**Investigating Known Pathogenic Variants for Familial Hypercholesterolemia in a Samoan Population**

by

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Abstract

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**Abstract**

Familial Hypercholesterolemia (FH) is a genetic condition that makes it difficult for an individual to remove excess cholesterol from their blood. As a result, these individuals have elevated LDL-cholesterol levels and their risk of developing premature cardiovascular disease increases 20-fold. FH affects 1 in 250 people globally; however, 90% of cases remain undiagnosed. Although the prevalence of cardiovascular disease in Samoa is relatively high, the prevalence of FH and previously identified risk alleles for FH in Samoa are unknown. FH is treatable and, in other countries, implementation of screening programs has identified at-risk individuals and enabled them to receive lipid-lowering medications. No studies have been done to estimate the prevalence of FH risk alleles and subsequently, the usefulness of a screening program.

In this study, I estimated the frequency of known FH pathogenic variants in three genes for FH (*LDLR*, *APOB*, and *PCSK9)* in 3475 Samoan adults, aged 24.5 – 65, from a population-based research study, and assessed whether these FH risk alleles were associated with LDL-cholesterol levels over 190 mg/dL—a hallmark of FH. In addition, I assessed the proportion of individuals with elevated LDL-cholesterol levels, as well as the effects of comorbidities, such as obesity, on LDL- and total cholesterol levels.

Six known pathogenic FH variants were present among the 3475 individuals in this study. However, individuals with four of these variants did not have elevated LDL-cholesterol levels of >190 mg/dL, although individuals who were heterozygous for variants *APOB* rs760832994 or *PCSK9* rs371488778 had LDL-cholesterol levels greater than 170 mg/dL. However, the mean of LDL-cholesterol levels among all participants was 130 mg/dL, which is above the recommended maximum of 100 mg/dL.

Finally, 135 individuals (3.9% of the participants) had LDL-cholesterol levels >190 mg/dL. Although other factors that contribute to high LDL-cholesterol levels must be taken into account, such as diet, exercise, and comorbidities like diabetes, this result is a good indication that at least one individual in this sample may have a previously unknown or novel pathogenic variant for FH based on the global prevalence of FH of 1 in 250 people. The public health significance of this study is that, based on these results, implementing a genetic screening program in Samoa for FH is not advisable at this time; however, because almost 4% of the population has elevated LDL-cholesterol levels, a screening program based off of cholesterol testing may be beneficial.

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Preface

I would like to thank the following individuals for whose help, support, and guidance were essential in the creation of this essay. To Dr. Candace Kammerer for her help in developing this project from a fledgling desire to write about familial hypercholesterolemia to a research project worth investigating and writing about, as well as all of her kind words, encouragement, patience, and guidance throughout the entire process. To Dr. Ryan Minster for providing me with all the necessary data from the Samoan project, helping untangle the dense information, and answering all of my questions. To Jacob Leisey-Bartsch for his statistical acumen and R-program prowess which greatly aided me during my data analysis and figure making.

Finally, to my family and friends who have supported me endlessly throughout my academic career and continue to be my biggest supporters and advocates. Without you all, none of this would have been possible. Thank you from the bottom of my heart.

# Cardiovascular Disease

Cardiovascular disease (CVD) is a group of diseases that affect both the heart and blood vessels and includes diseases, such as coronary artery disease and acute coronary syndrome (Sanchis-Gomar et al., 2016). The burden of CVD affects an estimated 128 million people worldwide (Dahlof, 2010). About 610,000 people in the United States (US) die every year from heart disease and it is the current leading cause of death in both men and women, accounting for one-third of all total deaths (CDC, 2017). The lifetime risk of developing CVD for those over 40 years old is 49% in men and 32% in women (Sanchis-Gomar et al., 2016).

As a result of the high incidence and prevalence of heart disease, the economic cost has been estimated to be $199.2 billion in healthcare costs and $130.5 billion in lost future productivity due to premature heart disease and mortality in 2013-2014. CVD accounted for 14% of healthcare expenditures in the US and was estimated to be higher than the expenditures for other diseases, such as cancer (Benjamin et al., 2018).

The risk factors for CVD include genetic susceptibility, hypertension, abnormal lipid profiles, obesity, smoking, stress, lack of physical activity, and poor diet. Many of these factors contribute to more than 90% of myocardial infarctions (MI), but are modifiable or preventable (Yusuf et al., 2004). Dahlof (2010) estimated that 70% of people have multiple risk factors which can interact to increase a person’s risk of CVD. Kurian and Cardarelli (2007) performed a systematic review of 13 studies that looked at different cardiovascular risk factors, and reported that Black Americans had a twofold higher prevalence of hypertension compared to White Americans. Both Black and Mexican Americans had an increased prevalence of diabetes compared to White Americans with an odds ratio (OR) of 2.39 and 3.24, respectively. With regards to smoking, these reviewers stated that smoking was lower in Mexican Americans compared to Black and White Americans, although Native Americans had a higher prevalence of smoking with an OR of 1.78 compared to White Americans (Kurian & Cardarelli, 2007).

Many treatments are available to help prevent or treat CVD by tackling different risk factors. CoA-reductase inhibitors, also known as statins, are an effective primary and secondary preventive measure to reduce high cholesterol levels by 60-70% (Borge G Nordestgaard et al., 2013) and subsequently reduce relative risk of overall mortality by approximately 13% (Briel, Nordmann, & Bucher, 2005; Mills et al., 2008). Other treatments for individuals with extremely high lipid levels (hyperlipidemia) include apheresis, which can decrease LDL-cholesterol levels by 50-75% (Borge G Nordestgaard et al., 2013). Many other treatments and surgeries are also available to help treat other risk factors or the presence of heart disease.

## The Pathology of Coronary Artery Disease

Coronary artery disease (CAD) is the most common type of CVD in the US (CDC, 2017). Atherosclerotic cardiovascular disease (ASCVD) and CAD occurs from atherosclerosis, which is the thickening and narrowing of arteries. The process of atherosclerosis begins in a person’s childhood and gradually progresses throughout a person’s lifetime (Insull, 2009). Atherosclerosis is difficult to detect in patients as they typically remain asymptomatic; however, patients with atherosclerosis and CAD typically have worse prognosis compared to those without in terms of surviving a myocardial infarction (Sanchis-Gomar et al., 2016).

