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The APOE ε4 Allele Association with Cholesterol Related Phenotypes in the Mexican American Population

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THE *APOE* ALLELE ε4 ASSOCIATION WITH CHOLESTEROL RELATED PHENOTYPES IN THE MEXICAN AMERICAN POPULATION

A Thesis

by

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ABSTRACT

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Diseases linked to high cholesterol have been well studied and several diseases-associated candidate genes have been suggested. Among these, the apolipoprotein E gene (*APOE*) gene associated with lipid metabolism and lipid-related traits was reported in the non-Hispanic population. There are three types of alleles *APOE* ε2, ε3, and ε4. The *APOE* ε4 allele has been identified as a biomarker for Alzheimer's disease (AD) and cardiovascular disease (CVD). In recent years, there have been limited studies regarding the association between the *APOE* gene and hypercholesterolemia in the Mexican American population. Therefore, the aim of this study is to investigate the frequencies of the *APOE* alleles and study their relationship with cholesterol levels and lipid-related traits in the Mexican American population. For the first time, we have demonstrated that *APOE* ε4 allele is associated with hypercholesterolemia in the Mexican American population. Further research is required to confirm the support of our current findings.

DEDICATION

Dedicated to my family, Jesus Alen Lozano, Cindia M. Lozano, and Ricardo Lozano and boyfriend. Everyone has helped me through this journey and wouldn't have been able to do it without everyone. Thank you to everyone who believed in me.

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CHAPTER I

INTRODUCTION

Gaps in Scientific Research

There is plenty of scientific research that has been explored in non-Hispanic/Latino Whites, however, there is a lack of diversity and inclusivity in clinical or translational research involving the Latino/Hispanic population, such as the Mexican American (MA) population. As of 2016 the Hispanic population has accounted for 18% or approximately 57.4 million of the nation's population since 1970 and has increased to approximately 60.4 in 2019, making it one of the fastest growing populations in the United States (Bustamante et al., 2020; Flores, 2017). One of the major issues is that the Hispanic/Latino population is categorized into one large group when there are sub-populations. An example of this is present in the information provided by the U.S. Census Bureau. The U.S Office of Management of Budget (OMB) used the term Hispanic and Latinos disregarding the specific race such as Cuban, Mexican, Puerto Rican, etc., (US Census Bureau, 2022).

The NIH Revitalization Act of 1993 was signed to include women and minorities in clinical research, but recruitment was still an issue. A couple of studies in the 1990s reported the difficulty in recruiting for intervention research and trying to find a plan that would effectively include Latinas consisted of a lot of flexibility and a well-thought-out plan (Naranjo & Dirksen, 1998; Pletsch et al., 1995).

In a previous study, a problem that researchers faced with the recruitment of Hispanic women was their high value in their culture and familism, and these beliefs and values prevented them from participating (Naranjo & Dirksen, 1998). Hispanic culture, values, and family are held to a high standard, therefore, traveling for a study was not an option for them. *Machismo* known as the social-cultural scripts of a male and female in the Hispanic community includes the belief that a woman should remain in their traditional role and that males have more dominance and power over them (Nuñez et al., 2016). There have been several factors observed that impact the participation of the Hispanic/Latinos population, these factors include: *machismo*, immigration status, fear of losing any government assistance, and other fears they have if they participate in research studies (Amaro, 1988; Arevalo et al., 2016; Naranjo & Dirksen, 1998; Nuñez et al., 2016).

Moreover, there is a lack of biomedical research reports among minorities, specifically the Latino population (Ceballos et al., 2014). In addition, limited studies on cholesterol-related phenotypes and genes were observed in the Hispanic/Latino as compared to the non-Hispanic population (see Figure 1). With limited Hispanic/Latino representation in basic research and clinical studies, more specifically in the Mexican American population, reports with a limited sample size might result in false positive or negative findings (Button et al., 2013). The Hispanics/Latinos are a genetically diverse population making their genotyping essential for improving our knowledge of diseases and treatment (Conomos et al., 2016; Lu et al., 2014). Therefore, one of the aims of this study is to help close the research gaps of the apolipoprotein E (*APOE*) ε4 allele in association with cholesterol-related phenotypes in Mexican Americans.

Statement of Purpose

Elevated cholesterol level was associated with an increased risk of all-cause mortality, such as Cardiovascular Disease (CVD) (Zhang et al., 2019). Hypercholesterolemia is defined as high plasma cholesterol levels along with the normal plasma triglycerides (TG) in the blood (Martinez-Hervas & Ascaso, 2019). Health conditions such as diabetes, CVD, hypertension, and AD have all been associated with hypercholesterolemia (Ibrahim et al., 2022; Mahley, 2016; Rhee et al., 2017). Data from 2005 to 2008 obtained by the National Health and Nutrition Examination Survey, Mexican Americans ages 20 and over demonstrated high total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) (Mozaffarian et al., 2015; Rodriguez et al., 2015). A previous study containing a population-based cohort of 16,415 U.S Hispanics/Latinos supported that this population had a higher prevalence of abnormally elevated cholesterol or fats in the blood (Rodriguez, Daviglus, et al., 2014). There have been multiple genes associated with the abnormal lipid and/or lipid-related phenotypes; among these candidate genes, *APOE* has been well observed in both Hispanics and non-Hispanics (Chang et al., 2010; Lozano et al., 2021; Satizabal et al., 2018).

