCNQ1* can form hetero-multimers with two other potassium channel proteins such as KCNE1 and KCNE3 (https://www.ncbi.nlm.nih.gov/gene/3784). This protein is also produced in the kidney, lung, stomach, and intestine, where it is involved in the transport of molecules through cell membranes (Jespersen et al. 2005). Analogously to the channel Kir 6.2 encoded by *KCNJ11, KCNQ1* is an ATP-dependent potassium channel that is expressed in the pancreas. It is associated more with changes in insulin secretion than with alterations in the repolarization of the membrane in the pancreatic cell β (Kim 2014). In two independent studies was found that carriers of the risk allele "T" in *KCNQ1* rs2237895 have a decrease in insulin secretion in pancreatic islets of human cells, and also suggesting modifications in imprinting and methylation(Travers et al. 2012; Wang et al. 2014). However, Gamboa-Melendez et al. not found an association for *KCNQ1* rs2237895 with T2D in Mexicans from Mexico City (Gamboa-Meléndez et al. 2012).

5) ADRA2A rs553668

Alpha-2-adrenergic receptors are members of the G protein-coupled receptor superfamily (https://pharos.nih.gov/idg/targets/ADRA2C). They include three highly homologous subtypes: alpha2A, alpha2B, and alpha2C. These receptors have a critical role in regulating neurotransmitter release from

sympathetic nerves and adrenergic neurons in the central nervous system (https://www.ncbi.nlm.nih.gov/gene/150). Physiologically *ADRA2A* mediates adrenergic suppression of insulin secretion and lipolysis (Rosengren et al. 2010a). Clinica evidence supports insulin alteration, such as a clinical study performed in Italy including 1345 healthy subjects analyzed in a 6 year follow-up, finding that subjects with two alleles "A" showed an increased baseline fasting plasma glucose (FPG) and a significant worsening of fasting glucose (β = 0.48; 95% CI 0.10-0.86) and insulin secretion (β =-20.75; CI -32.67- -8.82) for homeostasis model assessment-insulin resistance(HOMA-IR) (Bo et al. 2012). In another hand, the allele frequency for *ADRA2A* rs553668 has not been reported previously in Mexicans from the center of Mexico such as Mexico City.

6) VEGFA rs2010963

This gene is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer (https://www.ncbi.nlm.nih.gov/gene/7422). It is an active growth factor in angiogenesis and endothelial cells that Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis, and induces permeabilization of blood vessels (https://www.selfdecode.com/gene/vegfa/). Mutations in this gene have been associated with proliferative and no proliferative diabetic retinopathy (Al-Kateb et al. 2007; Abhary et al. 2009). Furthermore, the *VEGFA* rs2010963 has been suggested to play a significant role in the proliferative diabetic retinopathy in subjects from India (Choudhuri et al. 2015) and in determining serum VEGF levels in a Chinese population with the same disease (Fan et al. 2014). However, this variant is not associated with T2D in the UK Caucasian population (Freathy et al. 2006a), remaining unknow the participation of this SNV in several T2D related traits in Mexicans.

7) IGF2BP2 rs4402960

This gene encodes a member of the family of IGFII mRNA binding proteins. The protein encoded by this gene contains four KH domains and two RRM domains, participating in the binding to the 5'UTR of insulin-like growth factor-2 mRNA (IGF2), regulating the translation of IGF2 (Rodriguez et al. 2010). The variant *IGF2BP2* rs4402960 was initially associated with T2D by three complete genome association studies (Jia et al. 2011). However, a later study (Sladek et al. 2007) found no association between variants of this gene and susceptibility to T2D. This disparity may be due to small sample size, population stratification and errors in the design of the studies. Recently in a meta-analysis confirmed an association of *VEGFA* rs4402960 with a high risk of T2D in Europeans, East Asians, and South Asians populations (Zhao et al. 2012).

8) PPARG rs1801282

The protein encoded by this gene is the PPAR-gamma and is a regulator of adipocyte differentiation (https://www.ncbi.nlm.nih.gov/gene/5468). The name Peroxisome Proliferator-Activator Receptor is due chemical binding induces the proliferation of peroxisomes, organelles that contribute to the oxidation of fatty acids. PPARG can be activated both by a diet rich in fatty acids and their metabolic derivatives in the body, serving as a lipid sensor (Lorenzo et al. 2001; Ackert-Bicknell and Rosen 2006). In a large meta-analysis including 32,849 T2D cases and 47,456 controls, was found a moderate level of heterogeneity attributable to substantial variation in gene effect size (I (2) = 37%) reflecting the genetic difference between the ethnic origin (Gouda et al. 2010).

9) PPARGC1A rs8192678

The protein encoded by this gene is a transcriptional coactivator that regulates the genes involved in energy metabolism. This protein interacts with PPAR gamma, which permits the interaction of this protein with multiple transcription factors (https://www.ncbi.nlm.nih.gov/gene/10891). Overexpression of PPARGC1A in β cells during fetal life in mice is sufficient to induce β-cell dysfunction in adults, leading to glucose intolerance (Besseiche et al. 2015). Additionally, in a recent meta-analysis in Chinese Han population allele, “A” in *PPARGC1A* rs8192678 was found in association with T2D (Jing et al. 2012).

10)SIRT1 rs7896005

In humans, sirtuins function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the Sirtuin family (https://www.ncbi.nlm.nih.gov/gene/23411). *SIRT1* is expressed in diverse tissues, and its action is throughout different substrates. Including FOXOs, PPARγ, PGC1-α, MyoD, and p53, resulting in various specific tissue effects. Also, increased glycogenesis and suppression of glycolysis in the liver, as well as reduction of adipogenesis in adipose tissue, up-regulation of insulin secretion in pancreatic β cells and increase in mitochondrial activity, oxidation of fatty acids and insulin secretion in skeletal muscle (Liang et al. 2009; Gillum et al. 2011). In Pima Native American populations*, SIRT1* rs7896005 has been associated with reduced acute insulin in response to intravenous glucose bolus (adjusted P= 0.045) (Dong et al. 2011).

11)UCP2 rs659366

UCPs separate oxidative phosphorylation from ATP synthesis with energy dissipated as heat also referred to as the mitochondrial proton leak. UCPs

facilitate the transfer of anions from the inner to the outer mitochondrial membrane and the return transfer of protons from the exterior to the inner mitochondrial membrane (https://www.ncbi.nlm.nih.gov/gene/7351). *UCP2* rs659366 is associated with T2D and diabetic retinopathy in Chinese from Shanghai (Shen et al. 2014).

12)UCP3 rs3781907

This gene's protein product is postulated to protect mitochondria against lipid-induced oxidative stress. Expression levels of this gene increase when fatty acid supplies to mitochondria exceed their oxidation capacity and the protein enables the export of fatty acids from mitochondria (https://www.ncbi.nlm.nih.gov/gene/7352). The variant *UCP3* rs3781907 has not been associated previously with T2D but in 2009 was studied in 507 overweight Finish population for T2D traits finding a modification of LDL cholesterol levels and T2D (Salopuro et al. 2009), at present, there are not replication results for this study.

13)CDKN2A/2B rs10811661

Whole genome association studies associated the *CDKN2A/2B* locus as a candidate gene for diabetes. *CDKN2A* and *CDKN2B* are inhibitory genes of CDK, and their product p16 inhibits CDK4 which regulates the replication of β cells in the pancreas (Hu et al. 2009; Bao et al. 2012). *CDKN2A/B* genes are expressed in adipocytes and pancreatic islets and play a role in β-cell function and regeneration. The variant *CDKN2A/B* rs10811661 located 125 kb upstream of these genes has been identified as a risk allele in a contemporaneous Mayan population (Lara-Riegos et al. 2015) and Mexican mestizos from Mexico City (Gamboa-Meléndez et al. 2012).

CHAPTER 3. MATERIAL AND METHODS

1) JUSTIFICATION

Mexico is a developing country with high costs attending chronic diseases that affect the general population, the most representative illness in this group is T2D. This disease has been increasing continuously in the last 20 years, causing an economic loss because a high proportion of complications and comorbidities demand constant attention and also decrease the productive life of the adult people and life expectancy (Estadisticas Diabetes, 2013). Despite to be a complex disease studied worldwide, in Mexico the research in this diseases is scarce. Studies performed in Europeans population signal genetic influences in the development of this group of disorders. However, the knowledge of this genetic influences in Mexicans is unknown, and despite to the existence of variants risk identified in other populations, its presence and participation in the Mexican mestizo from Mexico City has been studied recently, because of that, the implementation of studies in genetic epidemiology could bring results that improve future health policies and an increase in the health of this population(Adan et al. 2007). The design of family and case-control studies analyzing candidate genes or replicating previous studies is a first approach to the knowledge of the Mexican susceptibility to T2D because its low cost, high prevalence, and facility to be designed, represents an opportunity of research for the young investigators.

2) HYPOTHESIS

If there is a selected group of SNVs associated with T2D and related traits in European, Asian, and other Mexican population; then some of these SNVs can be associated with T2D in Mexican mestizos from Mexico City.

3) AIM

a) PRIMARY AIM

I. To identify SNVs associated with T2D and related traits in a convenience sample from Mexico City.

b) SECONDARY AIMS

I. To obtain the allelic and genotypic frequency for the variants *KCNQ1* rs2237892, *ADRA2A* rs553668, *VEGFA* rs2010963, *IGF2BP2* rs4402960, *PPARG* rs1801282, *PPARGC1A* rs8192678, *SIRT1* rs7896005, *UCP2* rs659366, *UCP3* rs3781907*, IGF2BP2* rs4402960 and *CDKN2A/2B* rs10811661.

II. To analyze if one of the SNVs *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *SIRT1* rs7896005 and *UCP2* rs659366 are associated with T2D related traits in Mexican mestizos from Mexico City.

III. To analyze if one of the SNVs *PPARG* rs1801282, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *ADRA2A* rs553668, *KCNQ1* rs2237892, *SIRT1* rs7896005, *IGF2BP2* rs4402960 and *UCP3* rs3781907 is associated with T2D in Mexican mestizos from Mexico City.

4) DESIGN OF THE STUDY

Epidemiologic, observational, descriptive, and cross-sectional study.

5) POPULATION

This study was designed in two steps:

a) Step 1: Selection of 415 patients with recent T2D diagnosis, according to de American Diabetes Association criteria and phenotypic data for age, BMI, weight, height, hip and waist perimeter, waist to hip ratio, SBP, DBP, glucose, CHOL, TRG, HDL-C, insulin, HbA1C, and HOMA-IR.

b) Step 2: Selection of 415 T2D patients and 415 healthy controls with complete phenotype data for sex, age, DBP, SBP, glucose, CHOL, TRG, HDL-C, insulin, BMI, TRG/HDL index, and HOMA-IR.

All the samples obtained from a DNA bank located inside the facilities of Laboratory 1, in The Department of Genetics and Molecular Biology at Center for Research and Advanced Studies from the National Polytechnic Institute. This laboratory began to recruited phenotypes and DNA proceeding from volunteers who come to the National Medical Center Century XXI (CMNSXXI), at the Mexican Institute for Social Security (IMSS) between the years 2010-2014. The objective of this DNA bank was to identify the variants participating in the susceptibility to develop T2D in the Mexican mestizo from Mexico City, for each analysis were selected the individuals with more complete phenotypes avoiding missing values as much as possible.

A medical history of each patient was performed previously, including anthropometric and biochemical measurements of glucose, CHOL, TRG, insulin, and HbA1C. The diagnosis of T2D confirmed according to the T2D clinical criteria published by the American Diabetes Association (American Diabetes Association 2017): fasting glucose greater than 126 mg/dl on more than one occasion, random glucose level higher than 200 mg/dl on at least two times or HbA1C higher than 6.5 %. Diagnosis of hypertension was made with SBP ≥140 mmHg and DBP ≥90 mmHg (Williams et al. 2009). All participants were unrelated and of self-reported Mexican mestizo ancestry. This definition applies to volunteers who have been born in Mexico City (Mexican Valley), as did both of his or her parents and all four grandparents, and are the product of miscegenation between Europeans, Native Americans,

and Africans(López-Beltrán and Deister). In recent studies using ancestry markers in this population has not been found significant stratification effect in diabetes (Gamboa-Meléndez et al. 2012) and cardiovascular studies (Posadas-Sánchez et al. 2017) in Mexico City. The present project was approved by the Institutional Review Board located at CMNSXXI.

6) EXCLUSION CRITERIA

a) Presence of clinically significant renal, respiratory, hematological, gastrointestinal, hepatic, neurological or other inherited disorder different to T2D capable of altering the glucose metabolism.

b) Volunteers born in other states of the country, with less than three generations living in Mexico City.

c) Self-administration of insulin or another medication to treat diabetes before the T2D diagnosis.

d) There is a familial relationship between participants.

e) There is the presence of substance abuse or alcoholism.

f) Pregnant women.

g) Volunteers with significant incomplete values in demographic or phenotypic data that could affect the statistical analysis.

7) SAMPLE SIZE AND POWER ANALYSIS

The patients were recruited in a four years period, due that the minimal number of participants was fixed in 400 individuals per group, finally were included 415 cases and 416 controls to be analyzed.

Once obtained the results, was performed a post-hoc power analysis with the software Quanto V 2.1 to identify those SNVs with a power >0.90, a statistical significance of 0.05, according to the OR obtained and taking in count the allelic frequency reported in the literature for Mexicans in each SNV, under the

models dominant, recessive, and additive. Concerning the traits analyzed were also calculated according to the median and standard deviation obtained.

