HHS Public Access

Author manuscript

J Community Med Public Health. Author manuscript; available in PMC 2019 August 30.

Published in final edited form as:

J Community Med Public Health. 2019 ; 3(1): .

Genetic Epidemiologic Analysis of Hypertensive Retinopathy in an Underrepresented and Rare Federally Recognized Native American Population of the Intermountain West

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Abstract

Understanding disease risk is challenging in multifactorial conditions as it can differ by environment, ethnicity and race. The Confederated Tribes of the Goshute Reservation are one of the most isolated populations in the United States. Retinal changes are a reliable indicator for systemic disease. We conducted a cross-sectional study to identify correlations between genetic data and epidemiological risk factors for blinding retinal disease in this tribe. As part of the "Supporting Prediction and Prevention Blindness Project (SPBPP)" in the Native American Population of the Intermountain West, we found that hypertensive retinopathy was the most prevalent retinal disease. We found that forty-two percent of the Goshute population was affected. Blood samples, fundus photos and intraocular pressure were obtained for all participants. In addition, a standardized questionnaire was administered. DNA and total cholesterol, HDL, LDL, VLDL, triglycerides and HbA1c were also evaluated. Our study interrogated genetic variants from the PAGE study (ARMS2 rs10490924, CFH rs800292, rs1061170) and additional studies that looked at previously associated genetic variants with retinal disease associated with cardiovascular disease. We conducted univariate and multivariate logistic regression in Stata v15.0. We found an association between hypertriglyceridemia and HTR (adj$p$ = .05) within the Goshute population. To the best of our knowledge, this is the first study to demonstrate the prevalence of hypertensive retinopathy in a Native American population. Moreover, our study is the first to demonstrate an independently predictive relationship between hypertriglyceridemia and hypertensive retinopathy in an American Indian population. This study furthers our knowledge about prevalent blinding eye disease within the most geographically isolated federally recognized native United States American tribe, for which nothing has been published with respect to any disease. Although, this study furthers our understanding about the prevalence of genetic epidemiological risk factors

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within this population, it has greater implications for the screening of blinding diseases in underserved populations in general. This study can inform public health on planning and delivering of quality, accessible and relevant care to this population.

Keywords

Epidemiology; Genetic; Hypertensive retinopathy; Isolated population; Native American

Introduction

Underserved and isolated populations have unique paradigms of disease risk and prevention. These populations have challenges to healthcare delivery and may offer a way to better understand disease etiology and risk in a more homogenous context [1–3]. Certainly, less heterogeneity will increase the likelihood for identification of common mediators. The United States Department of Health and Human Services states that there are currently 573 federally recognized tribes in the United States [4]. American Indian reservations in the United States are often geographically isolated and underserved environments; therefore, they face inherent barriers in access to adequate health care and preventive services [4]. Importantly, this includes annual routine eye exams, which could reveal the presence of systemic diseases such as diabetes and hypertension.

There is a lack of information on the unique genetic epidemiological characteristics that influence Hypertensive Retinopathy (HTR) risk within federally recognized tribes that are geographically isolated with respect to prevalence. Available information points to diabetes and cardiovascular disease as the predominant health burden within the federally recognized tribes of the United States. There is significant morbidity and mortality due to these conditions in this largely underserved population as compared to other ethnic populations including Caucasians of Northern European descent [4–7]. Further, there is significantly less information available on the blinding diseases that co-segregate with cardiovascular disease (hypertensive retinopathy) in general [8]. Moreover, it is established that in addition to not having access to adequate health care, an isolated community may have poor resources to maintain a healthy diet to prevent diseases such as hypertension and hypertriglyceridemia which increases risk for morbidity and mortality. Due to this, those who live on an American Indian reservation may experience food insecurity. Food insecurity increases the risk for obesity, diabetes and hypertension among American Indian populations [9].

It is well established that in both the working and aging population the majority of blinding diseases which affect the retina or the back of the eye such as hypertensive retinopathy and, diabetic retinopathy, have both strong environmental and genetic components [10–16]. These diseases can manifest in the retina and be diagnosed with a color fundus photo (picture of the retina), before they manifest systemically [8,17–22]. In fact, in many instances diabetes and/or hypertension may go undiagnosed in an individual until they have a retinal exam [23–25]. Furthermore, numerous studies indicate that the severity and characteristics of retinal disease in conditions such as hypertensive and diabetic retinopathy

have direct correlation with systemic risk for stroke and heart disease in both American Indian and Caucasian populations [21,26–29].

In general, research with respect to determining the prevalence of eye disease amongst the American Indians of the United States through studies including the Strong Heart Study (SHS) [30,31], the Population Architecture Using Genomics and Epidemiology (PAGE) study [32] and the Family Investigation of Nephropathy and Diabetes (FIND-eye) study [33]. Although this work has provided important information with respect to prevalence of visual impairment within American Indians of the United States in general, critically this research lacks designation of the individual tribes that each individual participant may belong to. However, with respect to diabetic retinopathy, prevalence has been reported for individual tribes, within American Indian populations. Tribes such as the Sioux, Hopi and Navajo American Indians have had prevalence of DR at 45.3%, 41.0% and 29.0% respectively [34,35]. While other studies on American Indians in general, reported findings of what type of retinopathy was present suggesting that HTR was not found or was found but not as prevalent as DR [36–39]. Research that treats ethnically, socially and geographically diverse populations as a homogenous group is limited from both theoretical and practical perspectives. Theoretically, it is important to understand the genetic and epidemiological variation between groups as diverse as American Indian tribes, as variation between tribes may elucidate clues in the causes and manifestations of disease. Practically, such research needs to inform service delivery for these populations, as understanding the variation at the tribal level will allow policy makers and those planning and delivering relevant health services to tailor those services to achieve maximum access, relevance and utilization by the members of the individual tribes. New initiatives developed at the broad “American Indian” level may fall short if not designed and tailored to meet the needs of a particular tribe [40,41].

The Confederated Tribes of the Goshute Reservation spans both the states of Utah and Nevada located in the Intermountain Mountain West of the United States. The Goshute tribe is one of 24 tribes in these states [42]. Of all the federally recognized American Indian tribes, they are considered the most geographically isolated in the United States [43].