The pathology results from the body’s immune response. As excess LDL-cholesterol leaves the blood and enters the intima, enzymes modify the LDL particles and become oxidized. The oxidized LDL particles send a signal to the body’s inflammatory response system to activate and secrete adhesion molecules and chemokines, which draws in immune cells, such as monocytes and neutrophils, into the intima. As the monocytes enter the intima, they mature into macrophages which take in the LDL particles and become foam cells. The accumulation of foam cells and debris forms a necrotic core, which is then enveloped by smooth muscle cells and collagen called the fibrous cap. Together, this creates plaques in the arterial walls of the intima which reduces blood flows. As a person ages, the fibrous cap of the plaque thins and weakens, which can lead to a rupture, resulting in thrombosis (blood clot), coronary artery disease, myocardial infarction, or sudden cardiac death (Insull, 2009; Weber & Noels, 2011).

The risk of atherosclerosis and CAD increases with the appearance of one or more risk factors such as hypertension, smoking, diabetes, hyperlipidemia, and genetic susceptibility. Treatments, such as statins, and preventative measures, such as lifestyle and behavioral modifications have been shown to reduce excess LDL-cholesterol in the blood and decrease the formation of plaques (Insull, 2009; Weber & Noels, 2011).

# Familial Hypercholesterolemia and CAD

Familial hypercholesterolemia (FH) is an autosomal dominant, genetic condition where excess low-density lipoprotein (LDL)-cholesterol is unable to be removed from blood. Therefore, the primary symptom of FH in adults is having LDL-cholesterol levels over 190 mg/dL, or total cholesterol levels over 310 mg/dL. Physical symptoms include xanthomas or corneal arcus. These physical symptoms occur as a result of excess cholesterol being deposited in certain parts of the body (Singh & Bittner, 2015).

FH increases an individual’s risk of developing premature CVD or CAD up 20-fold (Ahmad et al., 2015; Borge G Nordestgaard et al., 2013). Patients who are heterozygous for specific genetic variants will show clinical symptoms, such as elevated LDL-cholesterol levels or xanthomas. Homozygous or compound heterozygous individuals will have even more severe elevated LDL-cholesterol levels and most will experience a cardiac event by their mid-20s (Youngblom, Pariani, & Knowles, 1993). Furthermore, undiagnosed men with FH have a 50% increased risk of having a cardiac event by the age 50, while undiagnosed women have a 30% increased risk by the age of 60 (Youngblom, Pariani, & Knowles, 1993).

The presence of multiple symptoms and a family health history of heart disease is crucial to making a clinical diagnosis of FH. There are currently no universally agreed upon diagnostic criteria tools used by healthcare professionals to aid them in their diagnoses of FH; however, the ones most currently used are the Dutch Lipid Clinic Network criteria, the UK Simon Broome criteria, and the US Make Early Diagnosis to Prevent Early Death (MEDPED) criteria. The Dutch Lipid Clinic Network criteria uses a scoring system to weigh the appearance and lack of several symptoms to determine if a patient has FH. The UK Simon Broome criteria is one of the only tools that incorporates genetic testing and the US MEDPED looks solely at cholesterol levels cutoffs based on family health history of FH (Table 1). Although all of them differ in both structure and the symptoms included, the main similarities are the inclusion of elevated LDL-cholesterol and having a family health history of hypercholesterolemia (McGowan, Hosseini Dehkordi, Moriarty, & Duell, 2019).

Table 1. Comparison of FH Diagnostic Criterias Tools

|  |  |  |  |
| --- | --- | --- | --- |
| FH Characteristic | Dutch Lipid | Simon Broome | MEDPED |
| Family health history of premature CAD | + | + |  |
| Family health history of tendon xanthomas | + | + |  |
| Family health history of hypercholesterolemia | + | + | + |
| Patient premature CAD | + |  |  |
| Elevated LDL-cholesterol | + | + | + |
| Genetic mutation | + | + |  |

## Incidence and Prevalence of FH

In FH patients exhibiting elevated LDL-cholesterol levels, the incidence of CHD is 5.8 per 1000 person-years while the incidence of ASCVD is 14.9 per 1000 person-years (Masana et al., 2019). FH is considered a common genetic condition within the US, with an estimated prevalence of 1 in 250 people or 0.4%. However, other populations and groups have different prevalence rates. Akioyamen et al. (2017) performed a meta-analysis combining 19 different studies with a total sample size of 2,458,456 individuals from 28 countries. The researchers analyzed the prevalence of FH from these 28 countries and stratified individuals by age and sex. The researchers showed that the pooled global prevalence of FH is 0.40% in adults and 0.36% in children. Prevalence was similar between males and females at 0.42% and 0.45%, respectively. Older populations had higher prevalence rates; individuals aged 60-69 years had higher prevalence compared to younger individuals. Prevalence rates also differ among regions, ranging from 0.21% in European populations to 1.14% in African populations (Table 1) (Akioyamen et al., 2017).

Based on the global prevalence rates of FH as well as the number of clinical cases of FH, researchers estimated that less than 10% of individuals with FH have been diagnosed within the US (Ahmad et al., 2015), and less than 1% have been diagnosed globally (Akioyamen et al., 2017).

Table 2. Prevalence of FH by Continental Populations

|  |  |
| --- | --- |
| **Population** | **Prevalence Rates (%)** |
| North American | 0.38 |
| European | 0.21 |
| African | 1.14 |
| Australian | 0.5 |
| Asian | 0.46 |

## Lipid Metabolism and Genes Associated with FH

Normal lipid metabolism is comprised of different parts and elements. LDL-cholesterol particles consist of apoB molecules that form an envelope around different types of lipids. The apoB molecules on the LDL-cholesterol particles bind to LDL-receptors that are found on the surface of the liver. The resulting LDL-cholesterol particle-receptor complex is brought into the endosome compartment of the liver cell where the particle is subsequently catabolized into its smaller lipid and amino acid components. The LDL-receptor is then recycled back to the surface of the liver or taken to the lysosome and destroyed.

If any of these elements or processes are dysfunctional, LDL-cholesterol levels increase in the blood characterizing FH (Sharifi et al., 2017). Variants in any genes involved in these processes are categorized according to guidelines developed by the American College of Medical Genetics and Genomics (ACMG). The categories include: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (Richards et al., 2015). Known variants in three genes involved in lipoprotein metabolism, *LDLR, APOB,* and *PCSK9*, are estimated to cause 75-90% of FH cases (Goldberg et al., 2011).