8) EXPERIMENTAL STRATEGY

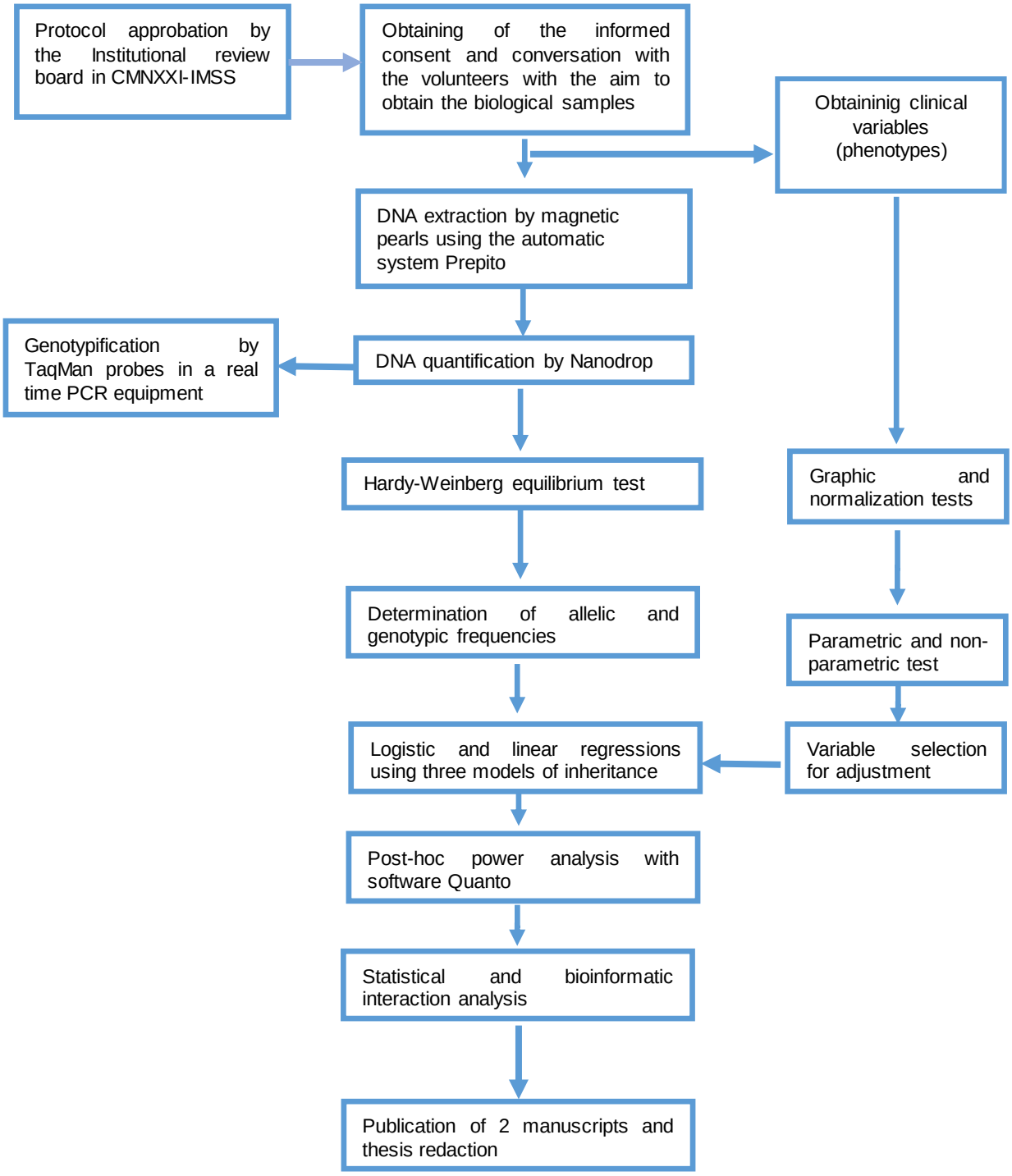

Figure 2. Experimental strategy.

9) ANTHROPOMETRIC VARIABLES

Quantitative and qualitative information was collected in the clinical history, and one database was constructed for further analyses. The anthropometric data were obtained through standardized measurement of height, weight, waist, and hip perimeter, using a calibrated clinical bascule and a measuring tape, taking into count the nearest centimeter as correspond for each variable. Also, the BMI was obtained by dividing weight (kg)/height2 (m2) and waist to hip ratio (WHR) by dividing waist (cm)/hip (cm). Blood pressure measurement was done with a stethoscope and a sphygmomanometer following a standardized technique according to the Eighth Joint National Committee (JNC-8) guideline on managing hypertension in adults (James et al. 2014). In all procedures, one or more health workers were always present.

10) BIOCHEMICAL VARIABLES

After eight hours fast, 5 ml of whole blood were extracted to obtain DNA, and the biochemical parameters TRG, CHOL, fasting plasma glucose(FPG), and HDL-C. Also, the biochemical variables were determined using The Biotechnica Automatic Analyzer BT3000TM and insulin was assessed using the Monobind™ human insulin ELISA Kit. Insulin resistance (IR) was determined using the HOMA-IR value >2.5. The TRG/HDL-C index for cardiovascular risk was calculated dividing the TRG by HDL-C values. A cardiovascular index higher than three was considered of risk.

11) SNV SELECTION

In the analysis concerning T2D traits were selected the variants *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *SIRT1* rs7896005 and *UCP2* rs659366, according to the next information:

Previous studies demonstrated that *CDKN2A/B* rs10811661 might confer increased risk for T2D by affecting beta cell function and has been associated with T2D in Asian and European subjects in a meta-analysis(Li et al. 2013).In another hand, *PPARGC1A* rs8192678 has been associated with T2D in people from Denmark and the north of China (Ek et al. 2001; Sun et al. 2006), and *UCP2* rs659366 is associated with T2D and diabetic retinopathy in Chinese from Shanghai (Shen et al. 2014). Furthermore, the *VEGFA* rs2010963 gene has been suggested to play a significant role in the proliferative diabetic retinopathy in subjects from India (Choudhuri et al. 2015) and in determining serum VEGF levels in a Chinese population with the same disease (Fan et al. 2014). VEGFA is not associated with T2D in the UK Caucasian population (Freathy et al. 2006b), remaining unknow the participation of this SNV in several T2D related traits in Mexicans. By last the *SIRT1* rs7896005 variant had a reduced acute insulin response nominally associated with T2D in the native Pima population (Dong et al. 2011). Consequently, all five SNVs could associate with T2D- related traits in the Mexican Mestizo.

In the case-control analysis, the variants *PPARG* rs1801282, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *ADRA2A* rs553668, *KCNQ1* rs2237892, *SIRT1* rs7896005, *IGF2BP2* rs4402960 and *UCP3* rs3781907 selected according to an extensive review of the literature in Pubmed, choosing eight common variants with a minor allele frequency (MAF) >0.05. Four of the variants (*ADRA2A rs553668, IGF2BP2 rs4402960, KCNQ1 rs2237892,* and *PPARG rs1801282*,) were associated previously with T2D by GWAS in European and/or Asian populations(Saxena et al. 2007; Scott et al. 2007; Zeggini et al. 2007; Kong et al. 2009, 2015; Rosengren et al. 2010b; Chen et al. 2013; Fuchsberger et al. 2016). The four variants *SIRT1 rs7896005, UCP3 rs3781907, PPARGC1A rs8192678*, and *VEGFA rs2010963* associated with

T2D traits in Europeans and Asian populations, but not tested for association with T2D specifically in Mexicans from Mexico City

12) DNA EXTRACTION AND GENOTYPIFICATION

The DNA was extracted using Qiagen's Gentra Puregene Blood® kit (Qiagen, Hilden, Germany) from human leucocytes and quantified by NanoDropTM 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) verifying a 260/280 relationship >1.800. DNA integrity was assessed using agarose gel (1%) electrophoresis dyed with ethidium bromide and running for 30 minutes to 70 volts. Genotyping was performed using 11 TaqMan minor groove binder probes (Applied Biosystems, Foster City, CA, USA) with fluorescent reporters FAM™ and VIC® dyes (**Table 3**) in a 96 wells PCR real-time system, Step One PlusTM system (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The genotypes obtained were analyzed using standardized criteria according to the TaqMan® Genotyper software (Life Technologies, Carlsbad, CA, USA). Ten percent of the samples were genotyped twice in a double-blinded randomized way to avoid errors in genotyping.

13) BIOINFORMATIC ANALYSIS

Interaction analysis was performed using GeneMANIA, a web interface which finds other genes that are related to a set of input genes, using an extensive collection of functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity(Zuberi et al. 2013).

Table 3. Characteristics of the SNVs elected.

Gene	Reference	MAF	TaqMan Probe Sequence
KCNQ1	rs2237892	T=0.1694/370	GGGAGCTGTCACAGGACTTTGCCACC[C/T]GGGGTGAGGGGCCTAGAAACCCCTC
ADRA2A	rs553668	A=0.2903/634	GCAGGGCTGCCCTTAGCATTTTTCTT[C/T]AAAAAGAGAGAGAGAGTTGGGAATG
VEGFA	rs2010963	C=0.3429/749	GCGCGCGGGCGTGCGAGCAGCGAAAG[C/G]GACAGGGGCAAAGTGAGTGACCTGC
IGF2BP2	rs4402960	T=0.342/747	CAGTAAGGTAGGATGGACAGTAGATT[G/T]AAGATACTGATTGTGTTTGCAAACA
PPARG	rs1801282	G=0.0659/144	AAACTCTGGGAGATTCTCCTATTGAC[C/G]CAGAAAGCGATTCCTTCACTGATAC
PPARGC1A	rs8192678	T=0.2658/1331	CTGAAATCACTGTCCCTCAGTTCAC[C/T]GGTCTTGTCTGCTTCGTCGT
SIRT1	rs7896005	G=0.3741/817	CTAGCTAGTTCCTATAAAGGTAGAAG[A/G]TTTAATTTTACAAATTATGCCATGC
UCP2	rs659366	T=0.4089/2048	TGACCCGTCCTGTGGGGGTAACTGA[C/T]GCGTGAACAGCCAACAATT
UCP3	rs3781907	G=0.2855/1430	GAGCTCCACCTCTGGGGCAACCCCT[G/A]CCACATCCTGCCTGTGGT
IGF2BP2	rs4402960	T=0.3890/1948	AGTAAGGTAGGATGGACAGTAGATT[G/T]AAGATACTGATTGTGTTTGC
CDKN2A/2B	rs10811661	C=0.206/450	GCAGCTCACCTCCAGCTTTAGTTTTC[C/T]CATGACAGTAAGTCTATTACCCTCC

14) STATISTICAL ANALYSIS

a) T2D traits analysis, a subgroup of 415 patients with T2D

Statistical analyses were performed with IBM SPSS Statistics 24.0 (SPSS, Inc., Chicago, Illinois, USA). Baseline results were expressed as the mean ± standard deviation (SD), and median (25th–75th percentiles) and genotype frequencies were calculated by direct counting. The Hardy-Weinberg chi-square test was used to evaluate the deviation of equilibrium. Comparison of between-group differences in clinical and anthropometrical variables was performed with ANOVA or the Kruskal-Wallis test, and categorical variables were analyzed with the chi-square or Fisher's exact test.

Using the software Quanto 1.2.4 (University of Southern California, Los Angeles, California, USA) the main genetic effect (β_g) was calculated with a statistical power of 80% and $\alpha = 0.05$, taking into account the global minor allelic frequency informed in dbSNP: a database of single nucleotide polymorphisms (http://www.ncbi.nlm.nih.gov/snp). Also, was used the mean and standard deviation for each trait after normalization of the values, using the dominant, recessive and additive models of inheritance. Linear regression analysis was used to test the association of variants and diabetes traits under the three models of inheritance. Those variables with non-normal distribution were rank-based inverse normal transformed to avoid confounding factors due to the preponderance of one sex and obesity patients, and the linear regression analysis adjusted for age, sex, and BMI. Additionally, an association test among paired SNVs was performed, and an interaction analysis between candidate genes associated with the paired SNV analysis was performed with Gene Mania software (www.genemania.org). Additionally, a power analysis was performed in the most unbalanced variable. A value of P between <0.05 and >0.01 was considered to have nominal significance, and in post-Bonferroni correction 0.05/5, a P≤ 0.01 was significant

b) Case-Control association study with T2D:

Statistical analysis for association was performed with IBM SPSS Statistics 24 software (SPSS Inc., Chicago, Il, USA). Clinical characteristics of cases and controls for quantitative values were analyzed according to normality distribution using the U-Mann-Whitney or T-student test. Differences in allelic frequencies with previous data informed in the literature for Mexican-Americans and deviation from Hardy–Weinberg equilibrium test (HWE) in control group was calculated by Pearson's Chi-square test; significant $p<0.05$ difference with Mexican-Americans was considered. It was tested the association between the variants and T2D affection status as categorical variables using logistic regression in the models dominant, recessive, and additive, adjusting to age, sex, and BMI. The best model was selected according to the Akaki and Bayesian information criteria; multiple comparisons adjustment was performed using Bonferroni correction. Taking in count the models, covariates, and variants analyzed; to avoid type, I error a significant P value < 0.001 was considered; the nominal association was contemplated for $P < 0.05$ and >0.001. To avoid type II error was performed a power analysis with the software Quanto version 1.2.4 (California, University of Southern California) in the results obtained by the model of inheritance; a power >0.9 was considered robust. According to Shapiro-Wilk test, quantitative variables were inverse rank transformed and linear regression models were used to analyze association with T2D related traits, under the additive, dominant and recessive models and were adjusted for age, sex, and BMI. Bonferroni correction was made for multiple comparisons and $P<2.3x10^{-4}$ was considered significant in the linear regression analysis, and the nominal association was found for $P <0.05$ and $>2.3x10^{-4}$.