The *APOE* gene plays different important roles in lipoprotein metabolism, it has shown to be a connection with the maintenance of homeostasis of cholesterol and responsible for the transportation of lipids (Mahley, 2016). There are three alleles attributed to the *APOE* gene: ε2, ε3, and ε4. *APOE* ε3 allele has been studied and identified as a biomarker for several health conditions such as Alzheimer's disease (AD), CVD, and hypercholesterolemia in Hispanics and non-Hispanic population (Lozano et al., 2021; Mahley, 2016; Yamazaki et al., 2016). Previous studies have shown that the *APOE* ε4 allele frequency is higher in Central Africa with 40%, Oceania coming second with 37%, Africa American at less frequency with 31%, and lastly

Australia with less than 30% (Corbo & Scacchi, 1999; Husain et al., 2021). However, much lower levels (less than 10%) were found in the Mediterranean and China populations (Egert et al., 2012; Hu et al., 2011; Husain et al., 2021).

As of July 3, 2022, there have been limited studies regarding the *APOE* ε4 allele and its association with cholesterol-related phenotypes in the Hispanic Population found in PubMed. In addition, when searching keywords on PubMed such as *APOE* ε4 in Mexican Americans or APOE Cholesterol in Mexican Americans there was zero to one article containing either or keywords. Therefore, the purpose of this research is to identify and investigate the association between the *APOE* ε4 allele and cholesterol-related phenotypes in the Mexican American population. As a result, our expectations are to be able to close the gaps in *APOE* allele status and its associations with cholesterol-related phenotypes in the Mexican American population.

CHAPTER II

LITERATURE REVIEW

Cholesterol

What is Cholesterol? Cholesterol is the byproduct of the liver and can also be obtained from the consumption of animals. Cholesterol circulates throughout the blood, and as the levels of cholesterol increase so does the risk of developing cardiovascular disease (American Heart Association, 2020). There are two types of cholesterol made in the body; Low-density lipoprotein (LDL) considered to be the "bad cholesterol," and High-density lipoprotein (HDL) described as "good cholesterol." LDL causes blockages in the arteries whereas HDL's function is to carry the "bad cholesterol" away from the arteries (American Heart Association, 2020). Triglycerides (TG) is understood to be the most common type of fat and are found in the body, high levels of TG, LDL, and a low level of HDL in the blood are all risk factors for CVD (American Heart Association, 2020). A previous study demonstrated plasma lipid differences between non-Hispanic Whites, non-Hispanic Blacks, and Hispanics, with Hispanics having a higher TG/HDL-C (Willey et al., 2011). Limited studies have been observed in the Hispanic/Latino population.

Hypercholesterolemia has contributed to other diseases such as CVD, stroke, Alzheimer's disease (AD), and type 2 diabetes (T2DM**)** (Rhee et al., 2017; Satizabal et al., 2018; Yaghi & Elkind, 2015)**.** Therefore, it is important to understand the relationship between

hypercholesterolemia and other chronic health conditions (diabetes, thyroid problems, hypertension, or obesity) and its possible association with the AD-associated *APOE* ε4 allele. A previous study consisting of 16,415 US Hispanic/Latinos in collaboration with the Hispanic Community Health Study/Study of Latino (HCHS-SOL) concluded that dyslipidemia was highly prevalent in the Hispanic/Latino with Cubans (69.8%), being at a much higher risk followed by Central Americans (68.8%) and Mexicans (64.8%) (Rodriguez, Daviglus, et al., 2014).

A report from the Center for Disease Control and Prevention (CDC) reported that from 2015 to 2016 Hispanic men approximately 13.1% had a higher percentage of total high cholesterol (240 mg/dl or more) than non-Hispanic Black men (10.6%), non-Hispanics White men (10.9%) and Asian men. Conversely, Hispanic women had a lower percentage (9.0%) of total High Cholesterol (HC), than Non-Hispanic Black women (10.3%) (*High Cholesterol Facts*, 2021; Virani et al., 2021). Dyslipidemia was demonstrated to be highly prevalent in two-thirds of the HCHS/SOL population especially those above the age of 40 and older and in women than males (Rodriguez, Daviglus, et al., 2014). Their results further explained that age, gender, higher BMI, and low physical activity influence dyslipidemia among all Hispanics and across background groups (Dominicans, Central Americans, Cubans, Mexicans, Puerto Ricans, and South Americans) (Rodriguez, Daviglus, et al., 2014).

Furthermore, the Multiethnic Study of Atherosclerosis (MESA) reported that Mexican Americans had a higher prevalence of dyslipidemia along with the frequency of metabolic syndrome and an elevated risk for atherosclerosis (Allison et al., 2008; Rodriguez, Allison, et al., 2014)(Rodriguez, Allison, et al., 2014). Therefore, it is necessary to further understand if high cholesterol levels have effects on the following chronic disease or traits.

There are many cholesterol-associated disorders and/or phenotypes including following diabetes, Alzheimer's disease, hypertension, and thyroid disease.