### Low-Density Lipoprotein Receptor (*LDLR*)

The *LDLR* locus is located on chromosome 19 and has 18 exons that are transcribed and translated to form the LDL-receptor. Mutations in *LDLR* can result in alterations in the LDL-receptor that affect binding and uptake of LDL particles, or receptor synthesis, recycling, and transport—all of which can cause a buildup of LDL-cholesterol in the blood (Hobbs et al., 1992). Over 2900 FH causing variants were reported to be in *LDLR.* Of these reported variants, 93% encompass missense, nonsense, and frameshift variants, or large rearrangements . All nonsense and frameshift variants, and large rearrangements are considered to be pathogenic or likely to be pathogenic. Missense variants were the most highly reported; however, 15% of reported missense mutations were considered not to be pathogenic. 7% of the 2900 reported variants are listed as variants of unknown significance (VUS) and more information is needed to determine the pathogenicity (Leigh et al., 2017; Sharifi et al., 2017).

Different populations exhibit different ranges of FH causing mutations in the *LDLR* gene. In Greece, the majority of FH cases result from a small number of variants, whereas in the Netherlands and UK, over 200 different mutations have been reported in FH cases. Countries within Latin America and South Asia lack information on the molecular basis of FH; however, based on the cohort studies in Latin America that have been done, only a few of the variants found in these populations had previously been reported in the European population beforehand (Sharifi et al., 2017). Therefore, most of the variants were novel to Latin America.

### Apolipoprotein B (*APOB*)

The *APOB* locusis located on chromosome 2p and has 29 exons that are transcribed and translated to form apoB, the major apolipoprotein located on LDL-cholesterol particles and ligand for the LDL-receptor (Sharifi et al., 2017). Mutations in *APOB* result in decreased binding of apoB to the LDL-receptor, and consequently, the LDL-cholesterol particles are unable to enter the liver cells to be catabolized. Most mutations in *APOB* are found in a “hotspot” located in exon 26. One common variant in this region is p.Arg3500Gln (R3500Q) which accounts for 6-10% of FH cases in European populations and over 12% in Amish populations. Another variant, R3500W, has a prevalence of 1 in 393 in East Asian populations (Andersen, Miserez, Ahmad, & Andersen, 2016). *APOB* mutations result in an increase in LDL-cholesterol levels in the blood by a mean of 60 – 70 mg/dL. Thus, the effects of these mutations have less severe effects on LDL-cholesterol levels than mutations in the LDLR locus (Andersen et al., 2016).

### Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*)

The *PCSK9* locus is located on chromosome 1p and consists of 12 exons encoding an enzyme that regulates the degradation of LDL-receptors and prevents them from being recycled back up to the liver cell surface. The PCSK9 molecule is synthesized as an inactive proenzyme that must undergo cleavage in the endoplasmic reticulum to be transported and secreted from the liver. Secreted PCSK9 enzymes bind to the LDL-receptor and directs the receptor to the lysosome to be degraded (El Khoury et al., 2017).

More than 20 variants in *PCSK9* cause FH (Sharifi et al., 2017). These variants are gain-of-function mutations that increases the degradation of LDL-receptors and reduces the number of LDL-receptors on the surface of the liver by approximately 23% (El Khoury et al., 2017). The decrease in LDL-receptors causes more LDL-cholesterol particles to accumulate in the blood.

Conversely, loss-of-function mutations have been shown to enhance LDL-cholesterol clearance from the blood. These mutations are usually nonsense mutations and two variants, p.Tyr142Ter and p.Cys679Ter, were found in 2.6% of Black Americans and another variant p.Arg46Leu was found in 3% of European populations (El Khoury et al., 2017; Sharifi et al., 2017). These variants have been associated with a 15-27% reduction in LDL-cholesterol levels and a 47-88% reduction in the risk of CVD (El Khoury et al., 2017).

### Monogenic and Polygenic FH

Monogenic cases of FH result when a variant in one of the three FH genes is the primary cause of the increased LDL-cholesterol levels. Polygenic FH occurs when increased total cholesterol levels result from the additive influence of several genes with modest effect. Common variants in over 100 loci, such as *APOE, ABCG8,* and *SORT1* have modest effects on LDL-cholesterol levels (Sharifi et al., 2017).

When an individual with high LDL-cholesterol levels undergoes genetic testing for FH, a causative variant in one of the three FH genes is found in 60-80% of cases diagnosed with definite FH and in 30% of patients with probable FH. However, patients who do not have a known causative mutation may have a polygenic form of FH. Alternatively, their FH may be due to a novel variant in a gene that has not been identified yet (Sharifi et al., 2017).

## Treatment for FH

After an individual has been diagnosed with FH, immediate treatment includes using cholesterol-lowering drugs such as statins, ezetimibe, and bile acid-binding resin. These treatments have decreased LDL-cholesterol concentrations by 60-70%. For individuals with extreme LDL-cholesterol levels (due to having two copies of a genetic mutation or resistance to cholesterol-lowering drugs), weekly or bi-weekly lipoprotein apheresis may be necessary to remove excess LDL-cholesterol from their blood. Apheresis has been shown to decrease LDL-cholesterol levels by 50-75% (Borge G Nordestgaard et al., 2013).

Recent discoveries regarding the function of the *PCSK9* locus have resulted in new therapies that inhibit the effects of *PCSK9* on degrading LDL receptors. These *PCSK9* inhibitors include small molecule compounds and antibodies, such as evolocumab and alirocumab, that prevent the binding of *PCSK9* to the LDL-receptors. These inhibitors have been shown to decrease LDL-cholesterol levels by approximately 60% when taken alone or with another statin. In addition, these *PCSK9* inhibitors have been reported to reduce the need and frequency of lipid apheresis for patients with extremely high levels of LDL cholesterol (Ogura, 2018; Papademetriou et al., 2018).

Overall, early detection and treatment can help lower mortality risk from premature CHD or ASCVD by 48% (Sharifi, Rakhit, Humphries, & Nair, 2016).

# Screening Methods for FH

FH is clinically actionable and having an earlier diagnosis can improve health outcomes and reduce mortality from premature CHD and ASCVD (Green et al., 2013). As stated previously, however, fewer than 1% of individuals with FH are diagnosed globally. Current screening methods include cholesterol screening, genetic testing, and cascade screening. Cholesterol screening was also the primary method by which over 90% of currently known FH cases in the US were identified (Børge G Nordestgaard & Benn, 2017). Therefore, routine cholesterol screening through a primary care provider for LDL-cholesterol levels over 190 mg/dL can aid in a clinical diagnosis for FH.