CHAPTER 4. RESULTS

1) T2D-related traits results

a) Univariate analysis of risk factors for T2D in Mexican Mestizos

The present study was performed to determine differences in the clinical, anthropometrical, and biochemical parameters of 415 (301 women, and 114 men) unrelated Mexican Mestizos with T2D. **Table 4** shows the univariate analysis of the population divided by sex. Both groups (women and men) have a similar distribution in age, and there were no differences in SBP, DBP, glucose, TRG, CHOL, insulin, HDL-C and HbA1C levels. BMI was significantly higher in women (29.13±7.19) than in men (26.95±3.85) with $p< 0.001$, and HOMA-IR was also substantially higher in women [2.65(1.69-4.49)] than in men [2.26 (1.41-3.72)], with $p<0.001$.

b) Comparison of allelic frequencies with HapMap and 1000 Genomes Project

The five analyzed variants were in HWE. Allelic frequencies of the five variants are displayed in **Table 5** and were compared to those reported in HapMap and the 1000 Genomes Project for Mexicans. The frequencies of *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *UCP2* rs659366, *VEGFA* rs2010963, and *SIRT1* rs7896005 agreed with these projects.

Table 4. Biochemical and anthropometrical data of Mexicans with T2D.

Clinical Data	All patients (N=415)	Women (n=301)	Men (n=114)	P value
Age, years	57.85±11.67	57.51±11.60	58.76±11.81	0.74
BMI, Kg/m2	28.52±6.54	29.13±7.19	26.95±3.85	< 0.001*
Weight	68±13	66±13	73±13	< 0.001*
Height	154±10	151±7	164±9	0.014
WHR	0.91±0.07	0.89±0.07	0.95±0.06	0.565
Hip, cm	103±13	105±14	100±9	< 0.001*
Waist, cm	97±11	97±11	96±11	< 0.001*
SBP, mm Hg	133.91±22.02	134.13±22.84	133.35±19.71	0.474
DBP, mm Hg	75.17±11.42	74.51±11.69	76.92±10.48	0.8314
Glucose, mg/dl	129.08±63.52	128.92±60.90	129.52±29.96	0.8307
Cholesterol, mg/dl	191.64±40.80	196.16±41.67	180.15±35.60	0.1274
TRG, mmol/L	1.90(1.42-2.77)	1.93 (1.46-2.76)	1.08(1.19-2.84)	0.1897
HDL-C, mg/dl	39.39±10.89	41.20±11.16	34.96±8.39	0.0926
Insulin, μIU/ml	8.15±9.75	8.59±10.68	7.12±6.57	0.1499
HbA1C, %	8.26±2.58	8.23±2.60	8.35±2.53	0.4085
HOMA-IR	2.47(1.56-4.33)	2.65(1.69-4.49)	2.26(1.41-3.72)	<0.001*

*N=Total sample size, n= sub-group size, BMI - body max index, WHR - waist to hip ratio, SBP - systolic blood pressure, DBP - diastolic blood pressure, TRG – triglycerides, HDL-C - high-density lipoprotein cholesterol, HbA1C - glycated haemoglobin, HOMA-IR - homeostasis model assessment of insulin resistance, Variables are expressed as means ± SD and median (25th-75th percentile). *P-Value <0.05.*

c) SNV association with T2D traits

Comparison of genotype frequencies with traits is displayed in **Table 6**, and concerning obesity, in **Table 7** Genotyping frequencies of SNVs in patients with T2D are not associated with obesity. These frequencies will be useful as a reference for future studies.

Table 5. Allelic frequencies for six SNVs in different studies and patients with T2D.

dbSNP	Actual Project	HapMap MXL	1000GMXL	1000GCEU	1000GYRI	1000GCHB
rs10811661	T=0.9(749)	T=0.905 (105)	T=0.891 (114)	T=0.801(181)	T=0.976(285)	T=0.555(152)
	C=0.1(81)	C=0.095 (11)	C=0.109 (14)	C=0.199(45)	C=0.024(7)	C=0.445(122)
rs8192678	C=0.8 (665)	C=0.802 (93)	C=0.773 (99)	C=0.65 (147)	C= 0.949(277)	C=0.581(158)
	T=0.2 (165)	T=0.198 (23)	T=0.227 (29)	T=0.35(79)	T=0.051(15)	T=0.419(114)
rs7896005	A=0.68 (562)	A=0.612 (71)	A=0.617 (79)	A=0.288(65)	A=0.976(287)	A=0.826(223)
	G=0.32 (268)	G=0.388 (45)	G=0.383 (49)	G=0.712(161)	G=0.024(7)	G=0.174(47)
rs659366	C=0.53 (435)	C=0.561 (64)	C=0.562 (72)	C=0.633(143)	C=0.534(157)	C=0.544(147)
	T=0.47 (391)	T=0.439 (50)	T=0.438 (56)	T=0.367(83)	T=0.466(137)	T=0.456(123)
rs2010963	C=0.35(292)	----------	C=0.336 (43)	C=0.328 (65)	C=0.306(66)	C=0.447(92)
	G=0.65(538)	----------	G=0.664 (85)	G=0.672(133)	G=0.694(150)	G=0.553(114)

dbSNP - the single nucleotide polymorphism database, Actual Project - T2D patients of this study, Data obtained from: MXL - the HapMap project of USA residents with Mexican ancestry, 1000GMXL - 1000 Genomes Project from USA residents with Mexican ancestry, 1000 CEU - Utah residents with Northern and Western European Ancestry, 1000 YRI - Yoruba from Ibadan, Nigeria, CHB - Han Chinese from Beijing, China.

In **Table 6** is displayed the results of the association analysis of genotype frequencies and for quantitative traits; nominal significance was identified in *PPARG* rs8192678 for glucose (p=0.023) and TRG (p=0.013); *VEGFA* rs2010963 with DBP (p=0.012), CHOL (p=0.013) and significant association with HDL-C ($p=7x10^{-4}$); *SIRT1* rs7896005 for DBP (p=0.012) and insulin (p=0.01); and *UCP2* rs659366 for cholesterol (p=0.034), glucose (p=0.031) and TRG (p=0.028) were nominally significant.

Table 6. Metabolic traits of the samples stratified by genotype.

dbSNP Genotype		BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	CHOL (mg/dL)	Glucose (mg/dL)	TRG (mg/dL)	HDL-C (mg/dL)	Insulin (mU/mL)	HbA1C (%)	HOMA-IR
rs10811661	T/T	28(26-32)	131(120-148)	76(68.5-83)	195(169.5-218)	119(94-164.5)	171(127.5-245.5)	39(34-46)	9(6-13)	8(7-10)	2.82(1.81-4.43)
	C/T	28.5(25-31.75)	135(121-151)	77(69-82)	186(165-214)	122(97-155)	168(111-214)	38(33-44)	5(4-9)	8(7-10)	1.74(0.96-3.88)
	C/C	31(26-33)	137(120-138)	76(69-77)	211(197-217)	150(141-312)	168(149-272)	47(42-51)	4(3-6)	14(7-15)	2.22(1.18-2.38)
	P	0.966	0.096	0.988	0.089	0.093	0.08	0.086	0.111	0.133	0.089
rs8192678	C/C	28(26-31)	132(120-148)	76(68-82)	187(168-216)	121(95.5-172.5)	169(125.5-245)	38(33-45)	7(4-11)	8(7-10)	2.28(140-3.85)
	C/T	29(25.5-32)	134(123-148)	76(69-83)	199(171.5-220.5)	114(94-150.5)	173(131-254)	42(35-49)	10(7-14)	8(7-10)	3.00(1.86-4.68)
	T/T	27(25.5-30.5)	136(121.5-158.5)	79(70.5-85)	199(179.5-224.5)	130(97-192.5)	156(128-178)	42(33-45)	12.5(8-24.5)	8(7-10)	4.60(2.88-6.45)
	P	0.651	0.078	0.073	0.063	0.023*	0.013*	0.087	0.092	0.314	0.065
rs2010963	C/C	29(26-32)	137(123.5-148.75)	77(70-82.75)	183(164.75-214.75)	128(97-174.5)	149.5(109.75-248)	38(33.75-47.25)	4(3-9)	8(7-10)	1.74(0.97-3.79)
	C/G	29(26-33)	130(118-147)	74(67-81)	188(169-214)	119(96-168)	169(127-231)	38(33-44)	7(5-11)	8.5(7-10.75)	2.33(1.47-3.64)
	G/G	28(25-31)	134.5(122-148)	76(69-83)	199(170.75-224.25)	115.5(93.75-157)	174(132.75-252.25)	41(34.75-49)	10(7-14)	8(7-10)	3.05(1.90-4.90)
	P	0.175	0.06	0.012*	0.013*	0.14	0.081	$7x10^{-4}$*	0.105	0.416	0.09
rs7896005	A/A	28(25-31)	132(120-148)	76(67-80.25)	187.5(168-215.25)	122(95-172.25)	168(122.75-245.25)	38(33-45)	6(4-11)	8(7-11)	2.19(1.30-3.56)
	A/G	28(26-32)	135(122.25-147.75)	76(69-83)	195.5(168-217.75)	119.5(96.5-157.75)	174(131.25-245.5)	39(34-48.75)	9(6-13)	8(7-10)	2.65(1.79-4.58)
	G/G	28(25-32)	134(118.5-154)	79(69.5-86)	203(182-231)	110(92.5-166.5)	161(127-248.5)	42(35-45)	12(10-19.5)	8(6.5-10)	3.72(2.88-6.33)
	P	0.867	0.075	0.012*	0.065	0.07	0.051	0.106	0.01*	0.592	0.107
rs659366	C/C	29(26-32)	134.5(120-148)	76(68.75-80)	181(164-216)	117.5(94.5-156.5)	169.5(109.75-242)	32(28-45)	5(4-10)	8(7-10)	1.89(1.14-3.54)
	C/T	28(25-31)	130(120-147.25)	76(69-84)	199(170.5-217)	121(95.75-175.25)	173(130-248)	39(34-46)	8(5-12)	8(7-10)	2.68(1.82-4.17)
	T/T	28(26-32)	136(123-151)	76(69-82.5)	199(177-229)	118(94-155.5)	165(128.5-238)	42(35-49)	11(7-16)	8(7-10)	3.11(2.04-6.16)
	P	0.401	0.06	0.634	0.034*	0.031*	0.028*	0.06	0.17	0.286	0.104

*dbSNP- the single nucleotide polymorphism database. Medians (25-75 percentile), BMI - body max index, SBP - systolic blood pressure, DBP - diastolic blood pressure, CHOL – cholesterol, TRG – triglycerides, HDL-C - high-density lipoprotein-cholesterol, HbA1C - hemoglobin glycosylated, HOMA-IR - homeostasis model assessment of insulin resistance, *P-value <0.05.*

d) SNVs association with T2D traits by models of inheritance

All five SNVs were studied for association with several traits in linear regression under three models of inheritance (dominant, recessive, and additive). **Table 8** shows the nominal and significant variants associated with the best model of inheritance after the Bonferroni correction for multiple testing. *VEGFA* rs2010963 was significantly associated with HDL-C (p=0.007) in the additive model, and with nominal significance with HbA1C (p=0.020) in the dominant model, and DBP (p=0.032) in the recessive model; The *UCP2* rs659366 had nominal significance with SBP (p=0.025) in the additive model, and *SIRT1* rs7896005 was significantly associated with DBP (p=0.006) in the recessive model. All models were adjusted for Age + Sex + BMI, and they were designated following the best Akaike and Bayesian criteria.

Table 7. Single-nucleotide variants and genotype frequencies in T2D patients concerning obesity.

Gen and dbSNP	Genotype	UDW	NW	OW	CIO	CIIO	CIII0	TOTAL
rs10811661[a]	T/T	1	69	155	77	29	8	339
	C/T	0	14	29	18	7	3	71
	C/C	0	2	2	0	1	0	5
rs8192678	C/C	1	49	127	61	23	8	269
	C/T	0	32	50	2	13	3	100
	T/T	0	4	9	5	1	0	19
rs2010963	C/C	1	9	28	11	8	0	57
	C/G	0	40	71	46	17	4	178
	G/G	0	36	87	37	12	7	179
rs7896005	A/A	0	38	89	46	18	3	194
	A/G	0	35	78	20	15	7	155
	G/G	1	12	19	10	4	1	47
rs659366[a]	C/C	1	23	63	23	8	3	121
	C/T	0	41	78	16	22	5	162
	T/T	0	20	44	24	6	3	97

Polymorphisms associated with obesity in previous studies, UDW – underweight, NW normal weight, OW - over weight, CIO - class I obesity, CIIO - class II obesity, CIIIO - class III obesity

Table 8. Nominal and significant variants by trait and model of inheritance.

dbSNP	Model	Coef. β (95% CI)	Trait	P-value
rs2010963	Additive	-0.12 (-0.20 - -0.03)	HDL-C	0.007*
rs2010963	Dominant	-0.17(-0.30 - -0.03)	HbA1C	0.02
rs2010963	Recessive	0.05(0.00-0.09)	DBP	0.032
rs659366	Additive	0.02(0.00-0.05)	SBP	0.025
rs7896005	Recessive	-0.06(-0.11- -0.02)	DBP	0.006*

*dbSNP- the single nucleotide polymorphism database, Models adjusted by age + sex + BMI, DBP - diastolic blood pressure, HbA1C - glycosylated haemoglobin, HDL - high-density lipoprotein, SBP - systolic blood pressure, the coefficient β is shown (95% CI) with log-transformed values, Nominal significance between 0.01> and <0.05, *Significant P-value <0.01, after Bonferroni correction.*

e) SNV association with T2D traits by pairwise association test

Results of the association test among paired SNVs (**Table 9**) showed that all SNVs are independent of one another. Further, if the analysis is performed with all the SNVs independently of HOMA-IR, the results are the same, as SNVs are independent of one another. However, the low significant value was found for the pair rs8192678 and rs1081661 (Exact Pr ≥ Chi-Square 0.966).