Diabetes

Diabetes mellitus is described as a group of metabolic diseases that causes problems with the function of glucose in the body. There are two types of diabetes mellitus, type 1, and type 2. Type 1 is known to be autoimmune, caused by the destruction of the beta cells in the pancreas affecting mainly children (Daneman, 2006; Kharroubi & Darwish, 2015). While type 2 diabetes (T2DM) is commonly found in adults causing insulin resistance and elevated glucose in the blood (Kharroubi & Darwish, 2015). In addition, data reports from 2016 demonstrated that less than 1% of U.S adults had type 1 diabetes accounting for 1.3 million adults while (T2DM) had a much higher rate of about 9% accounting for approximately 21.0 million adults (Bullard et al., 2018). Information provided by the CDC reported that Hispanic/Latino (17%) are at a much higher risk of developing type 2 diabetes than non-Hispanic whites (8%) (*Hispanic or Latino People and Type 2 Diabetes*, 2022). A study done on the Mexican population reported that from the years1998 to 2002 there was an increase in the mortality rate of T2DM in Mexico an estimated to grow exponentially (Villalpando et al., 2010). With the number of individuals with diabetes increasing rapidly, diabetes has reached to be an epidemic (Zimmet, 2017).

There are many factors contributing to T2DM, some of these factors include obesity, age, weight, genetics, and dyslipidemia (Seo et al., 2011; Wada et al., 2016). A cross-sectional study concluded that subjects with high serum total cholesterol (TC) levels were correlated with a decrease in insulin secretory capacity, which was considered a risk factor for the onset of diabetes in the Japanese population (Wada et al., 2016). A study involving the Hispanic/Latino

population found that adults with a high risk of developing high cholesterol were those with hypertension and diabetes (Rodriguez et al., 2015).

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is known to be the most common type of dementia, according to the Alzheimer's Association, there are more than 6 million individuals living with AD (*Alzheimer's Facts and Figures Report | Alzheimer's Association*, 2022). In addition, according to the Alzheimer's Association Facts and Figures, death rates have also increased by 145% from the years 2000 to 2019 (*Alzheimer's Facts and Figures Report | Alzheimer's Association*, 2022). AD has been characterized by abundant levels of amyloid beta (Aβ) plaque along with hyperphosphorylated tau tangles in the brain (Feringa & van der Kant, 2021; Rajmohan & Reddy, 2017). Individuals that have developed AD at a younger age (less than 65 years) have been referred to have early onset AD (EOAD) (Feringa & van der Kant, 2021).

High levels of cholesterol and aging along with genetic biomarkers (e.g., *APOE* ε4) have been demonstrated to be a risk factor for the elevated plaque in the brain, essentially responsible for EOAD. A study done with 400 women from Guerrero, Mexico concluded that women with an expression of the *APOE* ε4 allelic variant had higher levels of LDL, and an increase in CVD (Cahua-Pablo et al., 2016). A previous study in rats containing both young (4 month old) and older rats (14 month old) revealed a negative effect on memory performance when provided with a high fat/high cholesterol diet, there was also an indication of an increase in the hippocampal p-Tau levels in those at an older age (Ledreux et al., 2016). In addition, a study conducted in rabbits containing a control group and high cholesterol diet group demonstrated that those rabbits that were fed 2% (high cholesterol diet group) had an overall different structured brain compared to the control group, those who had a normal routine diet (Jin et al., 2018). The changes that

were present in the brain, were similar to those found in humans with AD (Jin et al., 2018). Until now, there is no study of cholesterol associated with AD as well as AD-associated *APOE* gene associated with hyper cholesterol levels in the Mexican American Population.

Hypertension

Hypertension among the heterogeneous/diverse Hispanic/Latino population has not thoroughly been looked into, with limited information on this population (Elfassy et al., 2020). U.S Hispanics/Latinos with a Caribbean background had a higher incidence rate for hypertension (Elfassy et al., 2020). Furthermore, a study consisting of 36,000 participants demonstrated that young adults who have exposures to elevated diastolic blood pressure (DBP), systolic blood pressure (SBP), or both and raised levels of LDL were associated with a higher risk for a CVD (Zhang et al., 2019). In addition, a study with 4,680 participants from 17 population samples (Japan, Peoples Republic of China (PRC), United Kingdom (UK), and the USA) found that dietary cholesterol was directly related to SBP, and furthermore concluded that a reduction in dietary cholesterol intake along with other nutritional changes could help control BP levels in the general population (Sakurai et al., 2011). So far, there has been no report of the AD-associated *APOE* gene in relation to hypertension in the Mexican American population in PubMed.

Thyroid Disease

Thyroid disease includes a variety of diseases in the thyroid such as hyperthyroidism hypothyroidism, thyroiditis, or enlargement of the thyroid gland known as goiter. Thyroid disease and its association with cholesterol have been studied since the 1930s (MASON et al., 1930). A study consisting of 8,795 participants from various races/ethnicities discovered that 49.5% of those with hyperlipidemia (5.2%) that were previously screened had elevated levels of thyroid stimulating hormone (TSH) (Duntas & Brenta, 2018; Willard et al., 2014). Further

demonstrates that thyroid dysfunction has negative effects on lipid metabolism leading to hypercholesterolemia (Duntas & Brenta, 2018). Furthermore, no previous study has been reported on the AD-associated *APOE* gene in connection with thyroid disease in Mexican Americans.