The purpose of the study, Supporting Prediction and Prevention Blindness Project (SPBPP), was to identify correlations between genetic data, epidemiological risk factors, and eye diagnoses in an effort to identify disease causality. This could pave the way for potential interventions and treatments in this special population. Moreover, studying the epigenetics in this unique population may have broader implications for understanding complex diseases in other populations. We sought to identify correlations between genetic data, epidemiological factors, serum measurements, and blinding eye disease in the Goshute tribe. Due to this tribe’s geographic isolation, this approach holds the potential to examine a wide range of questions including how genes and the environment impact one’s overall health and social and cultural factors impact well-being and healthy aging. In addition, isolated populations may have very little differences in lifestyle factors, so any identified genes may likely be truly associated with the disease [44]. Our analysis specifically examined known determinants of morbidity and mortality and how these factors relate to the development of potentially blinding pathology in this population. Our study interrogated genetic variants

from the PAGE study (ARMS2 rs10490924, CFH rs800292, rs1061170) and additional studies that looked at variants previously associated with retinal phenotypes with overlapping pathophysiology to cardiovascular disease and diabetes [19, 45–49]. This was complemented by a detailed standardized questionnaire that assessed life style and clinical factors on risk of morbidly and mortality. This work has implications for understanding disease risk, preventing co-morbid disease states such as myocardial infarction, and screening for hypertensive disease, both retinal and systemic. Studying the epidemiology and genetics in this population may also have broader implications for understanding complex diseases in other populations. Further, this research is the first study of its kind to provide foundational health status genetic epidemiology in this federally recognized tribe in the United States.

Materials and Methods

Study cohort

The study protocol was reviewed and approved by the Institutional Review Board at the University of Utah and conformed to the tenants of the Declaration of Helsinki. Participants were enrolled in this study after giving informed consent. The study team visited the Confederated Tribes of the Goshute Reservation and explained the study and its benefits, to Tribal Elders and the broader Goshute. The Goshute Tribe subsequently passed a resolution supporting the study approved by the Business Council under Article VII, Section 1(e) and (f) of the Constitution of the Confederated Tribes of the Goshute Reservation. Study participants were recruited from the Confederated Tribes of the Goshute Reservation and surrounding area. Peripheral blood samples, blood pressure, intraocular pressure, and fundus photos were obtained for each patient. In addition, each participant completed a standardized validated questionnaire [20,50–52]. From the blood samples, DNA was extracted from the peripheral blood leukocytes and candidate Single Nucleotide Polymorphisms (SNPs) were genotyped. These genetic factors tested included SNPs from genes previously associated with AMD in the PAGE study (ARMS2 rs10490924, CFH rs800292, rs1061170), in addition to other studies observing retinal neovascular phenotypes (ARMS2 rs10664316, HTRA1 [rs11200638, rs2672598, rs1049331, and rs2293870], CFH [rs16840422, rs12144939, rs2284664, and rs12066959], ROBO1 [rs1387665, rs4513416, rs9309833, and rs7622444], and RORA [rs12900948, rs8034864, rs730754]) and serum biomarkers for angiogenesis (ROBO1 and RORA) [6,10,46]. Peripheral blood samples were sent to LabCorp (Laboratory Corporation of America Holdings, Burlington, NC) to obtain lipid panel (Triglycerides, HDL, VLDL, LDL) and Hemoglobin A1c (HbA1c, an objective measure of sugar levels) results.

Phenotypic analysis

Non-mydriadic fundus images were obtained from both eyes of all study participants. At least three independent ophthalmologic and retinal specialists reviewed each photograph to evaluate for the presence of retinal disease. The presence, absence and severity of blinding eye disease was evaluated by at least three ophthalmologists with retinal surgery training using standard methods including the Keith, Wagener, and Barker classification [53,54]. Inconsistent diagnosis was resolved through direct and/or phone conversation between at

least two evaluating physicians. Therefore, each study participant received a clinical-level evaluation for presence or absence of blinding eye disease, including hypertensive retinal disease.

Genotyping

Genetic factors tested for association with observed phenotypes included SNPs from genes previously associated with retinal diseases like AMD (ARMS2/HTRA1, CFH, ROBO1, and RORA) and serum biomarkers for angiogenesis (ROBO1 and RORA). Genotyped SNPs included ARMS2 rs10490924 and rs10664316; HTRA1 rs11200638, rs2672598, rs1049331, and rs2293870; CFH rs800292, rs16840422, rs1061170, rs12144939, rs2284664, and rs12066959; ROBO1 rs1387665, rs4513416, rs9309833, and rs7622444; and RORA rs12900948, rs8034864, rs730754. Genotyping was performed on leukocyte DNA that was purified using Qiagen extraction kits. All CFH, ROBO1, ARMS2, HTRA1 (rs11200638 and rs2672598), and RORA SNPs were genotyped using a combination of pre-designed and Custom Taqman SNP Genotyping Assays (Applied Biosystems). Each assay was run in a 15ul reaction containing 2x Taqman GTXpress master mix, 40x or 80x probe, and 10 ng of DNA. Thermal cycling was performed according to the manufacturer's protocol. The ABI 7500 Real-Time PCR System, with the accompanying software, was used to analyze the genotypes. Direct sequencing was used to genotype HTRA1 SNPs (rs1049331 and rs2293870) using previously reported oligonucleotide primers. Polymerase chain reaction (PCR) was used to amplify genomic DNA fragments in 25 ul reactions containing the following: 20 ng of genomic DNA, 1 U of Taq DNA Polymerase (Invitrogen), 10x PCR buffer, 1.6 mM $MgCl_2$, and 0.16 mM of each dNTP (Invitrogen), 0.4 uM of each primer, 0.8M Betaine, and 5% DMSO. The temperatures used during the PCR were as follows: 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, with a final annealing temperature of 72°C for 5 minutes. For sequencing reactions, PCR products were digested according to the manufacturer's protocol using ExoSAP-IT (USB). DNA sequencing was performed at the (omitted) DNA Sequencing Core Laboratory. Electropherograms were read independently by two evaluators without knowledge of the subject's disease status. Serum biomarkers (ROBO1 and RORA) were tested using ELISA kits from MyBioSource.com (catalog numbers MBS910208 and MBS921385) following the manufacturer's protocol. Allele frequency in each population was calculated for normal subjects, or those without a diagnosis of HTR.