Table 9. Association test among paired SNVs.

dbSNP		Exact Pr ≥ Chi-Square
rs8192678	rs10811661	0.9666
rs2010963	rs10811661	0.9301
rs659366	rs2010963	0.693
rs8192678	rs7896005	0.3482
rs659366	rs8192678	0.3005
rs659366	rs10811661	0.287
rs2010963	rs7896005	0.287
rs659366	rs7896005	0.2568
rs8192678	rs2010963	0.1948
rs7896005	rs10811661	0.1005

dbSNP - Single nucleotide polymorphism database.

Finally, because systemic arterial hypertension displayed an unbalanced distribution with a high coefficient of variation, to determine its influence on results, the power analysis was tested in the unbalanced distribution by dividing the variable of HBP (high blood pressure) into three subgroups (**Table 10 and Figure 3**).

Table 10. Power analysis of arterial systemic hypertension categorical variable.

Average N per group	Number of groups	Total N	Alpha	Beta	Standard Deviation Between individuals	Effect Size, X Times Std. Dev.	Power
138.3	3	415	0.05	0.9474	0.55	0.005	0.0526
138.3	3	415	0.05	0.9396	0.55	0.010	0.0604
138.3	3	415	0.05	0.9064	0.55	0.020	0.0933
138.3	3	415	0.05	0.8461	0.55	0.030	0.1531
138.3	3	415	0.05	0.7558	0.55	0.040	0.2427
138.3	3	415	0.05	0.6376	0.55	0.050	0.3600
138.3	3	415	0.05	0.5015	0.55	0.060	0.4954
138.3	3	415	0.05	0.3635	0.55	0.070	0.6332
138.3	3	415	0.05	0.2403	0.55	0.080	0.7565
138.3	3	415	0.05	0.1436	0.55	0.090	0.8537
138.3	3	415	0.05	0.0772	0.55	0.100	0.9209
138.3	3	415	0.05	0.0370	0.55	0.110	0.9618
138.3	3	415	0.05	0.0158	0.55	0.120	0.9835
138.3	3	415	0.05	0.0060	0.55	0.130	0.9937
138.3	3	415	0.05	0.0020	0.55	0.140	0.9979
138.3	3	415	0.05	0.0006	0.55	0.150	0.9994
138.3	3	415	0.05	0.0002	0.55	0.160	0.9998
138.3	3	415	0.05	0.0000	0.55	0.170	1.0000
138.3	3	415	0.05	0.0000	0.55	0.180	1.0000
138.3	3	415	0.05	0.0000	0.55	0.190	1.0000
138.3	3	415	0.05	0.0000	0.55	0.200	1.0000
138.3	3	415	0.05	0.0000	0.55	0.210	1.0000
138.3	3	415	0.05	0.0000	0.55	0.220	1.0000
138.3	3	415	0.05	0.0000	0.55	0.230	1.0000
138.3	3	415	0.05	0.0000	0.55	0.240	1.0000
138.3	3	415	0.05	0.0000	0.55	0.250	1.0000

[a]*The total number of individuals tested was divided into three groups and means were compared*.

Results showed that a difference between means around 0.10 times had an SD= 0.55, which can be detected as significant with a probability of 0.92. Genetic effect calculated in **Table 11 and 12** showed good power (0.80) with an accuracy of approximately 10% in most cases. We marked in bold the percentages higher than 10%. The GeneMANIA bioinformatics analysis for interaction among*CDKN2A/CDKN2B* and *SIRT1* with *CDK6* and *MCM10* genes are displayed **Figure 4**. Results showed that *CDKN2A,*

CDKN2B, and *SIRT1* interact with *CDK6;* and *CDKN2A* and *SIRT1* interact with the *MCM10* homolog gene (**Figure 4**).

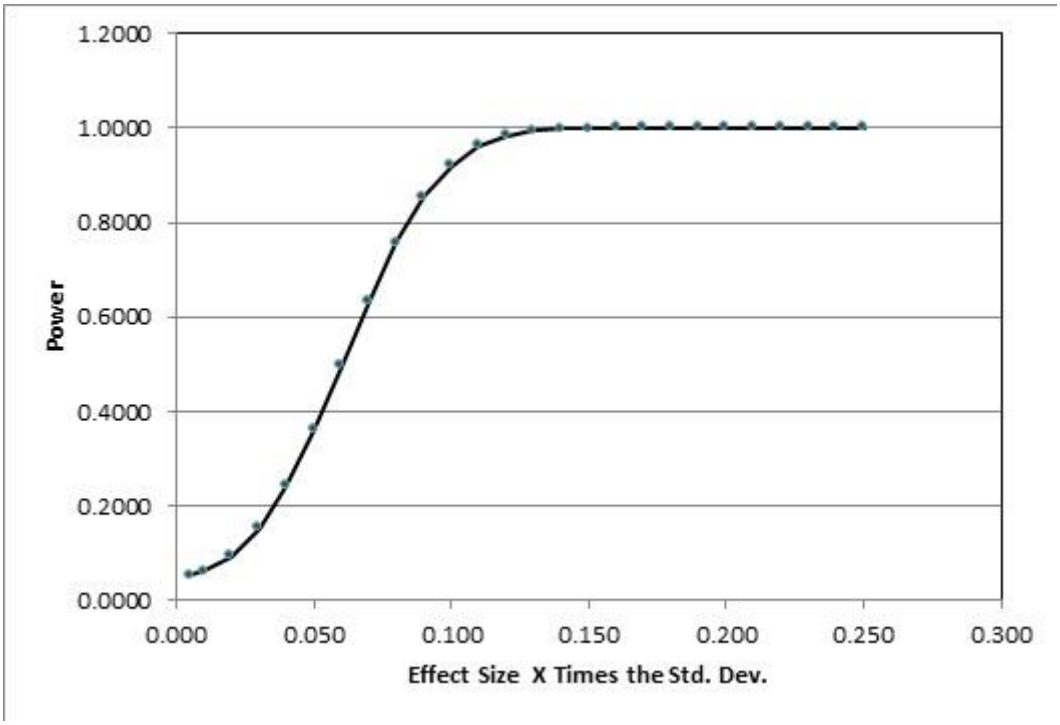

Figure 3. Unbalanced variable. These results are for the worst case that corresponds to the variable HBP and has a high variance coefficient and the most extensive unbalanced distribution with the gen rs10811661 in the three groups. A difference between means around 0.10 times the standard deviation can be detected as significant with a probability of 0.92.

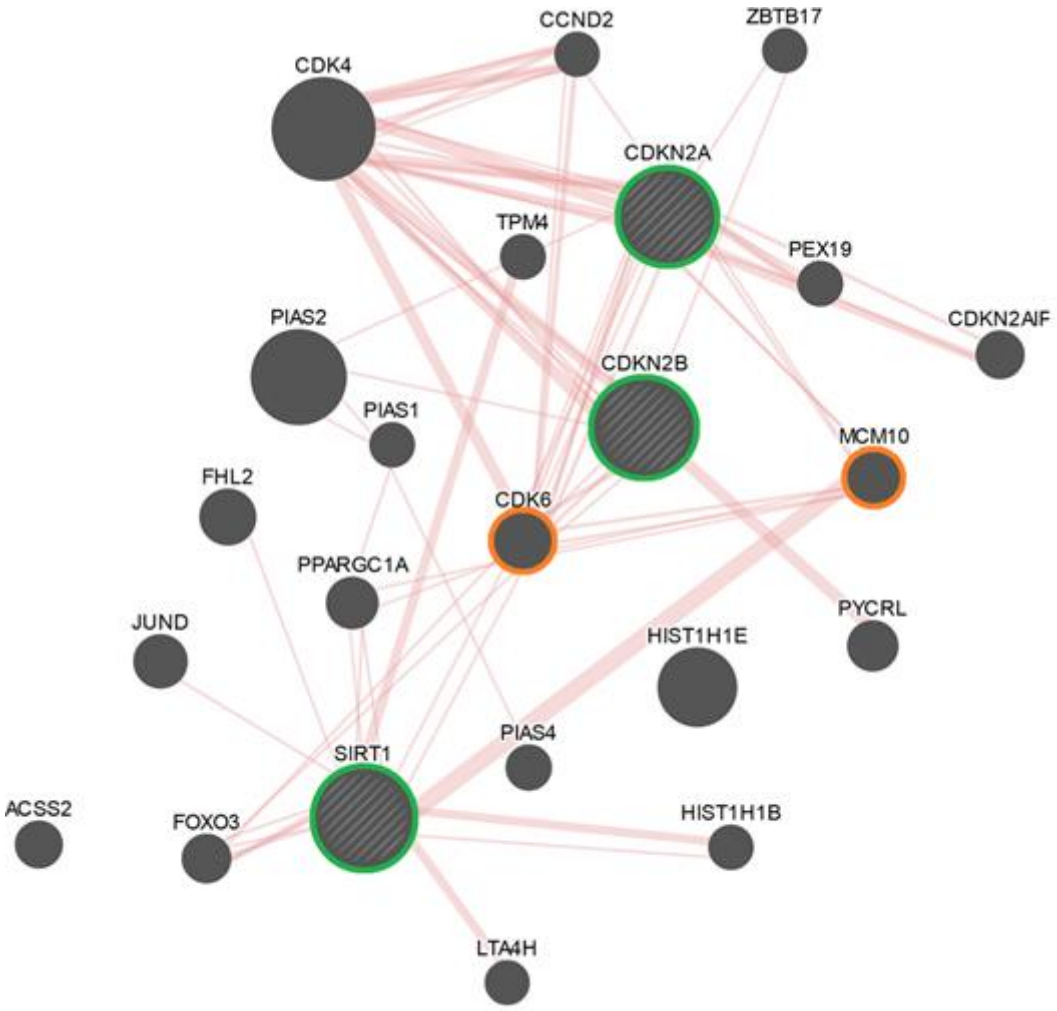

Figure 4. Interaction. Physical interaction analysis for CDKN2A, CDKN2B, and SIRT1. This figure displays the report of the three genes (green circle) by the GeneMANIA bioinformatics, showing that CDKN2A, CDKN2B, and SIRT1 interact with CDK6, and CDKN2A and SIRT1 interact with the product of homologous gene MCM10.

Table 11. Effect β_g calculated with Quanto software by regression coefficient according to three models of inheritance.

			DBP (mmHg)	Glucose (mg/dL)	HbA1C (%)	CHOL (mg/dL)	HDL-C (mg/dL)	SBP (mmHg)	TRG (mg/dL)	HOMA-IR	BMI (kg/m^2)	Insulin (mU/mL)
dbSNP	MAF	N	406	406	408	406	406	406	406	355	409	357
		Mean	77.4	102	11.2	179	49.8	143	154	2.20	31.3	6.96
		SD	11.41	63.56	2.582	40.78	10.84	22.04	165.6	3.960	5.071	9.687
rs10811661	0.10	Additive	3.80	**21.2**	0.861	**13.6**	3.61	7.35	**55.2**	1.62	1.69	3.95
		Dominant	**16.22**	**90.3**	3.670	**58.0**	**15.41**	**31.33**	**235.4**	6.89	7.21	**16.86**
		Recessive	4.11	**22.9**	0.931	**14.7**	3.91	7.95	**59.7**	1.75	1.83	4.28
rs2010963	0.35	Additive	2.39	**13.3**	0.541	8.5	2.27	4.62	**34.7**	1.02	1.06	2.49
		Dominant	4.92	**27.4**	1.114	**17.6**	4.68	9.51	**71.4**	2.09	2.19	5.12
		Recessive	3.27	**18.2**	0.739	**11.7**	3.10	6.31	**47.4**	1.39	1.45	3.40
rs659366	0.47	Additive	2.29	**12.7**	0.517	8.2	2.17	4.42	**33.2**	0.97	1.02	2.38
		Dominant	3.89	**21.7**	0.880	**13.9**	3.70	7.51	**56.5**	1.65	1.73	4.04
		Recessive	3.59	**20.0**	0.813	**12.8**	3.41	6.94	**52.1**	1.53	1.60	3.73
rs7896005	0.32	Additive	2.45	**13.6**	0.554	8.7	2.32	4.72	**35.5**	1.04	1.09	2.54
		Dominant	5.32	**29.6**	1.204	**19.0**	5.06	**10.28**	**77.2**	2.26	2.37	5.53
		Recessive	3.24	**18.0**	0.732	**11.6**	3.07	6.25	**47.0**	1.38	1.44	3.37
rs8192678	0.20	Additive	2.85	**15.9**	0.646	**10.2**	2.71	5.51	**41.4**	1.21	1.27	2.97
		Dominant	8.23	**45.9**	1.863	**29.4**	7.82	**15.91**	**119.5**	3.50	3.66	8.56
		Recessive	3.36	**18.7**	0.761	**12.0**	3.19	6.49	**48.8**	1.43	1.49	3.50

dbSNP - the single nucleotide polymorphism database. MAF - minor allele frequency, N - number of individuals per variable, SD - standard deviation, DBP - diastolic blood pressure, HbA1C - glycated haemoglobin, CHOL – cholesterol, HDL-C - high-density lipoprotein cholesterol, SBP - systolic blood pressure, TRG – triglycerides, HOMA-IR - homeostasis model assessment of insulin resistance, BMI - body max index. All the traits were rank-based inverse normal transformed. Accuracy higher than 10% are marked in bold numbers.