AD associated - *APOE* Gene

Among all cholesterol-associated genes, our interest is in the apolipoprotein E (APOE) found in chromosome 19q13.32. APOE plays a role in the regulation of lipid metabolism and provides regulation of plasma cholesterol equilibrium in the body (de Chaves & Narayanaswami, 2008). It is a glycoprotein containing 299 amino acids and synthesized and secreted in several tissues including the brain and liver (Getz & Reardon, 2009; Mahley & Rall, 2000). APOE participates in many other functions such as in the oxidative process, inflammation, and the central nervous system physiology (Mahley & Rall, 2000; Raghavachari, 2020). It is part of the chylomicrons, very low-density lipoproteins (VLDL), and intermediate-density lipoprotein (IDL) (Khalil et al., 2021). Interestingly, APOE has been thought to be protective against the buildup of plaque by promoting a process known as cholesterol efflux (Getz & Reardon, 2009; Liu et al., 2013).

The three alleles for *APOE* are ε2, ε3, and ε4. In a previous review, the frequencies of these alleles are as followed: ε2 (7%), ε3 (78%), and, ε4 (15%) (de Chaves & Narayanaswami, 2008). However, a study among the Hispanic/Latino population reported the following allele frequency for Mexicans: ε2 (2.86%), ε3 (86.16%), and ε4 (10.98%) further demonstrating that Dominicans had the highest frequency for *APOE* ε4 allele (17.45%) (González et al., 2018). The six genotypes found in the human subjects were the following homozygous phenotypes: ε4/ ε4, ε2/2, and ε3/ ε3, and heterozygote phenotypes: ε4/3, ε3/2, ε4/2 (Mahley & Rall, 2000). The

difference between these isoforms is the location of cysteine and arginine in residues 112 and 158 (Mahley & Rall, 2000). For example, ε2/2 is cysteine for both residues 112 and 158, ε3/3 has cysteine for residue 112 and arginine for residue 158, and lastly, ε4/4 contains arginine in both residues 112 and 158 (Liu et al., 2013; Mahley & Rall, 2000). *APOE* ε2 allele is known to be an uncommon allele, unlike the ε3 allele which is the most common type.

APOE ε4 allele has been demonstrated to be a high-risk factor for neurodegenerative disorders and CVD (Feringa & van der Kant, 2021; Huang et al., 2004; Mahley, 2016). Carrying one or more *APOE* ε4 alleles heightens the risk for hypercholesterolemia as well as the development of AD (de Chaves & Narayanaswami, 2008). Likewise, individuals with the genotype *APOE* ε3/ε4 and ε4/ε4 were associated with elevated levels of LDL-C and blood pressure (Garcia et al., 2021; Shi et al., 2018). While the *APOE* ε2 allele has been observed as the protective allele in AD (Husain et al., 2021). However, the *APOE* ε2 allele has not been found to be completely harmless as stated in a previous article (Li et al., 2020). In addition, individuals with homozygous *APOE* ε2 alleles were shown to be at a higher risk for the development of familial type III hyperlipoproteinemia and premature atherosclerosis (de Chaves & Narayanaswami, 2008; Khalil et al., 2021).

With limited studies reported on the Mexican American population, and due to their genetic diversity, it is essential to determine if the *APOE* ε4 allele is associated with elevated-cholesterol related phenotypes, such as hypercholesterolemia. Furthermore, in this study, we further discuss the association of the *APOE* ε4 allele with hypercholesterolemia within the Mexican American population (Lozano et al., 2021).

CHAPTER III

METHODS

Methods

The collection of data (N=1,382) presented in this study is an analysis of the data collected by the Texas Alzheimer's Research and Care Consortium (TARCC, N=1,320) in combination with our own data, the Initial Study of Longevity and Dementia from the Rio Grande Valley (ISLD-RGV, N=62). Since over 90% of our studied population is Mexican American, the terms Mexican American/Hispanic/Latino were used interchangeably unless the Hispanic/Latino term was used in publications. This data is similarly presented in our recent publication (Lozano et al., 2021). The studies were approved by the corresponding Institutional Ethics Committees and Institutional Review Boards. Written consent was obtained from our participants or their legally authorized proxies before any of the data was collected as previous studies demonstrated (Grady, 2015; McMillan, 2020) and UTRGV- the Office of Sponsored Program (OSP) as one of our reports (Xu et al., 2021).

Our first collection of data was from the ISLD-RGV, the Mexican American subjects were matched by their demographic information (e.g., age, gender, and ethnicity). Figure 2 demonstrates the project workflow of the ISLD-RGV recruitment (some of the content in Figure 2 is still part of our ongoing study). Subjects with hypercholesterolemia, diabetes, or other chronic disorders, and healthy subjects were recruited from the Rio Grande Valley (RGV). The

RGV includes areas throughout Cameron, Hidalgo, Starr, and Willacy Counties located in Texas. The recruitment took approximately a year, and our participants were recruited from local adult daycare centers and communities. The participants were asked to answer questionnaires that were provided either in English or Spanish during the interview process. These questionnaires included information about the lifestyle of the participants (Godwin et al., 2008), medical history modified, and questions regarding familism (Steidel & Contreras, 2003). Signed letters from several independent adult day care centers were obtained, permitting us access to the facility for recruitment. The recruitment took place in the RGV and was conducted by Kimberly Moreno and Victoria Padilla. In addition, saliva samples were obtained for genetic study. Unfortunately, due to COVID-19, recruitment was slowed down, and measures of precaution were taken.

The Texas Alzheimer's Research and Care Consortium provided us with their collected data. Previous publications have demonstrated in details the collection of TARCC's data and further information on this diverse population (O'Bryant et al., 2010; Royall & Palmer, 2019). The participants of TARCC had to go through several examinations, including medical evaluation and further clinical interviews.