Statistical analysis

We performed both a univariate (unadjusted) and multivariate (adjusted) analysis within three populations. One population included all the participants (Ranchers and American Indian participants), the second analysis was only considering the American Indian population, and the last analysis was within only the population of participants with a diagnosis of hypertensive retinopathy. In this we were able to determine both the unique risk when considering American Indian's with hypertensive retinopathy as well as the risk factors that the total population may be exposed to due to their environment. Our outcome variable for the first and second analysis was hypertensive retinopathy. Individuals without the condition were the controls and individuals with the condition were the cases. In the final analysis the American Indian population was the cases and the Rancher population was the

controls. Genotyped SNPs were tested for deviation from Hardy-Weinberg Equilibrium (HWE) using the Chi-square test in unaffected subjects (those without retinal disease) only. SNPs were tested for association using the minor allele, as defined by the allele occurring less frequently in the unaffected subjects, assuming an additive genetic model. Epidemiological definitions were as follows: Smokers were defined as those who had smoked greater than 100 cigarettes during their lifetime; BMI ≥ 25 kg/m^2 was considered overweight; BMI ≥ 30 kg/m^2 was considered obese; HbA1c 5.7–6.4 mmol/L was considered elevated; HbA1c > 6.4 mmol/L was considered diabetic; total cholesterol 200–239 mg/dL was considered moderately elevated; total cholesterol 240 mg/dL and higher was considered high; LDL 130–159 mg/dL was considered moderately elevated; LDL 160–189 mg/dL was considered high; LDL 190 mg/dL and higher was considered very high; healthy HDL levels were considered above 40 mg/dL for men and above 50 mg/dL for women; and VLDL > 40 mg/dL was considered high. For Triglycerides, borderline high was considered 150–199 mg/dL, high was considered 200–499 mg/dL, and very high was 500 mg/dL or above. All risk factors were tested for association with observed phenotypes using logistic regression in Stata v15.0 [55]. To determine the most predictive model for observed for phenotypes, a multivariate analysis was conducted for all participants using all measured risk factors showing association at P<.05. Specifically, a univariate analysis was conducted and only risk factors with an association at P<.05 were included in the multivariate model. This same analysis was conducted between the Rancher population with hypertensive retinopathy and the American Indian population with the condition. The analysis with only the American Indian population was similar, but the multivariate model was determined by risk factors showing associations to hypertensive retinopathy at P<.10 and were only found statistically significant in the multivariate model at P<.05 in the presence of other risk factors. Furthermore, allele frequencies were calculated for normal subjects, those without a diagnosis of HTR in both of the populations.

Results

We recruited 64 individuals for participation in this study: 14 Northern European descent-ranchers from Ibapah and 50 individuals of American Indian descent. Of the 14 Ranchers recruited, the average age was 53.14 (range 21.37 to 94.21 years) and 7 (50%) were males. The average BMI in the Rancher population was 37.38. Within this group 29% were smokers, 29% were considered obese, 7% met criteria for diabetes, 21% for High Blood Pressure (HBP), 36% for high cholesterol and 20% worked outside. Additionally, 5 individuals (36% total) had elevated HbA1c (≥ 5.7 mmol/L) not meeting the definition of diabetes. The average RORA protein level was 49.352, with the average ROBO1 level being 25.415 in the Ranchers. Hypertensive retinopathy was present in 8 (57%) of the Rancher population (Table 1). Of the 50 American Indians recruited, the average age was 44.3 (range 22.77–78.68) and there were 31 (62%) males. The average BMI in the American Indian population was 29.48. Within this group, 64% were smokers, 72% were considered obese, 38% were considered diabetic, 32% were considered to have High Blood Pressure (HBP), 28% were considered to have high cholesterol and 43% worked in an outside environment. Additionally, 37 individuals (74%) had an HbA1c elevated above normal though not meeting the definition of diabetes (≥ 5.7 mmol/L). In comparison to other American Indian tribes, the

prevalence of diabetes was lower in the Goshute population. Both the Navajo and the Sioux American Indians have prevalence of diabetes that are higher at 40% and 76% respectively. Though the Goshutes do have higher rates of diabetes compared to that of the U.S. population and Caucasians who both have a prevalence of 21% [56]. The average RORA protein level was 44.117, with the average ROBO1 protein level being 3.367 in this population. Mild hypertensive retinopathy was present in 21 (42%) of American Indians (Table 1).

Observing the individuals who were homozygous for the risk alleles within the SNPs associated with mild HTR, the American Indian population had higher frequencies of ARMS2 rs10490924 (G allele) in those with mild hypertensive retinopathy (67%) and those without (39%) compared to the Rancher population. In addition, they also had a higher frequency of the ARMS2 rs10664316 (delAT) risk allele when observing those who were homozygous for the risk allele in both the population with hypertensive retinopathy (48%) and those without (22%). The Rancher had higher frequencies of CFH rs2284664 (C allele) and CFH rs800292 (G allele) in both the population with mild hypertensive retinopathy (75%) and those without (33%) compared to the American Indian population. This was also true for CFHR2 rs12066959 (G allele) in Ranchers with mild hypertensive retinopathy (75%) and those without (50%), as well as HTRA rs2672598 (C allele) in Ranchers with mild hypertensive retinopathy (50%) and those without (67%). Moreover, there was a higher frequency of Ranchers who were homozygous for the RORA rs8034864 (C allele) risk allele compared to the American Indian population, in both the Ranchers with mild hypertensive retinopathy (50%) and without hypertensive retinopathy (33%). The Ranchers with a diagnosis of mild hypertensive retinopathy had a higher frequency of the CFH rs1061170 risk allele when observing individuals who were homozygous for the risk allele (50%) compared to the Ranchers without the disease and the American Indian population which had no individuals who were homozygous for the risk allele (Table 2).