Table 12. Effect β_g calculated with Quanto software as a percentage of the mean according to three models of inheritance.

			DBP (mmHg)	Glucose (mg/dL)	HbA1C (%)	CHOL (mg/dL)	HDL-C (mg/dL)	SBP (mmHg)	TRG (mg/dL)	HOMA-IR	BMI (kg/m^2)	Insulin (mU/mL)
dbSNP	MAF	N	406	406	408	406	406	406	406	355	409	357
		Mean	77.4	102	11.2	179	49.8	143	154	2.20	31.3	6.96
		SD	11.41	63.56	2.582	40.78	10.84	22.04	165.6	3.960	5.071	9.687
rs10811661	0.10	Additive	4.9	**20.9**	7.7	7.6	7.3	5.1	**35.7**	**73.6**	5.4	**56.8**
		Dominant	**20.9**	**88.9**	**32.8**	**32.4**	**31.0**	**21.9**	**152.4**	**314.0**	23.0	**242.2**
		Recessive	5.3	**22.6**	8.3	8.2	7.9	5.6	**38.7**	**79.6**	5.8	**61.4**
rs2010963	0.35	Additive	3.1	**13.1**	4.8	4.8	4.6	3.2	**22.5**	**46.3**	3.4	**35.7**
		Dominant	6.4	**27.0**	9.9	9.8	9.4	6.7	**46.3**	**95.3**	7.0	**73.5**
		Recessive	4.2	**17.9**	6.6	6.5	6.2	4.4	**30.7**	**63.3**	4.6	**48.8**
rs659366	0.47	Additive	3.0	**12.5**	4.6	4.6	4.4	3.1	**21.5**	**44.3**	3.2	**34.1**
		Dominant	5.0	**21.3**	7.9	7.8	7.4	5.3	**36.6**	**75.3**	5.5	**58.1**
		Recessive	4.6	**19.7**	7.3	7.2	6.9	4.9	**33.7**	**69.5**	5.1	**53.6**
rs7896005	0.32	Additive	3.2	**13.4**	4.9	4.9	4.7	3.3	**23.0**	**47.4**	3.5	**36.5**
		Dominant	6.9	**29.2**	**10.8**	**10.6**	**10.2**	7.2	**50.0**	**103.1**	7.6	**79.5**
		Recessive	4.2	**17.7**	6.5	6.5	6.2	4.4	**30.4**	**62.7**	4.6	**48.3**
rs8192678	0.20	Additive	3.7	**15.6**	5.8	5.7	5.4	3.9	**26.8**	**55.2**	4.1	**42.6**
		Dominant	**10.6**	**45.1**	**16.6**	**16.4**	**15.7**	**11.1**	**77.4**	**159.4**	**11.7**	**123.0**
		Recessive	4.3	**18.4**	6.8	6.7	6.4	4.5	**31.6**	**65.1**	4.8	**50.2**

dbSNP - the single nucleotide polymorphism database. MAF - minor allele frequency, N - number of individuals per variable, SD - standard deviation, DBP - diastolic blood pressure, HbA1C - glycated haemoglobin, CHOL – cholesterol, HDL-C - high-density lipoprotein cholesterol, SBP - systolic blood pressure, TRG – triglycerides, HOMA-IR - homeostasis model assessment of insulin resistance, BMI - body max index. All the traits were rank-based inverse normal transformed. Accuracy higher than 10% are marked in bold numbers.

2) Case-Control results

a) Descriptive results

In this study were included 831 individuals, who were divided into two groups of 415 T2D patients (age mean 59.09± SD 11.65) and 416 healthy controls (age mean 50.58± SD 8.93). The clinical and anthropometric variables comparing cases and controls are shown in **Table 13.** The univariate analysis gives a significant *P*-value < $1x10^{-4}$ for sex, TRG, and HOMA-IR. The eight SNVs included were in HWE according to the Chi^2 test in the control group. Variants *ADRA2A* rs553668 (p=0.006) and *KCNQ1* rs2237892 (0.02) in the Chi^2 analysis showed a significant difference when were compared with the allelic frequencies for Mexican-Americans reported in the 1000 Genomes Project.

Table 13. Descriptive characteristics of Mexicans with T2D and control group.

Clinical Data	T2D (n=415)	Controls (n=416)	*P*-value*
Sex (F or M), %	72.53/27.47	45.91/54.09	**$1x10^{-4}$**
Age, years	59.09±11.65	50.58±8.93	0.277
DBP, mm Hg	75.97±11.40	73.69±9.80	0.353
SBP, mm Hg	135.57±22.01	116.76±13.95	0.310
Glucose, mg/dl	140.53±63.48	84.90±9.31	0.316
CHOL, md/dl	195.98±40.76	187.66±44.13	0.319
TRG, mg/dl	208.48±164.78	145.59±70.87	**0.001**
HDL-C, mg/dl	40.76±10.90	48.45±16.55	0.294
Insulin, µIU/ml	10.03±6.24	10.50±5.75	0.317
BMI, kg/m2	30,97±15.08	27.49±4.42	0.679
WHR	0.904±0.10	0.92±0.08	0.030
TRG/HDL-C[a]	5.13±2.64	3.90±1.91	0.474
HOMA-IR	4.38±4.27	2.14±1.30	**0.001**

Data expressed as means ± SD (standard deviation). F, female; M, masculine; DBP, diastolic blood pressure; SBP, systolic blood pressure; CHOL, cholesterol; TRG, triglycerides; HDL-C, high-density lipoprotein; BMI, body mass index; WHR, waist to hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance. [a] Heart risk score obtained dividing triglycerides by high-density lipoprotein. * P < 0.05 indicates statistical significance, all the variables were analyzed according to normality with U-Mann-Whitney or T-student test.

b) SNVs associated with T2D

Logistic regression performed, displayed significant association with T2D for *ADRA2A* rs553668 under recessive model (OR 4.39; 95% CI 2.91-6.63; $P<1x10^{-4}$; Power 0.999) and under additive model (OR 1.64; 95% CI 1.34-2.00; $P<1x10^{-4}$; Power 0.956), in both cases were included as covariates age, sex, and BMI. Variants *PPARG* rs1801282, *PPARGC1A* rs819267, *SIRT1* rs789600, *IGF2BP2* rs440296 and *UCP3* rs378190 were in nominal association (**Table 14**) for a P>0.001 and <0.05.

c) Association results for T2D related traits in healthy controls

In the analysis of the T2D related traits and genotypic association (**Table 15**) by linear regression adjusting for age, sex and BMI was not found a significant association when a strict Bonferroni correction. Including the number of models, the number of variants and variables in the multiple comparisons adjustment for a significant association considering $P<2.3x10^{-4}$. The variants with nominal association with T2D related traits were *ADRA2A* rs553668 with FPG and insulin; *PPARG* rs1801282 with DBP and SBP; *IGF2BP2* rs4402960 with DBP and CHOL; *PPARGC1A* rs8192678 with SBP; *VEGFA* rs2010963 with SBP, HDL-C and TRG/HDL-C; *SIRT1* rs789600 with SBP and HDL-C; *KCNQ1* rs2237892 with TRG, HDL-C, and TRG/HDL-C; the *P*-value for all these nominal association were between $2.3x10^{-4}$ and 0.05 and are shown in **Table 15** according to the most significant model of inheritance and considering their respective β values.

Table 14. T2D case-control association analysis.

SNV	MAF Case/Control/Allele	Model[a]	*P*-value and OR (95% CI)[b]		Power[b]	*P*-value and OR (95% CI)[c]		Power[c]
rs1801282	0.20/0.15/G	Recessive	0.000	3.62 (1.77-7.43)	0.934	0.002	3.21 (1.50-6.88)	0.831
rs8192678	0.22/0.29/T	Additive	0.001	0.69 (0.55-0.86)	0.369	0.005	0.70 (0.55-0.90)	0.329
rs2010963	0.36/0.37/C	Dominant	0.380	0.88 (0.67-1.17)	0.008	0.280	0.85 (0.64-1.14)	0.016
rs553668	0.45/0.32/A	Recessive	0.000	4.39 (2.91-6.63)	0.999	**0.000[d]d**	3.64 (2.33-5.69)	0.999
rs2237892	0.37/0.35/T	Recessive	0.160	1.30 (0.90-1.88)	0.026	0.140	1.37 (0.90-2.08)	0.050
rs7896005	0.27/0.39/G	Additive	0.000	0.56 (0.45-0.69)	0.938	0.002	0.55 (0.44-0.70)	0.953
rs4402960	0.15/0.20/T	Dominant	0.001	0.62 (0.46-0.83)	0.348	0.002	0.62(0.45-0.84)	0.620
rs3781907	0.15/0.20/G	Dominant	0.013	0.69 (0.51-0.93)	0.157	0.046	0.72 (0.52-1.00)	0.104

SNV, single nucleotide variant; MAF, minor frequency allele; OR, odds ratio; CI, coefficient interval.
[a] Most significant model.
[b] Values without adjustment.
[c] Adjusted values for age, sex, and BMI; significant P value ≤ 0.001.
[d] Significant in additive model (OR 1.64; 95% CI 1.34-2.00; P<0.000; Power 0.956).

Table 15. T2D related traits in control group.

SNV	FPG	Insulin	DBP	SBP	CHOL	TRG	HDL-C	TRG/HDL-C[a]
rs1801282	0.33 (-7.36 - 8.02) Dominant 0.93	3.21 (-0.82 - 7.24) Recessive 0.12	-6.58 (-12.62 - -0.54) Recessive 0.033	2.92 (0.50 – 5.34) Additive 0.018	-1.05 (-9.27 - 7.16) Additive 0.8	15.07 (-57.88 - 88.02) Recessive 0.69	0.95 (-1.65 - 3.54) Additive 0.48	-0.35 (-1.95 - 1.25) Dominant 0.67
rs8192678	-2.18 (-15.02 - 10.67 Recessive 0.74	0.41 (-0.83 - 1.65) Dominant 0.52	-0.9 (-2.77 - 0.96) Dominant 0.34	-3.44 (-6.45 - -0.43) Dominant 0.026	3.06 (-2.65 – 8.76) Dominant 0.29	1.45 (-13.06 – 15.95) Additive 0.85	-3.18 (-8.10 - 1.75) Recessive 0.21	0.72 (-0.71 - 2.14) Dominant 0.32
rs2010963	-7.52 (-17.68 - 2.65) Recessive 0.15	0.76 (-0.16 - 1.67) Additive 0.11	1.45 (-1.32 - 4.22) Recessive 0.3	4.64 (0.17 - 9.12) Recessive 0.043	-2.34 (-8.51 - 3.83) Additive 0.46	16.48 (-9.74 - 42.70) Recessive 0.22	2.78 (-5.46 - -0.10) Dominant 0.043	1.69 (0.25 - 3.14) Dominant 0.022
rs553668	-7.14 (-14.04 - -0.25) Dominant 0.043	1.3 (0.06 - 2.54) Dominant 0.04	-1.08 (-4.50 - 2.33) Recessive 0.54	8.12 (4.50 - 11.73) Recessive 0.31	-1.99 (-10.42 - 6.44) Dominant 0.64	-8.15 (-49.22 - 32.92) Recessive 0.7	-1.76 (-4.43 - 0.90) Dominant 0.19	0.84 (-0.60 - 2.27) Dominant 0.25
rs2237892	-4.92 (-11.84 - 2.01) Dominant 0.16	-0.69 (-1.94 - 0.57) Dominant 0.29	1.14 (-0.43 - 2.71) Dominant 0.15	0.97 (-3.29 - 5.24) Recessive 0.65	-2.79 (-11.24 - 5.66) Dominant 0.52	23.63 (1.01 - 46.25) Dominant 0.041	-2.68 (-5.35 - -0.01) Dominant 0.05	2.13 (0.70 - 3.56) Dominant 0.0038
rs7896005	-7.84 (-15.10 - -0.57) Dominant 0.035	0.51 (-1.43 - 0.40) Additive 0.27	-1.17 (-2.55 - 0.20) Additive 0.095	2.89 (-5.11 - -0.67) Additive 0.011	5.26 (-3.76 - 14.28) Recessive 0.25	-14.60 (-32.58 – 3.37) Dominant 0.11	-1.98 (-3.92 - -0.04) Additive 0.046	1.99 (-0.01 - 3.99) Recessive 0.052
rs4402960	5.58 (-12.29 - 23.44) Recessive 0.54	2.93 (-0.29 - 6.15) Recessive 0.076	3.04 (1.11 - 4.96) Dominant 0.0021	0.94 (-2.22 - 4.09) Dominant 0.56	9.22 (1.86 - 16.59) Additive 0.014	19.8 (-38.55 - 78.16) Recessive 0.51	3.97 (-2.89 - 10.82) Recessive 0.26	0.22 (-1.49 - 1.05) Additive 0.73
rs3781907	8.74 (-8.63 - 26.12) Recessive 0.32	-0.38 (-1.48 - 0.72) Additive 0.5	-1.63 (-6.36 - 3.09) Recessive 0.5	-0.9 (-3.58 - 1.78) Additive 0.51	-3.54 (-12.26 - 5.17) Dominant 0.43	9.75 (-10.10 - 29.59) Additive 0.34	-2.79 (-9.47 - 3.89) Recessive 0.41	0.97 (-2.64 - 4.58) Recessive 0.6

In each cell are displayed in descending order of appearance: coefficient β, 95% CI, most significant model, P value, and all the traits were rank-transformed.
SNV, single nucleotide variant; FPG, fasting plasma glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure; CHOL, cholesterol; TRG, triglycerides; HDL-C, high-density lipoprotein cholesterol; WHR, waist to hip ratio.
[a]Cardiac risk score: triglycerides divided by high-density lipoprotein.
Nominal P-value between $>2.3x10^{-4}$ and, <0.05
Significant *P*-value $\leq 2.3x10^{-4}$; all the models were adjusted to age + sex +BMI and inverse rank transformed.