Furthermore, once the information was collected and we combined the data from both TARCC and ISLD-RGV, including demographic information, medical history. In terms of diseases from the combined data, questions of medical history were originally coded as 0 (absent of disease) and 1 (present of diseases). An example of this is if a subject had the presence or absence of a phenotype such as hypercholesterolemia, a score of 0 or 1 was given. Other demographic information was also coded similarly. All diagnoses were based on either self-report and/or standardized enzymatic procedures. In addition, those with serum cholesterol levels of above 220mg/dL were defined to have hypercholesterolemia.

DNA Isolation and Genotyping

The saliva sample was collected to better study the genetic determinants such as *APOE* genotypes and alleles. The collection of DNA was done by two different methods, blood samples, and saliva samples. TARCC provided us with the blood sample data from their 1,320 participants, as for ISLD-RGV, 62 saliva samples were collected from our participants. Previous studies further explain TARCC's DNA isolation and genotype, for detail, please see (Hall et al., 2012; Royall & Palmer, 2019).

For ISLD-RGV, consent forms were collected before saliva samples were taken from participants. Each participant provided us with approximately 2 ml of saliva. To collect saliva samples, we used a self-collecting kit Oragene DISCOVER (OGR-500) (DNA Genotek, Ottawa, ON, Canada), from the company DNA Genotek Collection (*DNA Genotek - DNA Saliva Collection - Research - Oragene OGR-500*, n.d.). The samples then were stored at 4°C to be ready for DNA extraction and isolation. The DNA isolation was performed using the protocol described in DNA Genotek PrepIT®•L2P (DNA Genotek, Ottawa, ON, Canada).

TaqMan single nucleotide polymorphism (SNPs) assays were used for the genotyping of ISLD-RGV using SNPs rs7412 and rs429358. Conversely, the *APOE* genotypes for TARCC were based on the data of Affymetrix Genome-Wide Human SNP Array 6.0 (Texas Alzheimer's Research and Care Consortium, n.d.). Next, *APOE* genotypes were grouped by the absence of alleles (ε2–, ε3–, and ε4–) or their presence (ε2+,ε3+, and ε4+). Data was also present as heterozygous (participants carrying different alleles) or as homozygous (participants carrying the same alleles). A total of 1,320 participants out of 1,382 were *APOE* genotyped, we are currently still working on genotyping the remaining samples.

Statistical Analysis

Statistical analysis was then performed according to *APOE* ε2, ε3, and ε4 carriers, noted as ε2+, ε3+, and ε4+ and non-carriers noted as ε2-, ε3-, and ε4-. Some of the variables that were used to conduct the statical analysis were BMI, age, education, hypercholesterolemia, etc., in our population. For statistical analysis to be performed we used the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM®SPSS, Chicago, IL USA). With SPSS a two-tailed *t*-test and x^2 analysis were used to determine the potential risk factors. Statistical significance was demonstrated with a $\boldsymbol{p < 0.05}$. Multivariable logistic regression was used to adjust for all the potential risk factors (e.g., sex, age, and education). This was to determine if the *APOE* ε4 allele was independently associated with any of the phenotypes that were being tested such as cholesterol-related traits. The odds ratio (OR) was used to determine the risk value between the *APOE* ε4 allele and the phenotypes. From our previous study, including (Xu et al., 2009) a power analysis was conducted using the Genetic Power Calculator, resulting in a 94% power based on the total sample of subjects (Purcell et al., 2003) (https://zzz.bwh.harvard.edu/gpc/ (accessed on November 2021)).

CHAPTER IV

RESULTS

The results present in this study have previously been published in our current manuscript (Lozano et al., 2021). This study consisted of a total of 1,382 participants, out of those only 1,320 samples were genotyped with the *APOE* gene.

A total of 860 (65.2%) participants presented with high cholesterol while 460 (34.8%) of the participants represent the non-hypercholesterolemia group. Table 1 demonstrates the demographic characteristics of those participants with hypercholesterolemia and those with non-hypercholesterolemia. This consists of information regarding age, gender, BMI, and other cholesterol-related phenotypes. In our published study, there was a total of 589 (68.5%) females and 271 (31.5%) males with high levels of cholesterol. The statistical significance observed in this table are age, hypertension, thyroid disease, and diabetes. The mean age of those with hypercholesterolemia is 70.41 ± 9.12 years which was noted to be statistically significantly older than those without hypercholesterolemia 67.20 ± 9.85 (***p* <0.05**). Those with hypercholesterolemia were at a much higher risk for hypertension, thyroid disease, and diabetes (***p* <0.05**). As for gender, BMI, and education, there was no statistical significance between those with hypercholesterolemia and those without hypercholesterolemia.

As aforementioned, the study consisted of a total of 1,382 Hispanic participants total, with over 90% of the participants being Mexican American. After further dividing the data

between females and males, we discovered that the majority of our participants were female (69.8%) than males (30.2). The frequencies of the *APOE* alleles consisted of the following *APOE* ε2 (3.3%), ε3 (83.9%), and ε4 (12.8%) as seen in Table 2. A demonstration of similar frequencies among the Hispanic population was observed in previous reports (González et al., 2018; Texas Alzheimer's Research and Care Consortium, n.d.).