Multivariate (adjusted) analyses were conducted for both the total population and the American Indian population to determine which risk factors might be independently associated (unadjusted) with hypertensive retinopathy. Our multivariate analysis for the total population included age and high blood pressure; these risk factors from the univariate analyses (Table 3) met our significance criteria for entry into the multivariate model (i.e. significance at $p<.05$). We found that age, which was a statistically significant risk factor of hypertensive retinopathy in the univariate analysis was not associated with hypertensive retinopathy in the multivariate model (OR, 1.034; 95% CI, .988–1.083; $p = .145$). Only high blood pressure was found to be moderately associated with hypertensive retinopathy in the multivariate compared to participants that did not have a diagnosis of mild hypertensive retinopathy (OR, 3.667; 95% CI; .951–14.141; p=.059). A multivariate analysis was conducted for the American Indian population using the risk factors LDL and Poor Triglycerides which were identified to be statistically significant risk factors for hypertensive retinopathy in our univariate analyses at $p<.10$ (Table 4). LDL, which was found to be significant in the univariate analysis was no longer associated with mild hypertensive retinopathy in the multivariate model (OR, 1.025; 95% CI; .994–1.05; p=.114). Poor Triglycerides remained associated with mild hypertensive retinopathy after adjusting for other risk factors in the multivariate analysis (OR, 4.228; 95% CI .998–17.912; p=.050). A

multivariate analysis was conducted for all participants diagnosed with mild hypertensive retinopathy that included the American Indian and Rancher populations. The risk factors that were included in this analysis included the SNPs CFH rs1061170 (C allele), ROBO1 rs4513416 (G allele), in addition to the risk factor alcohol consumption. These risk factors were found to be statistically associated with mild hypertensive retinopathy in the univariate analyses at p<.05 as seen in Table 5 (p =.009, p =.019 and p=.026, respectfully). Alcohol consumption, which was found to have an association with mild hypertensive retinopathy during the univariate analysis, was no longer statistically associated to mild hypertensive retinopathy in the multivariate analysis at p<.05 (OR, 14.947; 95% CI, =.578–386.389; p=. 103, Table 6) Similarly, SNP ROBO1 rs4513416 (G allele) was no longer a statistically significant protective factor against HTR (OR,.104; 95% CI,.005–2.332; p=.154,Table 6) in the American Indian population, after controlling for other factors (i.e., significant at p<.05). The SNP CFH rs1061170 (C allele) remained a protective factor against hypertensive retinopathy in the American Indian population compared to the Rancher population after accounting for other factors (OR, .039; 95% CI, .002-.841; p=.038, Table 6), in the multivariate analysis (i.e. significance p<.05).

When the risk allele frequencies were assessed for 19 SNP's previously found to be related to retinal disease to determine if there was statistical difference between the risk allele frequencies between the American Indians and the Ranchers of Northern European decent, the risk allele T for the SNP CFH rs12144939 was found to be moderately statistically significant (OR .118; 95% CI, .013–1.03; p=.053) between the populations, with the American Indian's having a lower frequency (T ~ 15% vs. 60%). There were no other SNP allele frequencies that were statistically different between the two populations (Table 7).

The allele frequencies for the SNPs examined within the American Indians without mild hypertensive retinopathy, compared to those within four previously studied diverse populations including, a family based cohort from New England (NESC), a case-control cohort from Central Greece, a case-control study from Seoul National University Bundang Hospital and a cohort from Timor-Leste can be found in Table 8 [57].

The American Indian population was similar to the Timor and Korean population in which both populations had lower frequencies of the protective T allele, compared to the NESC and the Greek population for SNP CFH rs12144939. The American Indian's had a frequency most similar to the Timor, with a frequency of 15% in comparison to the Timor frequency of 14.6% as shown in Table 9.

Discussion

Understanding risk for multifactorial conditions is challenging and numerous strategies have been employed [11,12,21]. Assessment of a homogenous population may allow for more impactful results. Furthermore, isolated populations often have less access to quality medical care services and preventative care services, therefore understanding their unique risk is important to devise the most effective and targeted screening strategies.

Diabetes within the American Indian population is considered to be a major public health problem with about 16% diagnosed with the chronic disease, as compared to only 8.7% in non-Hispanic whites. American Indians have the highest rate of age-adjusted diabetes of all racial/ethnic minority groups [58,59]. All individual who have diabetes are at risk for Diabetic Retinopathy (DR) [37,38].

Our study did not find DR as the major eye disease affecting this population, but found mild hypertensive retinopathy to be the eye disease that had the highest prevalence. The hypertensive retinopathy phenotype was extensively evaluated by at least three retinal surgeons using the Keith, Wagener and Barker classification. The finding of mild hypertensive retinopathy as the prevalent retinal disease in this population is further supported in that we did not find an association with history of diabetes, Hba1c, and VLDL (Table 3). While there is a great deal known about DR in the American Indian population, little is known about hypertensive retinopathy in this population. Hypertensive retinopathy is not only a potentially blinding condition but is also correlated with other serious co-morbid diseases such as stroke, myocardial infarction and renal disease [10,21,28,60]. While we did not find an association hypertension with mild hypertensive retinopathy in American Indians or Ranchers separately, we report a borderline association within the entire heterogeneous population. This is supported by other studies that show that hypertension is a risk factor for hypertensive retinopathy [22,61]. We were not able to conduct an analysis with the potential association between hypertensive retinopathy and stroke as others have reported in our study population because no one in this population reported having a history of stroke [10,21,28,60]. The average age for the Ranchers in our study was 53.15 years old and the average age of the American Indians in our study was 44.3 years old. A person's risk for stroke increases with age [62]. With the risk of stroke doubling every decade after the age of 55 [63]. This could have been why we did not observe stroke in either population studied. When considering solely the American Indian population, hypertriglyceridemia alone is the strongest predictor of hypertensive retinopathy. Therefore, statistically significant predictors of hypertensive retinopathy risk differ based on the population studied. This is to the best of our knowledge this is a novel finding within an American Indian population. Previous studies have shown an association between hypertensive retinopathy and hypertriglyceridemia in populations from Nepal and India. In addition, the studies conducted on the Nepalese and the populations from India were conducted with patients that had already been diagnosed with hypertension at the time of the study and were seeking care [13,64,65].