CHAPTER 5. DISCUSSION

Mexico is a country with more than 120 million people (INEGI 2015), all of them are at risk to develop at least one complex diseases. One of the most representative conditions is T2D which affects at least 9 million of people, is the third cause of death in Mexico and the leading cause of death in women and the second in men (World Health Organization 2016). Compared with the attention obtained in developed countries to treat T2D, in Mexico, a patient with the disease has more probability to die because of complication derived of poor glycemic control, the high obesity levels, and the prevalence of the disease in patients without a diagnosis (Alegre-Díaz et al. 2016). One of the theories that try to explain the recent high prevalence in diagnosis for T2D is Neel's hypothesis, which establishes the selection of genotypes associated with a thrifty metabolic efficiency and survival in selected populations (Neel 1999). One of the most representatives examples of this hypothesis is the Pima population which was divided in two by the war USA-Mexico. Because new limits between these countries, when the dietary habits change in the Pima population from the USA, the number of people with T2D increased in this subpopulation, and the Pimas who remain in Mexico conserve the same dietary habits through the years (Knowler et al. 1993). Similar examples have been found for Chinese (Chun et al. 2011), and Indian people (Gujral et al. 2015) who migrated to the United States recently. With the aim to study the role of genetic factors and the interaction with environmental factors in the occurrence of disease population as T2D, the genetic epidemiology surged as a new discipline recently (Bishop 1994). This discipline requires robust statistical methodologies to explain the results obtained and avoid false positive associations. In a review has been stated a 3.6% rate for replication studies based on previous positive association (Hirschhorn et al. 2002). However recently has been augmented this lack of positive results for replication studies and can be due to a poor p value=0.05. Consequently has been stated that a p-value=0.005 has an 80% possibility to obtain positive results in replication studies (Gorroochurn et al. 2007), when this criterion applyied to the information reported in the literature is found only the genes associated *SLC16A11*

rs13342232 ($p=5.5X10^{-12}$) *TCF7L2* rs7903146 ($p=2.5X10^{-7}$), *KCNQ1* rs2237897 ($p=4.9X10^{-16}$), *CDKN2A/2B* rs10811661 (p=0.003), *TNF-alpha* rs1800629 ($p=5x10^{-4}$), *ADRB3* rs4994 (p=0.002), *TCFL2* rs122553 (p=0.002), *IRS1* rs1801278 ($p=1x10^{-4}$) and *ABCA1* rs9282541 (p=0.001)(S?nchez-Pozos and Menj?var 2016), remaining a lot of pending genes without been studied or replicated, the main problem in this context is the lack of research in the area, there is no doubt about the multiple benefits of GWAs, whole genome and exome sequencing, but in genetic analysis for Mexico, remains a gap of fifteen years in research to be fulfilled.

In this study were selected the variants *KCNQ1* rs2237892, *ADRA2A* rs553668, *VEGFA* rs2010963, *IGF2BP2* rs4402960, *PPARG* rs1801282, *PPARGC1A* rs8192678, *SIRT1* rs7896005, *UCP2* rs659366, *UCP3* rs3781907, *IGF2BP2* rs4402960, and *CDKN2A/2B* rs10811661 associated with T2D or related traits.This work was divided into two parts, in the first analysis was genotyped T2D patients with recent T2D diagnosis and the complete phenotype data and in the second part was performed a case-control study in patients with previous T2D diagnosis and healthy controls.

T2D related traits in people with a recent T2D diagnosis

In this first part of the study the selected the variants *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *SIRT1* rs7896005, and *UCP2* rs659366 because have been associated with T2D in Chinese Han, Pima and Maya (Freathy et al. 2006b; Barroso et al. 2006; Al-Kateb et al. 2007; Dong et al. 2011; Gamboa-Meléndez et al. 2012; Shen et al. 2014; Lara-Riegos et al. 2015) for the study of traits related to diabetes according to their genotypic distribution in a subset of patients with T2D.

CDKN2A/B genes are expressed in adipocytes and pancreatic islets and play a role in β-cell function and regeneration. The variant CDKN2A/B rs10811661located 125 kb upstream of these genes has been identified as a risk allele in Mexican mestizo from Mexico City (Gamboa-Meléndez et al. 2012). Our results showed no statistically significant difference in allele frequency of this variant compared to HapMap and 1000 Genomes Project, or with any of the

traits related to T2D. Future analysis of CDKN2A/B rs10811661 in multi-ethnic Mexican cohorts to elucidate its implication and impact on T2D.

PPARGC1A is a coactivator of nuclear receptors and other transcription factors that regulate mitochondrial biogenesis, respiration, hepatic gluconeogenesis, and muscle fiber type switching (Barroso et al. 2006; Maver et al. 2008; Jemaa et al. 2013; Vázquez-Del Mercado et al. 2015). The frequencies obtained for *PPARGC1A* rs8192678 had a difference of 2.2% compared with 1000 Genomes Project and a difference of 10% with Mexicans from West Mexico (Jalisco State) (Garza-Veloz et al. 2011). The distribution difference obtained between these populations could represent different ancestry and migration history between central and western Mexico. Stratification of genotype frequencies resulted in significant nominal values for glucose (p=0.023) and TRG levels (p=0.013). This variant has been associated with T2D in populations from China (Lin et al. 2010), and Denmark (Ek et al. 2001), however, it has not been studied in Mexican populations for T2D related traits. Consequently, association studies for this gene must be replicated.

VEGFA rs2010963 has been associated with diseases, such as amyotrophic lateral sclerosis (Lambrechts et al. 2003). Allelic frequencies of this variant in Mexicans reported in a case-control study with preeclampsia showed no evidence of an association of *VEGFA* alleles or haplotype frequencies with this disease. However, diabetes complications, such as retinopathy with vascular implications, has been associated with rs2010963 in case-control studies (Stathopoulou et al. 2013; Fan et al. 2014; Shen et al. 2014; Choudhuri et al. 2015). Furthermore, metabolic traits analyzed in T2D patients of this study showed a significant P value for low HDL-C levels [Coef β (95% CI); -0.12 (–0.20 - –0.03), P=0.007] in the additive model and after correcting for multiple comparisons. The impact of the risk allele in the additive model could predispose people with T2D to cardiovascular disease. A Slovenian group of 143 subjects with T2D and myocardial infarction (MI) was compared with 228 diabetic subjects without the disease, and the *VEGFA* rs2010963 was associated with MI (Petrovic et al. 2007). These results agree with our results, suggesting an impact of this variant on low HDL-C levels and in the development of MI in patients with T2D. The present findings strengthen the contribution of *VEGFA* to variation in blood lipid levels, mainly for HDL-C.

Additionally, a nominal association with HbA1C (p=0.020; dominant model) and DBP (p=0.032; recessive model) was also observed in our study. These results support the affectation in patients with T2D and heart diseases.

SIRT1 is a stress-response and chromatin-silencing factor, and in Pima Native American populations, *SIRT1* rs7896005 has been associated with reduced acute insulin in response to intravenous glucose bolus (adjusted P= 0.045) (Dong et al. 2011). This variant displayed a significant association with DBP [Coef β (95% CI); -0.06(-0.11- -0.02), p=0.006] in the recessive model, suggesting a protective role. This project represents the first study testing the association between *SIRT1* rs7896005 with DBP in Mexicans. In mice, has been reported, *SIRT1* overexpression in vascular smooth muscle cells and is reported to reduce SBP (Gao et al. 2014) but not DBP.

UCP2 separates oxidative phosphorylation from ATP synthesis with energy dissipated as heat, also referred to as the mitochondrial proton leak (Jastroch et al. 2010). Allele frequencies displayed a difference of 3% with the reports from the international projects for Mexicans from Los Angeles, with no significant difference. A recent meta-analysis using three cohorts from Europeans (20,242 individuals) shows that *UCP2* rs659366 was associated with liver dysfunction because of gamma-glutamyl transferase levels varied by genotype with interaction with waist-to-hip ratio and body mass index (Vimaleswaran et al. 2015). However, in the present study, no association to any of the T2D-related traits was found. This result may be due to a different ancestry between the European population and the Mexican Mestizo population of this study.

Diabesity is used to refer to a form of diabetes that typically develops due to obesity; however, only a fraction of people with obesity develop diabetes (<10%) (Chadt et al. 2000). In this study, *CDKN2A/B* rs10811661, *PPARGC1A* rs8192678, and *UCP2* rs659366 that have been associated with obesity and T2D did not show association with obesity in the Mexican Mestizo population. Furthermore, when the group of patients with T2D was divided and analyzed by binary variable obesity in the logistic regression, no association with SNVs was found (results not included). Association of the CDKN2A/B rs10811661 with obesity and diabetes has been replicated in low-medium size samples. Therefore, the lack of association in this study may be due to the ancestry of the population under study, the sample size, or the stratification in the analysis.

Nevertheless, some studies have shown that most obese, insulin-resistant individuals do not develop hyperglycemia, maintaining a healthy β cell level despite obesity (Kahn 2001; Abbasi et al. 2002). Furthermore, only three loci have been found to share a robust association (*FTO, MC4R,* and *QPCTL/GIPR*) and genetic correlation between T2D and obesity (Grarup et al. 2014); therefore, the need remains to study traits as new sources of covariance for diabesity.

According to the results of the association test among paired SNVs, for *PPARGC1A* rs8192678 and *CDKN2A/B* rs10811661 (0.9666 for exact Pr ≥ Chi-Square), a bioinformatics analysis for genetic interaction was performed to support these results. However, a direct interaction between these SNVs was not found, but identification of intermediary genes, like the rich human β-cell proliferation *CDK6* (Fiaschi-Taesch et al. 2010) interaction with these two SNVs at *SIRT1* and *CDKN2A/2B* locus*,* was observed. *CDK6* codifies for a kinase regulated by cyclin D. A mutation in this kinase has been reported to reduce cell proliferation and impair cell motility and polarity (https://www.ncbi.nlm.nih.gov/gene/1021). A stronger interaction was observed between *CDKN2A* and *CDK6* than between *CDKN2B* and *CDK6* (**Figure 4**). A strong interaction was also found between *SIRT1* and *CDKN2A* with *MCM10*, which is required for both initiation and elongation during chromosomal DNA replication (Izumi et al. 2000). These interactions support a role of regulatory factors during β-cell-cycle progression in the pathogenesis of T2D, and as a result, this may influence insulin synthesis and secretion.

Case-control association study with T2D

In this part of the study was tested the association between the variants *PPARG* rs1801282, *PPARGC1A* rs8192678, *VEGFA* rs2010963, *ADRA2A* rs553668, *KCNQ1* rs2237892, *SIRT1* rs7896005, *IGF2BP2* rs4402960 and *UCP3* rs3781907 with T2D in a case-control study from Mexico City. The results obtained in this study supported the association of variant *ADRA2A* rs553668 with T2D. The individuals carrying two “A” allele for this variant had an increased risk to develop the disease, this result is supported by an OR 3.64

(95% CI 2.33-5.69) and an increased allele frequency of the minor allele which increases the probability to identify a genetic effect under a recessive model; the variant was significant also under an additive model with a lower OR 1.64 (95% CI 1.34-2.00), in both cases the power calculated was higher than 0.999 and 0.956 respectively. In a previous meta-analysis performed in 2012 were included 40828 subjects from different ethnicities to verify risk for T2D for carriers of *ADRA2A* rs553668 variant, finding a significant difference only in Europeans under the recessive model (Chen et al. 2013), this result is consonant with the recessive model obtained in this study.

Physiologically ADRA2A mediates adrenergic suppression of insulin secretion and lipolysis (Rosengren et al. 2010). This information is supported by clinical evidence such as a clinical study performed in Italy including 1345 healthy subjects analyzed in a 6 year follow-up, finding that subjects with two alleles "A" showed an increased baseline FPG and a significant worsening of fasting glucose (β = 0.48; 95% CI 0.10-0.86) and insulin secretion (β =-20.75; CI -32.67- -8.82) for HOMA-IR (Bo S et al., 2012). In a similar analysis performed in the present study, where 416 control subjects were included in linear regression and adjusted for age, sex, and BMI, it was obtained nominal values for FPG and Insulin under a dominant model in both cases for the variant *ADRA2A* rs553668.