Table 3 demonstrates the *APOE* ε4 allele status on demographics and cholesterol-related phenotypes in our studied Mexican American population. There were 1042 subjects without the *APOE* ε4 allele (78.9%) and 278 subjects with at least one *APOE* ε4 allele present (21.1%). In this table, those with at least one *APOE* ε4 allele showed statistical significance in age and hypercholesterolemia. The mean age of those with the *APOE* ε4 allele present was 70.75 ± 9.45, older than those without the *APOE* ε4 allele (**p <0.03**). There were 211 subjects (24.5%) with at least one *APOE* ε4 allele and hypercholesterolemia, and 67 (14.6%) subjects with the allele present but without hypercholesterolemia, making it statistically significant compared to those without the allele present (**p <0.005**).

In this study, table 4 represents a multivariable logistic regression analysis conducted to further confirm our results. After controlling any of the confounding variables (e.g., gender, education, and *APOE* ε allele), there was a statistically significant association present between *APOE* ε4 allele and hypercholesterolemia **(p <0.005**). The group sample with hypercholesterolemia consisted of 860 participants and those without high cholesterol levels were 460. The odds ratio represented in our current publication was 1.9-fold higher (1.36-2.67) at a 95% confidence interval compared to those without the *APOE* ε4- allele (Lozano et al., 2021). There was no association found between the *APOE* ε3 allele, BMI, and education and hypercholesterolemia as reported in table 4.

CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

Overall, this study demonstrates the association between the *APOE* ε4 allele and high cholesterol levels in Mexican American population after controlling the predictor (gender, BMI, *APOE* ε allele, and education). The findings of this study have recently been published in the Journal Genes by MDPI. In our studied population, *APOE* allele distributions are as follows *APOE* ε2 (3.34%), *APOE* ε3 allele (83.90%), and *APOE* ε4 allele (12.76%), which have been demonstrated similar allele distributions in previous studies in Hispanics (Blue et al., 2019; González et al., 2018).

In terms of *APOE* ε4 in association with high cholesterol levels, a study conducted in the Algerian population, as a non-Hispanic population has also demonstrated that *APOE* ε4 is a risk for hypercholesterolemia (Boulenouar et al., 2013). In addition, a case-control study illustrated that participants residing in Valencia, Spain who were carriers of at least one *APOE* ε4 allele were at a higher risk of developing hypercholesterolemia (Corella et al., 2000).

A study that consisted of 1,997 Mexican Amerindians (MA) participants, demonstrated partial support for our results (Martínez-Magaña et al., 2019). MA participants carrying an *APOE* ε4 allele demonstrated to have higher cholesterol levels (Martínez-Magaña et al., 2019),

which is similar to our findings, although our study's main focus is on TC and its association with hypercholesterolemia (Lozano et al., 2021). A study done on the Kashmiri population demonstrated that patients with cardiovascular disease (CAD) who carried an *APOE* ε4 allele had notable high levels of total cholesterol (TC) and LDL, further demonstrating the significant association between this allele and cholesterol levels (Afroze et al., 2016). Overall, *APOE* ε4-associated elevated cholesterol was demonstrated among several ethnic populations.

As aforementioned, the *APOE* ε3 allele is a neutral allele, there was no association between the *APOE* ε3 allele and hypercholesterolemia, obesity, or cardiovascular diseases in the Hispanic population (de Chaves & Narayanaswami, 2008; Lumsden et al., 2020) and in our current study (Lozano et al., 2021).

In terms of the disease protective *APOE* ε2 allele, several studies have explained that the *APOE* ε2 allele is considered a protective allele. *APOE* ε2 is able to lower the risk of developing CVD by lowering the levels of LDL (Anuurad et al., 2006; Wolters et al., 2019). Furthermore, in our published study, we observed no association between the *APOE* ε2 allele and cholesterol-related phenotypes in our studied population (Lozano et al., 2021).

We are aware of some strengths and limitations of this study. One of the strengths of our study is that it has been the first report to demonstrate a significant association between *APOE* ε4 allele and hypercholesterolemia in Mexican American population (Lozano et al., 2021). The study presented had several limitations, first limitation consisted of the sample size. Our current study has a total of 1,382 participants, making this sample size considerably small. In addition, our statistical power was high (94%). Since our study sample was limited our statistical power can decrease leading us to possibly obtain type II error (Button et al., 2013; Smith et al., 2002), in the future we would require a much bigger sample size for more dependable results. Our

second limitation is that the Hispanic population is extremely diverse, and the term Hispanic is used as a way to combine all the Hispanic ethnicities in one term (US Census Bureau, 2022). Previous studies have shown the Hispanic population is diverse, this diversity can include things such as beliefs, culture, genetics, and much more (Conomos et al., 2016).

Our future direction includes increasing the sample size of our study, we have currently collected approximately 83 more participants from the RGV, bringing the total to 145 samples and counting. We plan on continuing to analyze the existing data to know and further understand how the variations in health, lifestyle, and familism are correlated with cholesterol-related phenotypes and other traits. Once there is an understanding of the known genetic variants and their interactions with lifestyles and familism we can go further by genotyping the new recruiting subjects with *APOE* gene, other candidate genes, or Whole Genome Sequencing (WGS)/Whole Exome Sequencing (WES).