The presence of hypertensive retinopathy has also been associated with increased mortality [21]. Therefore, detection of hypertensive retinopathy is important not only for preservation of vision, but also for systemic health and disease prevention. The precise molecular genetic risk and pathophysiologic basis for hypertensive retinopathy are not yet elucidated, though the fundamental mechanisms are related to a sustained elevation in systolic blood pressure resulting in compromise of the retinal vascular integrity [60]. It is important to identify individuals at risk for this condition both for preservation of vision but moreover due to the serious and life threatening co-morbid conditions. This is true for all populations but certainly for underserved groups without adequate preventive care. The American Indians in this study are part of The Confederated Tribes of the Goshute Reservation. This is a tribe

that is geographically, genetically and socially isolated population allowing for a unique ability to identify correlations between genetic data, epidemiological risk factors, and ocular pathology in this homogenous population. Thus, our work is important for both better understanding of unique factors affecting this tribe as well as gaining insights into multifactorial disease.

This has important implications for disease prevention and screening. Furthermore, this may represent different pathophysiology with a final common pathway though this needs to be analyzed with further study. Hypertriglyceridemia has been linked to diet, through sugar consumption and poor exercise habits [66]. The American Indian population may have poor triglyceride levels due to diet. They may not have access to healthy nutritious foods that can contribute to healthy triglyceride levels. The rancher population may be able to access more nutritional food sources by crops that they grow themselves. The American Indian population has limited access to nutritional food sources. The nearest grocery store to the Goshute tribe is located 61.83 miles away (Smiths) and there is only a single general store nearby [67,68]. Exercise also plays a role in triglyceride levels. 80% of the American Indian population reported working inside compared to 57% of the Rancher population. Having an occupation that requires a person to work indoors leads to less calorie expenditure, because there is less work related to physical activity [69]. Having an occupation indoors also leads to less sunlight exposure and decreased Vitamin D levels. Vitamin D levels have been found to have a protective factor against cardiovascular disease [70–72]. Moreover, it has been shown that participants who had decreased levels of Vitamin D were statistically more likely to develop retinopathy compared to those participants with higher Vitamin D levels [73]. Though, the association between Vitamin D levels and decreasing hypertension remain inconsistent [74–76]. We did not see an association between Vitamin D exposure and hypertensive retinopathy the population studied (Table 3).

Though the ROBO1 protein levels and the ROBO1 SNPs we observed in our study were found to be not statistically significant risk factors for our population, when observing hypertensive retinopathy, the expression of ROBO1 has been found to be altered in different ocular neovascular diseases such as age-related macular degeneration and corneal inflammation, in addition to other forms of retinopathy such as diabetic retinopathy (DR) and Retinopathy of Prematurity (ROP) [16,19]. Furthermore, RORA protein levels and RORA SNPs were also not found to be statistically associated with hypertensive retinopathy in our population study. Though we did not observe an association, RORA has been associated with risk of neovascular age-related macular degeneration [17–19].

While elevated blood pressure has previously been associated with hypertensive retinopathy, hypertriglyceridemia has not previously been shown to be associated with the presence of hypertensive retinopathy in an American Indian population. These data represent a novel association with hypertensive retinopathy within a Native American tribe. Our data suggest that consideration of these factors for recommendation of retinal screening is important. Therefore, our data suggests that a retinal exam may be an appropriate first screening step, which may lead not only to reduction in eye disease but also systemic disease and mortality.

There are limitations to this study. The study may be relatively underpowered, as factors significant in univariate analysis failed to achieve significance in the multivariate logistic regression models. Thus, our study provides only suggestive evidence that other factors, including age, LDL, alcohol consumption and ROBO1 rs4513416 SNP may have a role in hypertensive retinopathy. Considering our association with hypertension and hypertensive retinopathy, we still had 70% power to detect an association assuming a p-value <.05 [77–80]. We have used small sample sizes in the past for studies of this nature quite successfully and in particular when we compare these findings to other cohorts from different parts of the world with varying prevalence of posterior eye diseases. In this manner we can identify factors (and potentially their disease mechanisms) that are protecting or increasing risk for blinding diseases such as hypertensive retinopathy, diabetic retinopathy and/or age-related macular degeneration. Despite the limitations of our study, the fact that we found an association between hypertensive retinopathy and hypertension in the American Indians with our admittedly modest sample size is strong evidence with our admittedly modest sample size is strong evidence in favor of this association [21,22]. Employing the use of geographically isolated populations in the study approach may be a valuable tool for understanding the role and interaction of epidemiological and genetic factors in contributing to multifactorial diseases such as hypertensive retinopathy to inform better preventive care services and delivery.

This study investigates a genetically isolated population, which as previously stated is important to uncover truly associated disease susceptibility variants for complex diseases [81–84]. The Goshutes are a small isolated population, which can be important for genetic studies focusing on interactions between genetic and environmental factors [44,81]. Isolated populations, though they may have a small sample size, are important populations to study because they are important to the understanding of diseases and their components in a homogenous context that will help to create new advancement in public health [82,83]. Observing allele frequencies in isolated populations and how they are associated with disease can help determine the health effects on genetic isolation [44]. Small populations may have less environmental variability when they are compared to larger populations that can be more geographically diverse [44]. Within these isolated populations there is also a decrease in genetic drift [44]. Health effects at the local level include resource availability, which can effect both the population’s environment and their lifestyle, because these can cause health problems [83,84]. Examples of resources that may not be available in these populations are the proximity to healthy foods or certain medical advancements which then causes health inequity in these isolated populations [83].

Future work is needed to identify if these findings remain the same across all grade levels of hypertensive retinopathy, or only for specific grade levels of hypertensive retinopathy. We took a candidate gene based approach and selected variants that have previously shown an independent association with retinal disease. However, future studies will likely take an agnostic genetic approach to this question, thereby identifying previously identified as well as novel variants that may associate with hypertensive retinopathy.

Conclusions

Studies have examined eye problems within Native Americans, however little is known about variations in genetic epidemiological factors and disease risk in individual tribes. This study furthers our knowledge about prevalent blinding eye disease within the most geographically isolated federally recognized Native US American tribe, for which nothing has been published with respect to any disease. Although, this study furthers our understanding about the prevalence of genetic epidemiological risk factors within this population, it has greater implications for the screening of blinding diseases in underserved populations in general. Hypertensive Retinopathy is a condition that can cause blindness and is associated with morbidity and mortality risk. This is the first study to report the prevalence of hypertensive retinopathy in a Native American tribe and moreover the association of hypertriglyceridemia with hypertensive retinopathy. This study can inform public health on planning and delivering of quality, accessible and relevant care to this population.