In another hand, the allele frequency for *ADRA2A* rs553668 has not been reported previously in Mexicans from the center of Mexico such as Mexico City. In this study we obtain 0.45 for T2D cases and 0.32 for controls; the relative antecedent is an independent study where was found a frequency of 0.40 for healthy Maya people from Merida City and 0.39 for healthy people living in a small community located in the port of Yucatan (Domínguez-cruz and Muñoz 2016). The allele frequency obtained in our study had a significant difference with the 0.25 frequency reported in the 1000 genomes project for Mexicans living in Los Angles California P-value <0.05, (www.ensembl.org). Between the Latin people included in the 1000 genomes project are the Peruvians, with 0.41 frequency for the "A" allele, which is near to the frequencies obtained in the Maya population and the present study with participant from Mexico, in both cases, the two populations are far away from the 0.66 frequency for Chinese

Dai in Xishuangbanna, in China. Future replications studies genotyping the variant *ADRA2A* rs553668 in Mexicans from Mexico City are needed to reinforce this difference in frequencies; geographic differences with the Mexicans living in Los Angeles can be a source of the allelic frequency divergence detected in this project and the apparent concordance with contemporaneous Mayas.

The variants *PPARG* rs1801282, *PPARGC1A* rs8192678, *SIRT1* rs7896005, *IGFB2BP2* rs4402960, and *UCP3* rs3781907, remained in nominal significance for association with T2D, the significant part of them with nominal values near to 0.02, but only two of them with a statistical power>0.080. Being *PPARG* rs1801282 with an OR 3.21(95% CI 1.50-6.88, P=0.02) and power 0.831, one of the most relevant between this group, this variant has been studied previously in Mexicans from Guerrero (Martínez-Gómez et al. 2011)(Martínez-Gómez et al., 2011), Yucatan(Lara-Riegos et al. 2015) (Lara-Riegos et al., 2015) and Mexico City (Martínez-Gómez et al. 2011; Gamboa-Meléndez et al. 2012) (Gamboa-Meléndez et al., 2012; Martínez-Gómez et al., 2011) under diverse models of inheritance, however, the OR varies between 0.642,(95% IC:0.356–1.160) for people from Yucatan to OR 1.19, 95% IC 0.92–1.55, in people from Mexico City. *PPARG* rs1801282 variant would be expected to be associated with T2D since this gene encodes a nuclear receptor that regulates adipocyte differentiation, lipid metabolism, and insulin sensitivity.

Moreover, the variant *SIRT1* rs7896005 with an OR 0.55(95% CI 0.44-0.79, P=0.02) and, a power 0.953 was the second variant with the best nominal value. This variant was suggested to be a risk allele for T2D in Pimas living in the USA. According to a nominal association value for women (OR 1.37, 95% CI 1.14-1.65, P=0.02) and contrasting to men with a low protection value (OR 0.97, 95% 0.79-1.18, P=0.02) under an additive model. This gene encodes the human Sirtuins protein expressed in β cells regulating insulin secretion (Liang et al. 2009), consequently, would be interesting to determine, if differences in regulation of protein expression may be related to a protective effect or the risk of diabetes.

Concerning to the analysis of related traits, none of the variants were associated with any T2D related traits after rigorous Bonferroni correction ($P<2.3x10^{-4}$), however, in the control group there were two variants with lower P-values that are important to consider in future studies. *KCNQ1* rs2237892 and the heart risk TRG/HDL-C score had a coefficient β 2.13, 95% CI 0.70 - 3.56, p=0.0038, under a dominant model. TRG/HDL-C score is considered an indicator of LDL-C particle size and is used to identify T2D patients with an atherogenic profile, this index is considered the best predictor compared to the parameters analyzed (Boizel et al. 2000); for this reason, we examined this score. Also, when the control group was analyzed using heart risk as a categorical variable, the low P-value remained. KCNQ1 is expressed in pancreas and heart, and it has been associated with Long QT syndrome 1, Jervell and Lange-Nielsen syndrome 1, atrial fibrillation 3, short QT syndrome 2, and hypertension (Jespersen et al. 2005) implicated in cardiovascular diseases.

Also, *IGF2BP2* rs4402960 was the second variant with the best nominal values for the metabolic trait DBP (coefficient β 3.04, 95% CI 1.11 - 4.96, p=0.0021) under a dominant model in controls of this study. IGFBP2 is part of a family of proteins with mRNA-binding implicated in RNA stability, localization, and translation (Rodriguez et al. 2010a). The protein encoded by this gene is secreted into the bloodstream and has been previously associated promoting angiogenesis and with T2D risk by GWAS (Saxena et al. 2007; Scott et al. 2007). Then, it will be critical to determine if there is a role of this protein in the diabetes metabolism and cardiovascular risk.

One of the limitations of this study is the lack of ancestry markers to discard substructure. However, a rigorous selection of the individuals included in the study has been made, such as specific verification of self-classification as Mexican Mestizo traced back at least three generations behind, additionally and rigorous double-blinded genotyping was performed, and outliers with more than four deviation standards have been discarded, plus stringent statistical adjustments for multiple comparisons and covariates such as sex, age and BMI in logistic and linear models, to avoid false positive associations, and calculation of statistic power for the best outcome.

CHAPTER 6. CONCLUSIONS

Although it has been described in the literature in the past decades the high susceptibility to develop T2D in the Mexican mestizo, the participation of scientific groups in genetic studies for this population is scarce. The Mexican population from Mexico City has a high incidence of T2D and related traits. According to the data informed in population census and health surveys performed by governmental efforts, because of this, the first studies analyzing genetic association studies were designed, but the number of the studies conducted continues in a low proportion compared to the information published in the literature. In the analysis including in this work with T2D patients recently diagnosed was found an association of *VEGFA* and *SIRT1* with low levels of HDL-C and high levels of DBP respectively. Additionally, in the case-control analysis was confirmed a high association of *ADRA2A* with T2D, under top statistical criteria. These results highlight the need to study the Mexican population from Mexico City in diverse genetic approaches to elucidate the genetic participation of the variants associated in other populations and propose new variants and genes for Mexicans with origin in Mexico City.

CHAPTER 7. SARCOPENIA PROJECT: SHORT STAY AT SOUTH TEXAS DIABETES AND OBESITY INSTITUTE, UNIVERSITY OF TEXAS RIO GRANDE VALLEY.

Collaborators:

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	John Blangero
	Donna Lehman

As part of an international collaboration with CINVESTAV, during the three months in a short stay at University of Texas Rio Grande Valley, I was trained in advanced statistical techniques including Monte-Carlo simulations, structural equations, use of PLINK and SOLAR (Sequence Oligogenic Linkage Analyses Routines) software with focus in case-control and family association studies.

Additionally, were analyzed in a first approach data from The San Antonio Mexican-American Family Studies (SAMAFS) which is composed by the San Antonio Family Heart Study (SAFHS) and The San Antonio Family Diabetes/Gallbladder Study (SAFDGS) to identify heritability of sarcopenia as a T2D related trait. Because, the results obtained are under review and belong to different research groups, the complementary data including the results for the draft will include sarcopenia heritability and analysis of related traits, and can be consulted once that the paper will be published and there is agreement about the draft, analysis, results and diverse content for all the researchers involved in the diverse parts of the sarcopenia project. In the next lines are included general information about the first part of the draft considered for sarcopenia heritability

Introduction to the study of sarcopenia

Life expectancy is increasing worldwide; this change is currently affecting the epidemiological profile of developed countries and threat to affect more deeply in their finances to the developing nations. In the United States of America (USA) is projected that the number of people older than 65 years of age double from 46 million in 2015 to 98 million by 2060(Mark Mather, Linda A. Jacobsen 2015), which leads to an increasing number of individuals requiring healthcare services, because of a rise of chronic degenerative diseases, comorbidities, and its complications. The multi-ethnic composition of the country makes mandatory to study the genetic and environmental influences in the diverse populations that are part of its epidemiological landscape.

One disease decreasing quality of life mainly through diminishing the motion in the aged adult is sarcopenia (Greek σάρξ-sarx, "flesh" or πενία-penia," poverty"); This disease is considered as progressive and generalized loss of skeletal muscle mass and strength function associated with aging (Landi et al. 2017), because a lower mobility in the aged adult and influence in comorbidity; for example, one of the most prevalent comorbidity is the presence of sarcopenia plus obesity (SOB)(Baumgartner 2000; Roubenoff 2000; Kalinkovich and Livshits 2017), enhancing health damage altogether more than separated independently(Bouchard et al. 2009; Lee et al. 2015).

Heritability for fat-free mass as a first approach to the study of sarcopenia has been studied in S-leut Hutterites of South Dakota(Abney et al. 2001), estimating a narrow sense (h^2= 0.45) and broad sense heritability (H^2= 0.76), and recently in twins from the United Kingdom(Livshits et al.) adjusting heritability for covariates was found h^2=0.809±0.050. However, different candidate genes have been analyzed mainly for muscle strength trying to explain part of the muscle phenotype in the aged adult, but no significant genes and variants have been found(Garatachea and Lucía 2013), the genetic component for sarcopenia is pending to be elucidated.

Diverse modern methods have been used for the study of sarcopenia, such as dual-energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), computerized axial tomography scan (CAT) and magnetic resonance (MR). BIA is the most practical and portable device to measure under

standardized conditions the body composition in adults with sarcopenia and is accepted to estimate skeletal muscle mass by The European working group on sarcopenia in older people (EWGSOP) (Cruz-Jentoft et al. 2010) and The Asian working group for sarcopenia (Chen et al. 2014). EWGSOP consider skeletal mass index as skeletal index adjusted for the squared height (SM/height^2, kg/m^2), based on The Third National health and nutrition examination survey (NHANES-III) data on >60 years for men and women, when the skeletal mass was measured by BIA(Janssen et al. 2002, 2004).

In the south of Texas was designed in 1991, the San Antonio Family Studies (SAMAFS) to analyze the genetic and environmental influences in complex diseases for the Mexican-Americans population which is considered an ethnic group with a high risk to develop comorbidities associated with metabolic syndrome.

This study aims to analyze the heritability of sarcopenia and genetic correlations for T2D traits in Mexico-Americans.

Material and Methods

The individuals included in this research shape The San Antonio Mexican-American Family Studies (SAMAFS) which is composed by the San Antonio Family Heart Study (SAFHS) and The San Antonio Family Diabetes/Gallbladder Study (SAFDGS).

San Antonio Family Heart Study (SAFHS):

The SAFHS (PI: Dr. John Blangero) began in 1991 and included 1,431 individuals in 42 extended families at baseline (Mitchell et al. 1996; Comuzzie et al. 1997; Curran et al. 2007). Probands were 40 to 60 years old low-income Mexican Americans selected at random without regard to presence or absence of disease, almost exclusively from Mexican American census tracts in San Antonio, Texas. All first, second and third-degree relatives of the proband and the proband's spouse, aged 16 years or above, were eligible to participate in the study. In addition to the demographic (age and sex) and medical history

information, extensive phenotypic data have been collected including type T2D affection status (WHO 2000; Expert Committee on the Diagnosis and Clasification of Diabetes Mellitus 2002) (WHO criteria, 1999 and ADA criteria, 2002; and, participants who did not meet these criteria but who reported that they were under treatment with either oral antidiabetic agents or insulin and who gave a history of diabetes were also considered to have T2D), age of T2D onset, and T2D-related quantitative traits: fasting glucose (FG), fasting insulin (FI), CHOL, TRG, HDL-C, low-density lipoprotein cholesterol (LDL-C), SBP and DBP, adiponectin, leptin, creatinine, height, weight, BMI, hip and waist circumferences, medications (diabetes, hypertension, and dyslipidemia), female hormone use, 2-hour glucose (2-h G), 2-hour insulin (2-h I), urine albumin-to-creatinine ratio (and albuminuria, micro- and macro-), and estimated glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease (MDRD) study equation and the Cockcroft-Gault (CG) equation (Puppala et al. 2007). The SAFHS participants have been followed in a mixed longitudinal fashion, up to a maximum of five visits. For the study of sarcopenia baseline data were used.

San Antonio Family Diabetes/Gallbladder Study (SAFDGS):

The SAFDGS also began in 1991, and initially included 579 examined individuals distributed across 32 pedigrees as part of the San Antonio Family Diabetes Study (SAFDS, PI: Dr. Donna M. Lehman)(Duggirala et al. 1999). The sample size was expanded to 40 families and more than 900 individuals through two recalls. The second recall refers to the San Antonio Family Gallbladder Study (SAFGS, PI: Dr. Ravindranath Duggirala), which recruited new family members from the original SAFDS families and family members from 8 newly recruited families. The SAFDS and SAFGS are referred to as San Antonio Family Diabetes/Gallbladder Study [SAFDGS] (Hunt et al. 2005; Puppala et al. 2006). The probands for the SAFDGS were individuals with T2D identified in an earlier epidemiologic survey, the San Antonio Heart Study. Only low-income Mexican Americans identified in the San Antonio Heart Study as having T2D were eligible to be probands. These individuals were approached in random order without regard to how many T2D individuals were in their families

(i.e., no attempt was made to recruit multiplex families preferentially). Thus, the T2D probands in the SAFDGS constitute a population-based case series of T2D individuals. All first, second and third-degree relatives, aged 18 or above, were invited to participate in the study. The SAFDGS data collection protocol is very similar to that used for SAFHS, and T2D was defined according to the ADA and WHO criteria as described above. Also, numerous other phenotypes described above were measured. As part of our ongoing studies, some of the SAFDGS participants have been recalled. Thus, the SAFDGS participants have been followed in a mixed longitudinal fashion, up to a maximum of 4 visits. For the exploration of sarcopenia genetic influences, baseline data of SAMAFs were studied.