In addition to cholesterol screening, other methods to identify individuals with FH include family health history or genetic testing (Børge G Nordestgaard & Benn, 2017). Genetic testing and cascade screening have become more viable options as the genetic causes behind FH have been identified. By detecting known mutations and risk alleles in the three primary genes associated with FH, a genetic diagnosis can be made. Cascade screening is a systematic screening approach that involves testing all first-degree relatives of an index patient who was diagnosed with FH and has a known mutation (Ned & Sijbrands, 2011). All of the first-degree relatives who carry the known mutation are then classified as index patients themselves and subsequently, all of their first degree-relatives are tested. Cascade screening is effective within a family because affected individuals have a 50% chance of passing on the mutation to their children. This approach requires that the specific mutation within the family is known, but approximately 15% of people diagnosed with FH do not have a known mutation in one of the three primary genes (Ned & Sijbrands, 2011).

## Utility of Screening Studies

Compared to traditional cholesterol screening, genetic and cascade screening is estimated to be more cost-effective and also increases diagnoses of FH (Ned & Sijbrands, 2011; Borge G Nordestgaard et al., 2013; Sturm et al., 2018); however, few countries have adopted this screening method. Although relatively few studies have been done on the effectiveness of cascade screening, a five-year study of cascade screening in the Netherlands reported 37% of blood relatives of an index case had an FH mutation. Of those with an FH mutation, 39% received lipid-lowering medication during the first year, and 93% received medication the second year (Umans-Eckenhausen, Defesche, Sijbrands, Scheerder, & Kastelein, 2001).

Other researchers have investigated cholesterol screening rates specifically among people with FH using National Health and Nutrition Examination Study (NHANES) data from 1999-2014. Of those included in the NHANES dataset, 58% did not report their cholesterol levels. For those who reported having FH, cholesterol screening rates were 91.3% compared to 69.9% of the general population. Prevalence of statin use in adults with FH were reported to have increased over time; however in 2014, only 52.3% reported using statins to lower their LDL-cholesterol levels (Bucholz, Rodday, Kolor, Khoury, & de Ferranti, 2018). These results revealed a large discrepancy in the number of individuals with FH who are screened and the number who are subsequently treated for it.

## Health Disparities in FH Diagnoses

In addition to the disparity between identification of individuals with FH and subsequent treatment, other disparities exist for certain groups and populations. The CASCADE-FH patient registry is the only active national registry for FH patients in the US and between February 2014 to September 2016, it contained records from 3537 individuals of all races and ethnicities who were enrolled at 26 different sites throughout the entire US. With information from this registry, Amrock and colleagues (2017) showed that women were diagnosed, on average, four years after men and were 40% less likely to have a statin regimen after being diagnosed. The investigators also reported that Blacks were diagnosed at a later age compared to all other races and ethnicities, despite having prevalence rates that were comparable to Whites. In addition, Blacks and Asians were also less likely to achieve 50% LDL-cholesterol reduction after being diagnosed (Amrock et al., 2017).

# Background on American Samoa and the Independent Nation of Samoa

American Samoa and the independent nation of Samoa have undergone an epidemiologic transition as non-communicable diseases (NCD), such as CVD, diabetes, and obesity, have increased, while infectious diseases and maternal, neonatal, and perinatal mortality, have declined (Hawley et al., 2012). The increase in NCD is due in part to a shift in lifestyles and diet as these two nations experienced a rapid modernization, growing urbanization, and better healthcare access. However, there is also a shift towards a more sedentary lifestyle and increase in risk factors such as smoking, unhealthy eating habits, and alcohol consumption that has led to an increase in NCD rates.

Although the populations of American Samoa and the independent nation of Samoa are genetically homogenous due to continual interaction and migration, the two nations are under different stages of modernization (Hawley et al., 2014). American Samoa is more modernized than independent Samoa, and as a result, lifestyles and diet differ. Galanis et al. (1999) found that in American Samoa, there was increased energy uptake in the form of carbohydrates and protein and less fat and saturated fat intake, whereas in the lower socioeconomic status (SES) of Samoa, there was a higher intake of fat and lower energy intake of carbohydrates, sodium, and protein. In addition to differences in diet, Ezeamama et al. (2006) found that differences in SES also contributed to the presence of CVD risk factors. High SES correlated with higher odds of risk factors in American Samoa, but decreased odds of risk factors in independent Samoa (Ezeamama et al., 2006).

The WHO reported that chronic diseases were responsible for 71% of deaths in Samoa in 2002 with 37% due to CVD. They also reported that the percent of individuals who are overweight has increased over the past few decades; in 2005, 85% of men and 89% of women are overweight (World Health Organization, 2005). In addition, cholesterol levels have changed over the past decades as well. Total cholesterol and triglyceride levels have significantly increased, whereas HDL cholesterol levels have decreased since the 1990s compared to the 1970s and 1980s (McGarvey et al., 1993).

With the increase in obesity, percent overweight, and NCD in American Samoa and the independent nation of Samoa, developing and implementing interventions to decrease the burden of CVD and diabetes is critical. At least 80% of premature CVD, stroke, and diabetes can be prevented through behavioral interventions that emphasize a healthy diet, physical exercise, avoidance of alcohol and tobacco products (World Health Organization, 2005). For genetic risk factors, such as FH, implementing programs that can identify, diagnose, and prescribe treatments, such as statins, is important.

## Health Disparities in Samoan Populations

Compared to other Asian and Pacific Islander populations, specific health disparities exist in Samoan populations. Juarez et al. (2010) reported that 50% of Samoan participants were obese, the highest rate among Asian and other Pacific Islander populations in the US; relative to whites, the odds ratio for developing obesity was 2.8. Samoans also reported the highest mean numbers of poor physical and mental health days in the past 30 days at 5.4 and 4.4 days, respectively. Based on self-reported answers of how often participants were able to receive an appointment for regular or urgent care as soon as they wanted, Samoans had some of the lowest access to health care. Conversely, Puerto Ricans and Native Hawaiians had greater access to care (Juarez et al., 2010). Spending on health per capita in Samoa was $418 in 2014 and healthcare spending accounted for 7.2% of the GDP compared to a mean of $4018 for spending on health per capita and a mean of 11% of the GDP in healthcare spending in New Zealand, which also has a large proportion of Polynesian residents (World Health Organization, 2019).

Kaholokula et al. (2008) also looked at perceived barriers to healthcare treatment. Samoan participants expressed a high degree of confidence in Western-trained physicians and medicine compared to traditional Samoan practices regarding treatment for heart failure. Although they trusted physicians, some of the barriers that were cited included belief that their healthcare provider did not spend enough time getting to know them, financial burden when purchasing medicine or healthier foods, and competing demands, such as childcare, work, or family obligations (Kaholokula et al., 2008).