Acknowledgements

Research reported in this publication was additionally supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development and the Office of Research on Women's Health of the National Institutes of Health under Award Number K12HD085852; National Institutes of Health (EY014800), and an Unrestricted Grant from Research to Prevent Blindness, Inc., New York, NY, to the Department of Ophthalmology & Visual Sciences, University of Utah. The content is solely the responsibility of the authors and does not necessarily represent the official views of the national Institutes of Health. We especially want to thank the Confederate Tribes of the Goshute Reservation for their participation in this study. We also thank Dr. Randall Olson, MD., for his support of this work.

Funding: This work is supported by The ALSAM Foundation.

References

1. Andersen MK, Pedersen CE, Moltke I, Hansen T, Albrechtsen A, et al. (2016) Genetics of Type 2 Diabetes: The Power of Isolated Populations. Curr Diab Rep 16: 65. [PubMed: 27189761]
2. Grarup N, Moltke I, Albrechtsen A, Hansen T (2015) Diabetes in Population Isolates: Lessons from Greenland. Rev Diabet Stud 12: 320–329. [PubMed: 27111118]
3. Nair AK, Baier LJ (2015) Complex Genetics of Type 2 Diabetes and Effect Size: What have We Learned from Isolated Populations? Rev Diabet Stud 12: 299–319. [PubMed: 27111117]
4. Office of Minority Health (2018) Profile: American Indian/Alaska Native The Office of Minority Health, U.S. Department of Health and Human Services.
5. Howard BV, Lee ET, Cowan LD, Fabsitz RR, Howard WJ, et al. (1995) Coronary heart disease prevalence and its relation to risk factors in American Indians. The Strong Heart Study. Am J Epidemiol 142: 254–268. [PubMed: 7631630]
6. Dumitrescu L, Carty CL, Taylor K, Schumacher FR, Hindorff LA, et al. (2011) Genetic determinants of lipid traits in diverse populations from the population architecture using genomics and epidemiology (PAGE) study. PLoS Genet 7: e1002138.
7. Naqshbandi M, Harris SB, Esler JG, Antwi-Nsiah F (2008) Global complication rates of type 2 diabetes in Indigenous peoples: A comprehensive review. Diabetes Res Clin Pract 82: 1–17. [PubMed: 18768236]
8. Feehan M, Hartman J, Durante R, Morrison MA, Miller JW, et al. (2011) Identifying subtypes of patients with neovascular age-related macular degeneration by genotypic and cardiovascular risk characteristics. BMC Med Genet 12: 83. [PubMed: 21682878]

9. Jernigan VBB, Huyser KR, Valdes J, Simonds VW (2017) Food Insecurity among American Indians and Alaska Natives: A National Profile using the Current Population Survey-Food Security Supplement. J Hunger Environ Nutr 12: 1–10. [PubMed: 28491205]
10. Bhargava M, Wong TY (2013) Current Concepts in Hypertensive Retinopathy: The retinal physician is often the first to detect it. Retinal Physician.
11. Nolan CJ, Damm P, Prentki M (2011) Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet 378: 169–181. [PubMed: 21705072]
12. Deangelis MM, Silveira AC, Carr EA, Kim IK (2011) Genetics of age-related macular degeneration: current concepts, future directions. Semin Ophthalmol 26: 77–93. [PubMed: 21609220]
13. Badhu B, Dulal S, Baral N, Lamsal M, Shrestha JK, et al. (2003) Serum level of low-density lipoprotein cholesterol in hypertensive retinopathy. Southeast Asian J Trop Med Public Health 34: 199–201. [PubMed: 12971535]
14. Maghbooli Z, Pasalar P, Keshtkar A, Farzadfar F, Larijani B (2014) Predictive factors of diabetic complications: a possible link between family history of diabetes and diabetic retinopathy. J Diabetes Metab Disord 13: 55. [PubMed: 24860795]
15. Nwanyanwu KH, Newman-Casey PA, Gardner TW, Lim JI (2015) Beyond HbA1c: Environmental Risk Factors for Diabetic Retinopathy. J Clin Exp Ophthalmol 6: 405. [PubMed: 26973797]
16. Rama N, Dubrac A, Mathivet T, Ní Chárthaigh RA, Genet G, et al. (2015) Slit2 signaling through Robo1 and Robo2 is required for retinal neovascularization. Nat Med 21: 483–491. [PubMed: 25894826]
17. Silveira AC, Morrison MA, Ji F, Xu H, Reinecke JB, et al. (2010) Convergence of linkage, gene expression and association data demonstrates the influence of the RAR-related orphan receptor alpha (RORA) gene on neovascular AMD: a systems biology based approach. Vision Res 50: 698–715. [PubMed: 19786043]
18. Schaumberg DA, Chasman D, Morrison MA, Adams SM, Guo Q, et al. (2010) Prospective study of common variants in the retinoic acid receptor-related orphan receptor α gene and risk of neovascular age-related macular degeneration. Arch Ophthalmol 128: 1462–1471. [PubMed: 21060049]
19. Jun G, Nicolaou M, Morrison MA, Buros J, Morgan DJ, et al. (2011) Influence of ROBO1 and RORA on risk of age-related macular degeneration reveals genetically distinct phenotypes in disease pathophysiology. PLoS One 6: e25775.
20. Morrison MA, Silveira AC, Huynh N, Jun G, Smith SE, et al. (2011) Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. Hum Genomics 5: 538–568. [PubMed: 22155603]
21. Wong TY, Mitchell P (2004) Hypertensive retinopathy. N Engl J Med 351: 2310–2317. [PubMed: 15564546]
22. Grosso A, Veglio F, Porta M, Grignolo FM, Wong TY (2005) Hypertensive retinopathy revisited: some answers, more questions. Br J Ophthalmol 89: 1646–1654. [PubMed: 16299149]
23. Lenake M, Du Toit N (2014) The eye in systemic disease. South African Family Practice 56: 8–14.
24. Pinazo-Durán MD, Zanón-Moreno V, García-Medina JJ, Arévalo JF, Gallego-Pinazo R, et al. (2016) Eclectic Ocular Comorbidities and Systemic Diseases with Eye Involvement: A Review. Biomed Res Int 2016: 6215745.
25. Reena M (2014) Your Eyes Could Be the Windows to Your Health. American Academy of Ophthalmology.
26. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, et al. (2013) 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 31: 1281–1357. [PubMed: 23817082]
27. Jolly SE, Koller KR, Metzger JS, Day GM, Silverman A, et al. (2015) Prevalence of Hypertension and Associated Risk Factors in Western Alaska Native People: The Western Alaska Tribal Collaborative for Health (WATCH) Study. J Clin Hypertens (Greenwich) 17: 812–818. [PubMed: 25644577]