Conclusion

Our current study focuses on how the AD-related *APOE* gene is associated with socio-demographic information (e.g., sex, age, and education) and high cholesterol-related phenotypes among the Mexican American population. As a result, we have demonstrated that the *APOE* ε4 allele is associated with hypercholesterolemia in Mexican Americans. In our study, we have also determined *APOE* allele distributions which are like previous reports in Mexican American, or Hispanic/Latino populations. In addition, there was no association found between *APOE* ε2 or *APOE* ε3 alleles in cholesterol-related traits in this specific population. One of our main goals of this study is to bring light to the understudied Hispanic/Latino population. We expect that our current findings will offer further education in the understanding of genetics and cholesterol-related phenotypes.

Taken together, our findings highlight *APOE* ε4 carriers tend to have impaired cholesterol metabolism in Mexican Americans. In the future, a better understanding of the function of *APOE*-associated cholesterol can contribute to the development of novel therapeutic strategies for patients with elevated cholesterol levels. Further research on the Mexican American population is essential to confirm and support our findings.

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APPENDIX

APPENDIX

Figures

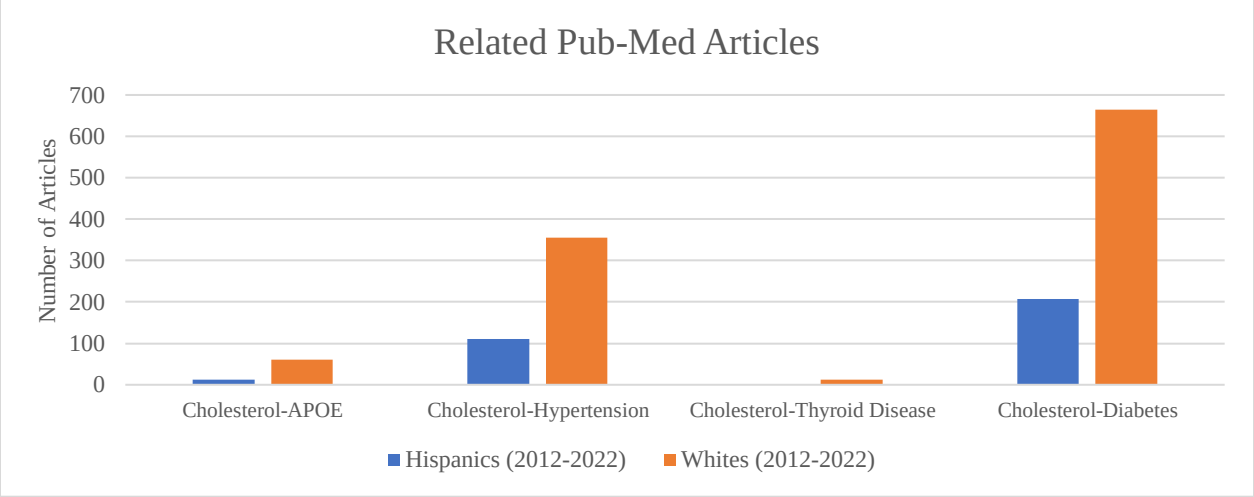

Figure 1: Publications of Cholesterol Related Issues between Hispanics and White

Keywords such as *APOE* association with Cholesterol disease, in the Hispanic and White population were used in the search engine PubMed. Filters like a timeline (2012-2022), specific text availability (abstract), and species (human) were used to get the overall results of articles on PubMed.