Ninety percent of premature deaths due to chronic disease that occur in low- and middle-income countries are preventable. Thus, interventions to address health discrepancies and improve access to healthcare in Samoa are needed. As a result, the WHO has designed a set of interventions targeted at noncommunicable diseases for primary health practitioners in low-resource settings to prevent future burdens from these diseases (Bollars et al., 2018). These steps include: identifying disease early, implementing protocols for referrals to health facilities for treatment and follow-up, and increasing knowledge of risk factors. The implementation process includes: contacting stakeholders to inform them of the specific intervention, creating training programs for health professionals, making screening results available to the community, and creating educational programs to teach risk management to the community.

## Prevention and Treatment for Heart Disease in Samoa

In Samoa, cultural beliefs and perceived notions of risk need to be considered prior to developing prevention and/or treatment programs. Siaki et. al (2012) looked at cultural beliefs and perceived risk for CVD and diabetes using data from 28 women and 15 men, aged 18-55. Their study showed that the Samoan participants had knowledge about different CVD risks and believed themselves to be at moderate or high risk for CVD based off of personal and family health history, health behaviors, and physical health; however, 87.3% of participants did not report taking any prescription medicines (Siaki et. al, 2012). Siaki et. al (2012) also reported that the participants’ perceived risk of developing CVD or diabetes compared to their actual risk was mostly accurate; over 50% of participants accurately categorized whether they were at low, moderate or high risk for developing the disease. Participants also recognized that other factors outside of family and personal health history played a role in developing CVD or diabetes, with most knowing the importance of physical and healthy eating habits. However, their ideal weight goal tended to be higher than the national recommendations issued by the National Institutes of Health, which may be a result of their cultural perspectives (Siaki et al., 2012).

Another study utilizing focus groups recruited 36 participants (22 Hawaiians, ten Samoans, and four individuals who provided home care for a native Hawaiian or Samoan) investigated the perceptions on heart failure and the healthcare system in Samoa. The investigators reported that although participants knew the importance of managing risks and symptoms through healthy lifestyle habits, several barriers prevented them from achieving a healthy lifestyle. One of the barriers was the change in traditional Samoan lifestyle diets (that were based on subsistence farming) to a more western lifestyle and diet. This lifestyle change made it difficult for the Pacific Islanders to acquire land to grow their own food; furthermore, processed foods were less expensive to purchase (Kaholokula et. al, 2008). Another barrier was denying the presence of heart failure. Older adult Polynesians have traditional beliefs about the power behind spoken words (*mana*) and believed that speaking of misfortunes, such as heart disease, gave it the ability to manifest or worsen. Alternatively, denying the presence disease may also have been a coping mechanism that enabled them to avoid negative psycho-social symptoms, such as depression or anxiety (Kaholokula et al., 2008).

# Hypotheses and Aims

CHD in Samoa is the number one cause of premature deaths in Samoa, accounting for 37% of deaths in 2002 and a mean of 3114.3 years of life lost (YLL) in 2017 compared to the mean of 3036.7 YLL in other low-middle socio-demographic index countries (IHME, 2019; WHO, 2019). However, the prevalence of FH and subsequently, its effect on CHD, in Samoa is unknown. Furthermore, the frequency of previously identified risk alleles for FH in the Samoan population is unknown. As described in the Introduction, FH is treatable and, in other countries, implementation of screening programs has identified at-risk individuals and enabled them to receive lipid-lowering medications. Although other studies in Samoa have estimated the prevalence of obesity, diabetes, and adiposity, no study has been done to estimate the prevalence of FH risk alleles and thus, determine the usefulness of implementing a screening program.

One aim of this project is to determine the frequency of 832 known FH risk alleles in *LDLR*, *APOB*, and *PCSK9* in the Samoan population. I hypothesized that a subset of the population-based study sample will carry a known risk allele, and that their cholesterol levels will be over 190 mg/dL. In order to test this hypothesis, I will use data from a genome-wide association study of 3475 Samoan adults.

A second aim of this project is to determine whether having a known FH risk allele correlates with the FH phenotype of elevated LDL-cholesterol levels over 190 mg/dL. In addition, I will determine what proportions of individuals with elevated LDL-cholesterol levels have an FH variant.

Finally, the third aim of this paper is to determine, based on the frequency of FH risk alleles and correlation to FH phenotypes, whether a genetic screening program for FH would be useful in Samoa. If the frequency of FH is relatively high, implementation of a screening program would benefit the Samoan population. If more individuals are diagnosed with FH prior to experiencing an adverse cardiac event, they could be placed on a statin regiment that would lower their risk of developing CVD.

# Data Sources

The dataset analyzed for this study came from participants recruited from 33 villages within the four main census regions of the Independent Nation of Samoa. Researchers were allowed by the Ministry of Health to spend two to three days in each village to complete their sample collection from participants. Recruitment and measurements were taken between February and July 2010. This dataset was originally collected as part of a genome-wide association study (GWAS) looking at obesity-related traits.

Of the 3504 participants between the ages of 24.5 and 65 years old, 29 were excluded based on not meeting the study inclusion criteria, which included age and pregnancy status since this may affect the original purpose of the GWAS study researching obesity traits. This resulted in a total of 3475 eligible participants for the dataset. 99.4% completed questionnaires on socio-demographic information, medical health history, alcohol and tobacco consumption, physical activity, household assets inventory, and acculturation assessment. A food frequency questionnaire was also administered to measure food intake, cooking habits, and fruit and vegetable consumption. In addition to these two questionnaires, biometric measurements were taken which included height, weight, body mass index (BMI), blood pressure, and blood glucose. 84.6% gave a fasting serum sample after being asked to fast for ten hours overnight to measure cholesterol levels. 91.1% gave blood samples for DNA extraction, with the first phase of genotyping being completed in November 2011. Genotype information was sequenced using different transcripts available from the gnomeAD browser. *LDLR, APOB,* and *PCSK9* has multiple transcripts available and SNPs may vary between transcripts for the same gene depending on the differences in transcription and translation start sites and end sites, or differences in splicing that affect which exons are part of the transcript. Information on medication and treatment usage was unavailable from the dataset and all analyses performed was done irrespective of treatment.

The numbers of study participants broken down by age, sex, obesity, and other select characteristics are presented in Table 3. Although efforts were made to recruit participants that were representative of the entire Independent Nation of Samoa, the final study population does not accurately reflect the total population proportion based on the 2011 census. The proportion of women in the study (58.6%) was higher than that in the general population proportion (48%). Older participants were also overrepresented compared to younger participants; 42.2% of participants were 60 – 64 years of age, but only 16.1% of participants were 24.5 – 29 years of age. Of the four main census regions, rural populations were overrepresented at 32.4% and 33.1% compared to the urban and semi-urban areas which represented 15.9% and 19.6% of the study.