Individuals participating in this study signed informed consent and were instructed about the perspectives, benefits, and utility of the research. Institutional Board Review of the University of Texas Health Science Center at San Antonio approved the study and all the procedures were performed according to the Helsinki Declaration and The Nuremberg Code.

Sarcopenia related traits measured by BIA

Sarcopenia was defined according to the EWGSOP considering skeletal mass measured by BIA interpreted as skeletal index adjusted for the squared height (SM/height^2, kg/m^2), based on statistical analysis of NHANES III data on older on >60 years for men and women, taking into count cut-off points by gender, considering the grades for men: severe sarcopenia ≤8.50 kg/m^2, moderate sarcopenia 8.51–10.75 kg/m^2 and normal muscle ≥10.76 kg/m^2; for women: severe sarcopenia ≤5.75 kg/m^2, moderate sarcopenia 5.76–6.75 kg/m^2, and normal muscle ≥6.76 kg/m^2(Janssen et al. 2002, 2004). Because the portability of BIA to measure body composition in family studies, percent of body fat (%BF) and kilograms of fat-free mass (FFM) were measured by this method (Valhalla Scientific, San Diego, CA). The T2D related traits included in the study were HDL-C, CHOL, TG, 2-h I, 2-h G, FI, FG, DBP, SBP, RFFM, FFM, %BF, BMI, WC, and Leptin.

Statistical Analysis

Univariate analyses were performed using SPSS Statistics 24 software (SPSS Inc., Chicago, Il, USA). To study normal distribution in quantitative traits Shapiro-Wilks test was used, traits with a non-normal distribution were log_{10} transformed or rank-based inverse normal transformed as appropriated. In a non-normal distribution, the use of a rank transformation is useful because replace the data by their ranks or average ranks in case of ties, generating perfectly normal distribution based in the rank transformation of residuals (Narinç and Aygün 2017),(Conover and Iman 1981). Because of variance component methods used to calculate genetic influences is based and start from the assumption of normal distribution, in all the analysis, outliers were identified and discarded when those were more significant than four standard deviations from a normalized data.

All the genetic analyses were performed in SOLAR Eclipse Version 8.3.X; this software counts with all the standardized routines to calculate, heritabilities, genetic, phenotypic and environmental correlations and also linkage analysis in extended pedigrees(Almasy and Blangero 1998). The analysis of continuous quantitative traits was performed using the variance component methods routines standardized in SOLAR for the study of complex phenotypes such as sarcopenia, this model assumes a normal distribution, and divide the total sarcopenia variance (σ^2_p) into genetic (σ^2_g) and environmental components (σ^2_e); $\sigma^2_p = \sigma^2_g + \sigma^2_e$ (Almasy and Blangero 2010); because there is an additive effect in the family approach to analyzing the genetic component for sarcopenia, heritability in narrow sense(Almasy and Blangero 1998) was calculated as $h^2 = \sigma^2_a + \sigma^2_p$. Pairs of traits were studied by a joint bivariate analysis (Czerwinski et al. 1999), to obtain correlations between traits using the next model: $\rho_p = \sqrt{h_1^2}\sqrt{h_2^2}\,\rho_G + \sqrt{(1-h_1^2)}\sqrt{(2-h_2^2)}\,\rho_E$ were ρ_p represent the phenotypic correlation between a selected pair of traits h_1 and h_2 and calculate the additive genetic (ρ_G) and environmental correlations (ρ_E) by likelihood-ratio using a p-value ≤0.05. The covariates age, sex, age^2, age x sex and age^2 x sex were evaluated in all the genetic analyses. Because of fat-free mass changes according to the

aging by decades in both sex, age was analyzed using six dummy variables per decade.

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APENDIX I. PUBLICATIONS AND MANUSCRIPTS

1) PUBLICATIONS RELATED TO THE THESIS

Accepted

a. Totomoch-Serra A, Muñoz M dL, Burgueño J, et al. Association of common polymorphisms in the VEGFA and SIRT1 genes with type 2 diabetes-related traits in Mexicans. Arch Med Sci 2018.

2) MANUSCRIPTS RELATED TO THE THESIS

Submitted

a. Totomoch-Serra A, Muñoz M dL, Revilla-Monsalve MC, et al. Association of ADRA2A rs553668 variant with type 2 diabetes in a case-control study for eight variants in Mexico City.

3) MANUSCRIPTS IN COLLABORATION AT CINVESTAV

In preparation

a. Domínguez-Cruz MG, Muñoz M dL, **Totomoch-Serra A**, et al. Genome-wide association study identifies five novel risk loci for type 2 diabetes in a Maya population.

b. Domínguez-Cruz MG, Muñoz M dL, **Totomoch-Serra A**, et al. Family-based association study of T2D susceptibility nucleotide variants, in Maya communities.

APPENDIX II. OTHER ACADEMIC AND RESEARCH ACTIVITIES DURING PH.D. STUDIES

1) STAY ABROAD

a. South Texas Diabetes and Obesity Institute, September-November 2016, Researcher host Dr. Ravindranath Duggirala

2) GRANTS OBTAINED

a. Source CONACYT I0017 – Call for Basic Science Research 2015., No. 258103, P.I. María de Lourdes Muñoz Moreno (2016-2018). Project title: Mito-epigenetics as a molecular marker in T2DM for a Mexican Population.

3) COURSES AND ASSISTANCE TO CONGRESS DURING THE PH.D. STUDIES

a. Update Course in Medical Genetics, Research Unit in Human Genetics. Pediatrics Hospital, XXI Century National Medical Center, UNAM, and Mexican Association of Human Genetics, February 10th to 15Th 2014, Mexico City, Mexico.

b. XXXIX National Congress in Human Genetics, November 2014, Juriquilla Queretaro, Mexico.

c. XLVII Theory and Practice Course in Human Genetics, Chemistry Faculty, UNAM, and Mexican Association of Human Genetics, June 29th to July 3^{rd,} 2015, Mexico City, Mexico.

d. Course in Basic Microscopy, Confocal Fluorescence, and sampling. CINVESTAV, LANSEC-CINVESTAV, INMA, ATL and NIKON, August 27th to 28th 2015, Mexico City, Mexico.

e. Online course in Pharmacogenomics, INMEGEN, 2015.

f. XXII Theory and Practice Course in Cytogenetics, Mexican Society of Genetics and UAM, Department of Health Science, September 7th to 11 2015, Mexico City, Mexico.

g. Course in Human Genetics Population, National Institute of Pediatrics, 21st September to November 30th, 2015, Mexico City, Mexico.

h. XL National Congress in Human Genetics, November 2015, Monterrey Nuevo León, Mexico.

i. Course in Introduction to "R" programming and Statistics, January 11st to 15th 2016, Juriquilla Queretaro, Mexico.

j. IX Update course in Medical Genetics, Hospital Angeles de México, Mexican Association of Human Genetics and UNAM, February 8th to 13rd 2016, Mexico City, Mexico.

k. 2nd International Human Migration Conference, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional and The University of Kansas, 40 horas, del 17 al 21 de octubre del 2017. Ciudad de México, México.

l. Transtorno Bipolar, Esquizofrenia y Transtorno por uso de sustancias, 20 puntos curriculares, del 2 de octubre al 10 de diciembre de 2017. Asociación Nacional de Médicos Generales y Familiares A.C., Curso en Línea plataforma Intramed.

APENDIX III. LIST OF TABLES

APPENDIX IV. LIST OF FIGURES

APPENDIX V. ABBREVIATIONS

ANOVA: Analysis of Variance.

BMI: Body Mass Index.

CHO: Cholesterol.

CINVESTAV: Center for Research and Advanced Studies.

CONACYT: The National Council of Science and Technology.

CONAPO: The National Population Council.

DBP: Diastolic Blood Pressure.

DNA: Deoxyribonucleic acid.

ENSANUT: The National Health and Nutrition Survey.

FPG: Fasting plasma glucose.

GWAS: Genome-wide association study.

HbA1C: Glycated hemoglobin.

HBP: High Blood Pressure.

HDL-C: High-Density Lipoprotein- Cholesterol.

HOMA-IR: Homeostasis model assessment – Insulin Resistance.

HWE: Hardy-Weinberg equilibrium.

IMSS: The Mexican Social Security Institute.

INEGI: National Institute of Statistics and Geography.

IR: Insulin resistance.

JNC-8: The eighth joint national committee on the prevention, detection, evaluation, and treatment of high blood pressure.

MAF: Minor Allele Frequency.

NGS: Next Generation Sequencing.

OMIM: Online Mendelian Inheritance in Man.

PDGF: Platelet Derived Growth Factor.

SBP: Systolic Blood Pressure.

SD: Standard Deviation.

SIGMA: Slim Initiative in Genomic Medicine for the Americas.

SNV: Single Nucleotide Variant.

STDOI: South Texas Diabetes and Obesity Institute.

T2D: Type 2 Diabetes.

TRG/HDL index: Triglycerides / High-Density Lipoprotein-Cholesterol ratio.

TRG: Triglycerides.

VNTR: Variable Number Tandem Repeat.

WHR: Waist to hip ratio.

APPENDIX VI. ARTICLE ACCEPTED FOR PUBLICATION

Show decision letter

AMS-07850-2018-02 **Association of common polymorphisms in the VEGFA and SIRT1 genes with type 2 diabetes-related traits in Mexicans**

Decision: **accept without changes**

Letter:

February 22, 2018
AMS-07850-2018-02
Association of common polymorphisms in the VEGFA and SIRT1 genes with type 2 diabetes-related traits in Mexicans

Dear Dr. Maria de Lourdes Muñoz,

I am pleased to inform you that your manuscript, entitled: Association of common polymorphisms in the *VEGFA* and *SIRT1* genes with type 2 diabetes-related traits in Mexicans, has been finally accepted for publication in Archives of Medical Science.

We would like to inform that your paper will be published after receiving publishing fee. In order to receive the invoice, please complete the form including data for invoicing, which is available in the payment bookmark. Failure to complete this form means that you choose not to obtain the invoice.

Please log in to your account:
https://www.editorialsystem.com/ams/article/115310/view/#payment
to proceed with the payment.

If you are paying by wire transfer, please indicate manuscript number the name of the contact author and the title. In case of any problems or questions, please do not hesitate to contact the technical editor at eshelp@termedia.pl. We would appreciate if you could confirm payment within 14 days in order to ensure your manuscript to be included in one of the forthcoming issues.

Thank you for submitting your work to our journal.

Kindest regards,
Prof. Maciej Banach, MD, Ph.D., FAHA, FESC, FNLA
Editor-in-Chief,
Archives of Medical Science
http://www.archivesofmedicalscience.com

Review 1:

This revised form of the article can be published

Review 2:

I do not have any more comments.

AMS
Archives of Medical Science

Association of common polymorphisms in the VEGFA and SIRT1 genes with type 2 diabetes-related traits in Mexicans

Type:

Research paper

Abstract:

Introduction
Type 2 diabetes (T2D), and related traits are highly prevalent in Mexico. This study aimed to test the association of CDKN2A/B, PPARGC1A, VEGFA, SIRT1 and UCP2 gene polymorphisms (rs10811661, rs8192678, rs2010963, rs7896005 and rs659366 respectively) with metabolic traits in 415 unrelated Mexican Mestizo with type 2 diabetes Mellitus (T2D) under three models of inheritance.

Material and methods
Unrelated Mexican Mestizos with T2D (300 females, and 115 males) were genotyped by TaqMan assays. Triglycerides, cholesterol, glucose, HDL, insulin and anthropometric measurements were determined in these patients; and HOMA-IR was evaluated.

Results
Body mass index (BMI) and homeostatic model assessment-insulin resistance indexes (HOMA-IRs) were significantly higher in women than in men. Distribution of allelic frequencies of all polymorphisms tested agreed with the 1000 Genomes Project and the International HapMap Project for Mexican-Americans. The five polymorphisms were in Hardy-Weinberg equilibrium, and the association analysis of genotype frequencies with the diabetes-related traits displayed significance in PPARGC1A (rs8192678) for glucose (p=0.023) and triglycerides (p=0.013); VEGFA (rs2010963) with diastolic blood pressure (DBP) (p=0.012), cholesterol (p=0.013) and HDL (p=0.0007); SIRT1 (rs7896005) for DBP (p=0.012) and insulin (p=0.01); and UCP2 (rs659366) for cholesterol (p=0.034), glucose (p=0.031) and triglycerides (p=0.028). Linear regression performed with three models of inheritance, adjusted by Age + Sex + BMI and corrected with the Bonferroni method showed significant association of rs2010963 with HDL in an additive model (p=0.007); and rs7896005 was significantly associated with DBP in the recessive model (p=0.006).

Conclusions
These results support VEGFA and SIRT1 association with cardiovascular traits in patients with T2D.

Keywords:

single nucleotide polymorphisms, high-density lipoproteins, diastolic pressure, Mexicans, type 2 diabetes mellitus