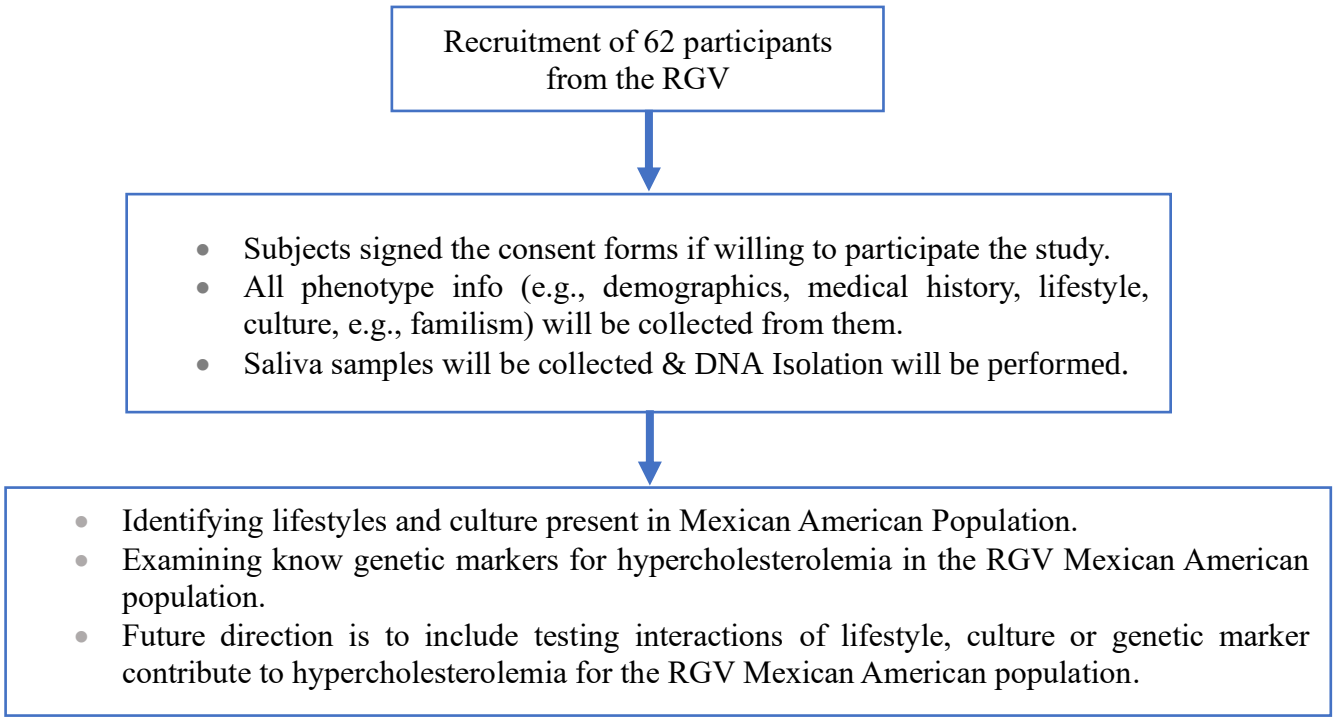

Figure 2: Project Workflow

Tables

Table 1: X^2 and t-test for Demographic and Health Characteristics in participants with Hypercholesterolemia vs non-Hypercholesterolemia.

	NON-HYPERCHOLESTEROLEMIA (N=460)	**HYPERCHOLESTEROLEMIA (N=860)**	***P* VALUE**
AGE (MEAN ± SD)	67.20 ± 9.85	70.41± 9.12	**< 0.001**
MALES (N, %) N=399	128 (32.08%)	271 (67.92%)	0.165
FEMALES (N, %) N=921	332 (36.05%)	589 (63.95%)	
BMI (MEAN ± SD)	30.37± 7.23	30.35± 6.24	0.956
HYPERTENSION (N, %) N=874	231 (26.43%)	643 (73.57%)	**< 0.001**
THYROID (N, %) N=273	73 (26.74%)	200 (73.26%)	**0.002**
DIABETES (N, %) N=489	93 (19.02%)	396 (80.98%)	**< 0.001**
EDUCATION (MEAN ± SD)	9.74± 4.66	10.45± 4.62	0.518

Body Mass Index (BMI), Standard Deviation (SD).

Table 2: Frequency of *APOE* Alleles in the Studied Mexican Americans.

***APOE* ε2**	***APOE* ε3**	***APOE* ε4**
3.3%	83.9%	12.8%

APOE ε2, ε3 or ε4: Carrying at least one copy of *APOE* alleles.

Table 3: *APOE* ε4 Allele Status on Demographics and Cholesterol-related phenotypes in the Mexican Americans population.

	***APOE* ε4 – (N=1042)**	***APOE* ε4+ (N=278)**	***P* value**
Age (mean ± SD)	69.33± 9.40	70.75± 9.45	**0.027**
Females (N, %) N=921	731 (79.37%)	190 (20.63%)	0.624
Males (N, %) N=399	311 (77.94%)	88 (22.06%)	
Education (mean ± SD)	10.17 ± 4.62	10.41 ± 4.74	0.236
BMI (mean ± SD)	30.44 ± 6.62	29.61 ± 6.35	0.064
Hypercholesterolemia (N, %) N=860	649 (75.47%)	211 (24.53%)	**< 0.001**
Hypertension (N, %) N=832	640 (76.92%)	192 (23.08%)	0.564
Diabetes (N, %) N=459	365 (79.52%)	94 (20.48%)	0.170
Obese (N, %) N=112	88 (78.57%)	24 (21.43%)	0.639

APOE ε4- represents absent of allele. *APOE* ε4+ represents present of at least one ε4 allele. Body Mass Index (BMI), SD (Standard Deviation).

Table 4: Logistic Regression between Hypercholesterolemia and APOE ε4

	Hypercholesterolemia group (N=860) vs. Non-Hypercholesterolemia group (N=460)	
	OR (95%CI)	***P* value**
Sex	0.86 (0.65, 1.13)	0.28
BMI	1.00 (0.99, 1.02)	0.53
***APOE* ε3+**	1.34 (0.59, 3.06)	0.484
***APOE* ε4+**	1.90 (1.36, 2.67)	**< 0.001**
Education	0.94 (0.75, 1.19)	0.61

Body Mass Index (BMI), Odd Ration (OR), Confidence Interval (CI).

BIOGRAPHICAL SKETCH

Stephanie Lozano is a first-generation college student and the first one in her family to complete a graduate degree. Stephanie graduated from Roma High School in the Spring of 2015 and started her Bachelor's in Biomedical Science in the Fall of 2015. During her time at UTRGV Stephanie joined several organizations and started to work on different research projects under the mentorship of Dr. Chun Xu. Stephanie was awarded the ENGAGE Scholar and was part of the MBRS-RISE program (Minority Biomedical Research Support Program- Research Initiative for Scientific Enhancement). Under Dr. Xu's mentorship, she was able to have several publications and was able to present at several conferences. Stephanie graduated with her bachelors in the summer of 2019 and decided to work as a pharmacy technician and medical coordinator for about a year before pursuing her Master's in Biochemistry and Molecular Biology. Stephanie Lozano admired the hard work of Dr. Chun Xu and decided Dr. Xu was the best fit to be the chair of her thesis committee. Under the supervision of Dr. Chun Xu, Stephanie has been able to publish the work she and her lab team have gathered throughout the years. As a graduate of the master's program, Biochemistry and Molecular Biology from the University of Texas Rio Grande Valley in July 2022, Stephanie plans on continuing her education.

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