Table 3. Select Characteristics of Samoan Sample Population Stratifed by BMI Categories

|  |  |  |  |
| --- | --- | --- | --- |
|  | Normal | Overweight | Obese |
| SexMaleFemale | 277177 | 561546 | 5871311 |
| Age24 – 3334 – 4344 – 5354 – 6364 – 73 | 143111101936 | 27127329523929 | 33251754446540 |
| Experienced Heart AttackNoYesN/A | 378076 | 93910158 | 158026292 |
| Previously Diagnosed with Heart DiseaseNoYesN/A | 45022 | 1090116 | 1873196 |

 Note: Polynesian cutoffs were used for BMI groupings

**Table 3 Continued**

The National Center for Biotechnology Information (NCBI) variation viewer was used to identify all pathogenic variants. The variation viewer catalogs biomedical and genomic information submitted from researchers who have uploaded information to dbSNP, dbVAR, and ClinVar from their peer-reviewed studies. The variation viewer has software available that allow users to search and analyze the data compiled, as well as the primary literature source to provide more details and information for each variant.

# Methods

This study uses the cholesterol levels measured from the fasting serum sample and genotyping data from the DNA samples in order to answer the proposed aims.

## FH Pathogenic Risk Variants

I identified a total of 28 potential pathogenic and pathogenic variants that are related to FH in *PCSK9*, 746 variants in *LDLR*, and 58 variants in *APOB* (Table 4) in the NCBI variation viewer. As described above, I restricted variants to those three genes that were implicated in FH and had pathogenic variants. The dbSNP name, position, variant type, coding change, and protein change were all recorded in an excel file for the pathogenic variants found in each gene.

 All variants found in *LDLR, APOB,* and *PCSK9* in the Samoan participants were pulled from the genotyping data of the Samoan participants. Variants were detected by comparing the Samoan genotypes against a reference allele to see if any participants had an alternative allele at certain positions in one of the three genes. The position and coding change of the alternative allele were recorded in an excel file for each variant found in each gene. In addition, based on the position and type of allele change, the presumed impact of the variant was also recorded, such as low impact for synonymous changes and high impact for insertions or deletions in a coding region (Table 5).

### Gene Analysis

Using Microsoft Excel, known variants in the Samoan dataset were compared to the known FH pathogenic variants from NCBI Variation Viewer. The SNP names (rsID) and chromosomal positions in the Samoan genotyping data were matched to the list of known FH pathogenic variants. Once a match was located using either the SNP name or chromosomal position, I looked in further detail to ensure the allele change in the Samoan dataset correlated with the same allele change in the pathogenic variant, resulting in the same protein change, insertion, deletion, or frameshift. Afterwards, I gathered the genotypes for the matches and plotted the total and LDL-cholesterol levels by genotype groups using box and whisker plots to determine whether there was an observed

difference in cholesterol levels between those with a known pathogenic variant and whether or not the mean cholesterol levels for those with a pathogenic variant were ≥190 mg/dL for LDL-cholesterol or ≥310 mg/dL for total cholesterol.

Table 4. Known FH Pathogenic Risk Variants in PCSK9, APOB, and LDLR from NCBI Variation Viewer

|  |  |
| --- | --- |
| Gene and Variant Type | Number of Variants |
| *PCSK9** Single Nucleotide Variant
* Insertion
* Indel
 | 282611 |
| *LDLR** Single Nucleotide Variant
* Deletion
* Insertion
* Indel
 | 74629710926309 |
| *APOB** Single Nucleotide Variant
* Deletion
* Indel
 | 5833817 |

Table 5. Impact of Variants Found in LDLR, APOB, and PCSK9 From Samoan Dataset

|  |  |
| --- | --- |
| **Impact of Variant** | **Number of Variants** |
| *LDLR* | 5606 |
| * Modifier
 | 5486 |
| * Low
 | 76 |
| * Moderate
 | 44 |
| * High
 | 0 |
| *APOB* | 408 |
| * Modifier
 | 337 |
| * Low
 | 23 |
| * Moderate
 | 47 |
| * High
 | 1 |
| *PCSK9* | 280 |
| * Modifier
 | 256 |
| * Low
 | 10 |
| * Moderate
 | 14 |
| * High
 | 0 |

## LDL-Cholesterol Levels and Covariates

Biometric data in the Samoan dataset was obtained and coded in an excel file and subsequently imported to Stata and R-statistical package for analysis. LDL-cholesterol levels were summarized in Stata to investigate the distribution of cholesterol levels, and to determine how many participants had cholesterol levels over 190 mg/dL. This information was also stratified by sex to assess any differences found between males and females. To assess any potential effects of covariates on LDL-cholesterol levels, a linear regression analysis was performed in R. Covariates previously reported to affect LDL-cholesterol levels include age, sex, and obesity. Obesity levels using Polynesian BMI cutoffs were used. Polynesian populations have a higher cutoff for BMI categories due to having a higher proportion of lean body mass for a given BMI. Using these cutoffs, overweight is defined as BMI = 26-32 kg/m2 and obesity is defined as BMI > 32 kg/m2.

# Results

I assessed the proportion of individuals with elevated LDL-cholesterols, as well as the effects of comorbidities, such as obesity, on LDL- and total cholesterol levels. Next, I compared variants in the Samoan genotyping dataset and the list of known FH pathogenic variants to obtain a list of possible pathogenic variants for FH among Samoan participants.

## LDL-Cholesterol Level Analysis

The average LDL-cholesterol levels among the 2937 participants were stratified by Polynesian BMI cutoffs. The mean LDL-cholesterol levels were higher among participants who were obese and overweight compared to normal BMI. Inversely, HDL-cholesterol levels were highest among participants who were of normal BMI compared to overweight and obese (Table 6). LDL-cholesterol levels were approximately normally distributed (Figure 1). The mean LDL-cholesterol level was 130 mg/dL, with a minimum of 21 mg/dL and a maximum of 324 mg/dL and 135 individuals had LDL-cholesterol levels over 190 mg/dL. Thus, 4.6% of the population would meet the LDL-cholesterol cutoff for FH. I observed no differences in mean LDL-cholesterol levels by sex: the LDL-cholesterols means were 129.5 mg/dL in males and 130.13 mg/dL in females (Figure 2).