28. Shantha GPS, Ashok Kumar A, Bhaskar E, Sivagnanam K, Srinivasan D, et al. (2010) Hypertensive retinal changes, a screening tool to predict microalbuminuria in hypertensive patients: a cross-sectional study. Nephrology Dialysis Transplantation 25: 1839–1845.
29. Cheung N, Rogers S, Couper DJ, Klein R, Sharrett AR (2007) Is diabetic retinopathy an independent risk factor for ischemic stroke? Stroke 38: 398–401. [PubMed: 17194880]
30. Lee ET, Welty TK, Fabsitz R, Cowan LD, Le NA, et al. (1990) The Strong Heart Study. A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 132: 1141–1155. [PubMed: 2260546]
31. Lee ET, Russell D, Morris T, Warn A, Kingsley R, et al. (2005) Visual impairment and eye abnormalities in Oklahoma Indians. Arch Ophthalmol 123: 1699–1704. [PubMed: 16344442]
32. Franceschini N, Carty C, Buzková P, Reiner AP, Garrett T, et al. (2011) Association of genetic variants and incident coronary heart disease in multiethnic cohorts: the PAGE study. Circ Cardiovasc Genet 4: 661–672. [PubMed: 22042884]
33. Arar NH, Freedman BI, Adler SG, Iyengar SK, Chew EY, et al. (2008) Heritability of the severity of diabetic retinopathy: the FIND-Eye study. Invest Ophthalmol Vis Sci 49: 3839–3845. [PubMed: 18765632]
34. Berinstein DM, Stahn RM, Welty TK, Leonardson GR, Herlihy JJ (1997) The prevalence of diabetic retinopathy and associated risk factors among Sioux Indians. Diabetes Care 20: 757–759. [PubMed: 9135938]
35. Rate RG, Knowler WC, Morse HG, Bonnell MD, McVey J, et al. (1983) Diabetes mellitus in Hopi and Navajo Indians. Prevalence of microvascular complications. Diabetes 32: 894–899. [PubMed: 6618018]
36. Mansberger SL, Gleitsmann K, Gardiner S, Sheppler C, Demirel S, et al. (2013) Comparing the effectiveness of telemedicine and traditional surveillance in providing diabetic retinopathy screening examinations: a randomized controlled trial. Telemed J E Health 19: 942–948. [PubMed: 24102102]
37. Lee ET, Lee VS, Kingsley RM, Lu M, Russell D, et al. (1992) Diabetic retinopathy in Oklahoma Indians with NIDDM. Incidence and risk factors. Diabetes Care 15: 1620–1627. [PubMed: 1468294]
38. Bursell SE, Fonda SJ, Lewis DG, Horton MB (2018) Prevalence of diabetic retinopathy and diabetic macular edema in a primary care-based teleophthalmology program for American Indians and Alaskan Natives. PLoS One 13: e0198551.
39. Chin EK, Ventura BV, See KY, Seibles J, Park SS (2014) Nonmydriatic fundus photography for teleophthalmology diabetic retinopathy screening in rural and urban clinics. Telemed J E Health 20: 102–108. [PubMed: 24219153]
40. Bylander J (2018) Meeting The Needs of Aging Native Americans. Health Affairs Blog.
41. (2012) Native Vision: A focus on improving behavioral health wellness for California Native Americans California Reducing Disparities Project. Native American Strategic Planning Workgroup Report.
42. Public Health Professionals Gateway (2017) Tribal Geography in Relation to State Boundaries. Centers for Disease Control and Prevention.
43. Dennis RD (1951) The Goshute Indians of Utah In: The History of Utah's American Indians. J. Willard Marriott Library, University of Utah.
44. Hatzikotoulas K, Gilly A, Zeggini E (2014) Using population isolates in genetic association studies. Brief Funct Genomics 13: 371–377. [PubMed: 25009120]
45. Woo SJ, Ahn J, Morrison MA, Ahn SY, Lee J, et al. (2015) Analysis of Genetic and Environmental Risk Factors and Their Interactions in Korean Patients with Age-Related Macular Degeneration. PLoS One 10: e0132771.
46. Restrepo NA, Spencer KL, Goodloe R, Garrett TA, Heiss G, et al. (2014) Genetic determinants of age-related macular degeneration in diverse populations from the PAGE study. Invest Ophthalmol Vis Sci 55: 6839–6850. [PubMed: 25205864]
47. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA (2011) Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ 342: d636. [PubMed: 21343206]