Table 6. Mean Cholesterol Levels Between BMI Categories

|  |  |  |
| --- | --- | --- |
|  | Mean (mg/dL) | Standard Deviation(mg/dL) |
| Total Cholesterol LevelsNormalOverweightObese | 185.26198.64 203.86 | 37.2939.6235.48 |
| LDL-Cholesterol LevelsNormalOverweightObese | 115.67129.00133.82 | 32.9935.5532.75 |
| HDL-Cholesterol LevelsNormalOverweightObese | 52.0446.4143.19 | 12.4411.0010.06 |

Note: Polynesian cutoffs were used for BMI groupings



Figure 1. Distribution of LDL-Cholesterol Levels (mg/dL)



Figure 2. LDL-Cholesterol Levels by Sex

## Association Between LDL-Cholesterol and Covariates

I performed a linear regression analysis on the combined effects of age, sex, and obesity on LDL-cholesterol levels (Table 7). Age, being overweight, and being obese had a statistically significant effect on LDL-cholesterol levels. Every one-year increase in age was associated with a 0.906 mg/dL increase in LDL-cholesterol levels (p = 2e-16). Being overweight was associated with an 11.507 mg/dL increase in LDL-cholesterol levels (p = 7.71e-09), while being obese was associated with a 15.444 mg/dL increase (p = 7.26e-16). Sex was not associated with LDL-cholesterol levels.

Table 7. Linear Regression Analysis of Covariates on LDL-Cholesterol Levels

|  |  |  |
| --- | --- | --- |
| **Covariate** | **Coefficient** | **P-value** |
| Age | 0.906 | 2e-16\* |
| Sex | -0.586 | 0.639 |
| Overweight | 11.507 | 7.71e-09\* |
| Obese | 15.444 | 7.26e-16\* |

Note: \*Statistically significant

## LDLR, APOB, and PCSK9 Analysis

In the Samoan genotyping data, different variants were pulled from the genotyping data and the impact of each variant was determined based on the position of the variant and the subsequent protein or sequence change that occurred (nonsynonymous protein change, frameshifts, null changes, etc). These variants were then compared to known FH pathogenic variants listed in the NCBI variation viewer. Based on chromosomal position and rsID SNP name, I detected fifteen matches in *LDLR*, one match in *APOB*, and three matches in *PCSK9*.

In *LDLR*, of the fifteen matched variants, only two unique rsID numbers and chromosomal positions were observed. Several of the matched variants have the same coding and protein changes due to the fact that *LDLR* has at least 8 different sequence transcripts available and all transcripts were included in the Samoan genotype dataset. All of the observed variants result in a synonymous amino acid change and are listed as benign in NCBI (Table 8). In *APOB*, the one matched variant (rs760832994) is a frameshift mutation and is known to be pathogenic for FH (Table 9). In *PCSK9*, rs371488778 and rs1159114 result in a pathogenic in-frame insertion and missense variant, respectively. The third match, rs11583680, is also a missense variant; however, it is listed as likely benign (Table 10).

Table 8. Matched Variants in LDLR

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SNP Name****(# of Individuals)** | **Chromosomal Position** | **Coding Change** | **Protein Change** | **Molecular Consequence** |
| rs2228671(n = 20) | 11,100,236 | c.333C>Tc.81C>Tc.81C>Tc.81C>Tc.81C>Tc.81C>Tc.81C>T | p.Cys111Cysp.Cys27Cysp.Cys27Cysp.Cys27Cysp.Cys27Cysp.Cys27Cysp.Cys27Cys | Synonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous Variant |
| rs140241383(n = 1) | 11,100,236 | c.1110C>Tc.354C>Tc.735C>Tc.477C>Tc.858C>Tc.858C>Tc.858C>Tc.456C>T | p.Ser370Serp.Ser118Serp.Ser245Serp.Ser159Serp.Ser286Serp.Ser286Serp.Ser286Serp.Ser152Ser | Synonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous VariantSynonymous Variant |

Table 9. Matched Variant in *APOB*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SNP Name (# of individuals)** | **Chromosomal Position** | **Coding Change** | **Protein Change** | **Molecular Consequence** |
| rs760832994 (n = 2) | 21,002,393- 21,002,397 | c.13028\_13029delAT | p.Tyr4343fs | Frameshift |

Table 10. Matched Variants in *PCSK9*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SNP Name (# of individuals)** | **Chromosomal Position** | **Coding Change** | **Protein Change** | **Molecular Consequence** |
| rs371488778(n = 1) | 55,039,880 | c.60\_65dupGCTGCT | p.Leu21\_Leu22dup | In-frame Insertion |
| rs11591147(n = 3) | 55,039,974 | c.137G>T | p.Arg46Leu | Missense Variant |
| rs11583680(n = 366) | 55,039,995 | c.158C>T | p.Ala53Val | Missense Variant |

## Matched Variants and LDL-Cholesterol Levels

Further analysis was performed on the matched results to see if the allele change corresponds with having total cholesterol levels over 310 mg/dL or LDL-cholesterol levels over 190 mg/dL (Figures 3 and 4). The number of Samoan participants with any of the known variants is too small to be definitive regarding the relationship between genotype and cholesterol, with the exception of the *LDLR* rs2228671 and the *PCSK9* rs11583680 variants. Neither of these latter two variants were associated with an increase in total or LDL-cholesterol levels. The one individual heterozygous for *PCSK9* rs371488778 variant, as well as the two individuals heterozygous for the *APOB* rs760832994 variant, had higher total and LDL-cholesterol levels than the remaining Samoan participants. The three individuals with the *PCSK9* rs11591147 variant have lower total and LDL-cholesterol levels compared to individuals without this variant.



Figure 3. Total Cholesterol Levels Grouped by Genotype



Figure 4. LDL-Cholesterol Levels Grouped by Genotype

# Conclusion

CVD affects an estimated 128 million people worldwide and is a leading cause of death in many developed countries (Dahlof, 2010). Several contributing factors to CVD is genetic susceptibility, hypertension, abnormal lipid profiles, obesity, stress, and lack of physical activity. FH is an autosomal dominant condition where excess LDL-cholesterol is unable to be removed from blood, resulting in adults having LDL-cholesterol levels over 190 mg/dL, or total cholesterol levels over 310 mg/dL. If cholesterol levels remain untreated, this increases an individual’s risk of developing premature CVD or CAD up 20-fold (Ahmad et al., 2015; Borge G Nordestgaard et al., 2013). Treatments, such as statins, ezetimibe, and bile acid-binding resin, have decreased LDL-cholesterol concentrations by 60-70% (Borge G Nordestgaard et al., 2013). Therefore, it is imperative that individuals with FH are screened and detected early to lower their risk of premature CVD and an adverse cardiac event. Compared to traditional cholesterol screening methods, genetic and cascade screening have been shown to be effective ways of increasing diagnoses of FH.

Samoan and other Polynesian populations have undergone an epidemiological transition where CVD, diabetes, and obesity have increased due to a shift towards modernization and growing urbanization. Thirty-seven percent of deaths in Samoa were due to CVD and in 2005, 85% of men and 89% of women are overweight (World Health Organization, 2005). However, there have been no studies looking at the prevalence of FH in the Independent Nation of Samoa or the frequency of FH risk alleles which may be contributing to the prevalence of heart disease in this country.

This study aimed to identify any known FH pathogenic risk variants in a Samoan population and whether or not it correlates with having an FH phenotype of LDL-cholesterol levels over 190 mg/dL. These aims were created in order to ascertain whether or not a screening program is necessary in Samoa. Another aim of this study was to observe comorbidities, such as obesity, to see if it has an interacting effect with FH variants on cholesterol levels.

Of the participants for whom genotype information was available, 393 had a potential pathogenic variant for FH based on information from the NCBI. In *LDLR,* 21 individuals had variants inrs2228671 or rs140241383; however, their cholesterol levels did not differ from the mean cholesterol levels of individuals who did not have these variants. The less frequent allele for both of these variants coded for the same amino acid as the common allele (Table 6); therefore, these variants were highly unlikely to have an effect.

Two individuals had a pathogenic variant in *APOB* (rs760832994) which resulted in a frameshift mutation. The LDL-cholesterol levels for these two individuals reach the clinical threshold for FH; however, there were not enough individuals with this mutation to calculate statistical significance. Based on both LDL-cholesterol levels and the presence of a known pathogenic variant, it is highly likely these two individuals have FH.

In *PCSK9,* there were 370 individuals with a variant in rs371488778, rs11591147, and rs11583680. The one individual with the in-frame insertion variant in rs371488778 (p.Leu21\_Leu22dup) had a higher cholesterol level than the mean level of those without the pathogenic allele; however, it does not reach the clinical levels of FH. Effects of other genetic and environmental factors may be causing the increased cholesterol levels for this individual. Alternatively, other factors may be mitigating the effects of the presumed pathogenic allele for FH. Nevertheless, because their LDL cholesterol level is elevated, this individual is at increased risk for cardiovascular disease. Interestingly, participants with the missense variant in rs11591147 (p.Arg46Leu) have lower total and LDL-cholesterol levels compared to those without the missense variant. This mutation is one of the known loss-of-function mutations found in 3% of European populations that contributed to the discovery of different *PCSK9* inhibitors. This mutation results in a protein change from arginine to leucine. Arginine has a positive electric side chain which makes it hydrophilic while leucine is hydrophobic. Of the 366 individuals with a mutation in rs11583680, all of them have similar cholesterol levels. Although this is a missense mutation, it results in a protein change from alanine to valine. These two amino acids are both hydrophobic, polar amino acids. The resulting change from alanine to valine resulted in a semi-conservative substitution that likely has minimal impact.

The mean LDL-cholesterol level in this sample was 130 mg/dL, which is above the recommended levels of under 100 mg/dL. This high level may be due to the change in lifestyle and diet in Samoa after modernization. When stratified by sex, the means of the LDL-cholesterol levels were very similar to each other, showing no significant difference in cholesterol levels between males and females. The plot of the overall distribution also revealed that 135 individuals (4.6% of the sample population) had LDL-cholesterol levels over 190 mg/dL, one of the hallmarks of FH. Although other factors that contribute to high LDL-cholesterol levels must be taken into account, such as diet, exercise, and comorbidities like diabetes, this is a good indication that at least one individual in this sample may have an unknown or novel FH pathogenic variant based on the global prevalence of 1 in 250 people. As described previously, the two individuals with the *APOB* frameshift variant rs760832994 most likely have FH. Future consideration should be taken to investigate the presence of potentially novel variants in the Samoan population. Intriguingly, one individual had an LDL-cholesterol level of 21 mg/dL. Loss-of-function variants in *PCSK9* have been shown to be protective against CAD and CVD, and this individual may also have a *PCSK9* variant. Although this study was only assessing pathogenic variants, future studies may look for protective variants in the Samoan population.

Besides genetic effects, covariates also play a role in raising LDL-cholesterol levels. Both age and obesity criteria had a statistically significant positive association with LDL-cholesterol levels. For every year increase in age, this was associated with a 0.906 mg/dL increase in LDL-cholesterol levels. This means that for a thirty-year age difference, that would be associated with a 27.18 mg/dL increase in LDL-cholesterol levels. Being overweight or obese is also associated with having higher LDL-cholesterol levels. Thus, an individual with an FH pathogenic variant may have increased (or decreased) levels of LDL-cholesterol depending on their specific suite of covariates. Other considerations that need to be taken into account are family health history of high cholesterol and current statin and treatment usage to control cholesterol levels; however, the information provided by this dataset is inadequate to determine the effect of these factors on cholesterol levels.

## Public Health Significance

Although the prevalence of CVD in Samoa is high, no studies have been done to assess the prevalence of FH in the Independent Nation of Samoa or the frequency of FH risk alleles. FH is treatable and identifying individuals with this disorder at an early age will enable them to be treated to lower their cholesterol levels and reduce their risk of early onset of CVD. In this study, I ascertained whether any known FH pathogenic risk variants were present in a sample of the Samoan population and also whether these variants were associated with LDL-cholesterol levels over 190 mg/dL. The longer-term goal of the study was to determine whether a genetic screening program for FH should be implemented. Because I detected only two or three individuals who had known pathogenic variants in genes associated with FH and also had elevated LDL-cholesterol levels, development of a genetic screening program would not be beneficial at this time. The low frequency of known pathogenic variants in the Samoan population may reflect the low global frequency found for these pathogenic variants in other populations. However, future considerations

The population of Samoa has high rates of obesity, diabetes, and CVD. Notably, the LDL-cholesterol levels are significantly higher than what is recommended by the WHO, 130 mg/dL versus 100 mg/dL, respectively. In addition, 3.9% of the population has LDL-cholesterol levels higher than the 190 mg/dL which is one of the characteristics of individuals with FH. Therefore, as yet unknown variants may affect development of FH in Samoans and future studies are needed to identify these potential variants. As stated above, a genetic testing screening program for FH in Samoa is premature; however, implementing a global cholesterol screening program to detect individuals in need of statins and heart medication to prevent the onset of CVD may be beneficial.

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