48. Shakeri H, Hadaegh H, Abedi F, Tajabadi-Ebrahimi M, Mazroii N, et al. (2014) Consumption of synbiotic bread decreases triacylglycerol and VLDL levels while increasing HDL levels in serum from patients with type-2 diabetes. Lipids 49: 695–701. [PubMed: 24706266]
49. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK (2016) Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights 11: 95–104. [PubMed: 27398023]
50. DeAngelis MM, Ji F, Kim IK, Adams S, Capone A Jr, et al. (2007) Cigarette smoking, CFH, APOE, ELOVL4, and risk of neovascular age-related macular degeneration. Arch Ophthalmol 125: 49–54. [PubMed: 17210851]
51. Kim IK, Ji F, Morrison MA, Adams S, Zhang Q, et al. (2008) Comprehensive analysis of CRP, CFH Y402H and environmental risk factors on risk of neovascular age-related macular degeneration. Mol Vis 14: 1487–1495. [PubMed: 18704199]
52. DeAngelis MM, Lane AM, Shah CP, Ott J, Dryja TP, et al. (2004) Extremely discordant sib-pair study design to determine risk factors for neovascular age-related macular degeneration. Arch Ophthalmol 122: 575–580. [PubMed: 15078676]
53. Keith NM, Wagener HP, Barker NW (1974) Some different types of essential hypertension: their course and prognosis. Am J Med Sci 268: 336–345. [PubMed: 4616627]
54. Henderson AD, Bruce BB, Newman NJ, Biousse V (2011) Hypertension-related eye abnormalities and the risk of stroke. Rev Neurol Dis 8: 1–9. [PubMed: 21769065]
55. (2017) Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.
56. Stahn RM, Gohdes D, Valway SE (1993) Diabetes and its complications among selected tribes in North Dakota, South Dakota, and Nebraska. Diabetes Care 16: 244–247. [PubMed: 8422783]
57. Morrison MA, Magalhaes TR, Ramke J, Smith SE, Ennis S, et al. (2015) Ancestry of the Timorese: age-related macular degeneration associated genotype and allele sharing among human populations from throughout the world. Front Genet 6: 238. [PubMed: 26217379]
58. (2017) Diabetes Report Card 2017 Centers for Disease Control and Prevention, US Department of Health and Human Services Atlanta, GA, USA.
59. McLaughlin S (2010) Traditions and Diabetes Prevention: A Healthy Path for Native Americans. Diabetes Spectrum 23: 272–277.
60. Tso MO, Jampol LM (1982) Pathophysiology of hypertensive retinopathy. Ophthalmology 89: 1132–1145. [PubMed: 7155524]
61. Klein R, Klein BE, Moss SE, Wang Q (1993) Blood pressure, hypertension and retinopathy in a population. Trans Am Ophthalmol Soc 91: 207–222; discussion 222–226. [PubMed: 8140692]
62. (2017) Stroke Risk. Centers for Disease Control and Prevention.
63. (2018) Brain Basics: Preventing Stroke. National Institute of Neurological Disorders and Stroke, U.S. Department of Health and Human Services.
64. Gupta RP, Gupta S, Gahlot A, Sukharamwala D, Vashi J (2013) Evaluation of hypertensive retinopathy in patients of essential hypertension with high serum lipids. Med J DY Patil Univ 6: 165–169.
65. Bastola P, Pun CB, Koirala S, Shrestha UK (2012) Fasting serum lipids and fundus changes in hypertensive patients. Nepal Journal of Medical sciences 1: 103–107.
66. DiNicolantonio JJ, Lucan SC, O'Keefe JH (2016) The Evidence for Saturated Fat and for Sugar Related to Coronary Heart Disease. Prog Cardiovasc Dis 58: 464–472. [PubMed: 26586275]
67. Butko B (2005) Greetings from the Lincoln Highway: Traveling America's First Coast-to-Coast Road. Stackpole Books.
68. McRae WC, Jewell J (2017) Moon Utah. Avalon Travel.
69. Church TS, Thomas DM, Tudor-Locke C, Katzmarzyk PT, Earnest CP, et al. (2011) Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. PLoS One 6: e19657.
70. Judd SE, Tangpricha V (2009) Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci 338: 40–44. [PubMed: 19593102]
71. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, et al. (2008) Vitamin D deficiency and risk of cardiovascular disease. Circulation 117: 503–511. [PubMed: 18180395]

72. Nijjar PS (2015) Vitamin D and Cardiovascular Disease: Where We Currently Are. American College of Cardiology.

73. Mutlu U, Ikram MA, Hofman A, de Jong PT, Uitterlinden AG, et al. (2016) Vitamin D and retinal microvascular damage: The Rotterdam Study. Medicine (Baltimore) 95: e5477. [PubMed: 27930528]

74. Mehta V, Agarwal S (2017) Does Vitamin D Deficiency Lead to Hypertension? Cureus 9: e1038. [PubMed: 28357170]

75. Chen S, Sun Y, Agrawal DK (2015) Vitamin D deficiency and essential hypertension. J Am Soc Hypertens 9: 885–901. [PubMed: 26419755]

76. Ullah MI, Uwaifo GI, Nicholas WC, Koch CA (2010) Does vitamin D deficiency cause hypertension? Current evidence from clinical studies and potential mechanisms. Int J Endocrinol 2010: 579640.

77. Dean AG, Sullivan KM, Soe MM (2013) OpenEpi: Open Source Epidemiologic Statistics for Public Health.

78. Dean AG, Sullivan KM, Soe MM (2010) Epi Info and OpenEpi in Epidemiology and Clinical Medicine: Health Applications of Free Software. Createspace Independent Pub.

79. Sullivan KM, Dean A, Soe MM (2009) OpenEpi: a web-based epidemiologic and statistical calculator for public health. Public Health Rep 124: 471–474. [PubMed: 19445426]

80. Papakostas TD, Morrison MA, Lane AM, Awh C, DeAngelis MM, et al. (2018) Genetic Risk Factors for Radiation Vasculopathy. Invest Ophthalmol Vis Sci 59: 1547–1553. [PubMed: 29625478]

81. Heutink P, Oostra BA (2002) Gene finding in genetically isolated populations. Human Molecular Genetics 11: 2507–2515. [PubMed: 12351587]

82. Kristiansson K, Naukkarinen J, Peltonen L (2008) Isolated populations and complex disease gene identification. Genome Biol 9: 109. [PubMed: 18771588]

83. Rudan I (2006) Health effects of human population isolation and admixture. Croat Med J 47: 526–531. [PubMed: 16909449]

84. Laitinen T (2002) The value of isolated populations in genetic studies of allergic diseases. Curr Opin Allergy Clin Immunol 2: 379–382. [PubMed: 12582319]

Table 1:

Characteristics of the total population and American Indian.

Risk Factor	American Indian Population (n=50)	Rancher Population (n=14)
Average age in years (range)	44.3 (22.77 – 78.68)	53.14(40.96 – 94.21)
Males (% total)	31 (62%)	7 (50%)
Smokers (% total)	32 (64%)	4 (29%)
Obese (% total)	36 (72%)	4 (29%)
Diabetes (% total)	19 (38%)	1 (7%)
Elevated HbA1c (% total)	37 (74%)	0 (0%)
High Blood Pressure (% total)	16 (32%)	3 (21%)
High Cholesterol (% total)	14 (28%)	5(36%)
Hypertensive Retinopathy	21 (42%)	8 (57%)
Outside Working Conditions	10 (20%)	6 (43%)
BMI	37.38 (23.1– 65.5)	29.48 (20.9 – 41.5)
Average RORA Protein level (range)	44.117 (3.373 – 191.872)	49.352 (14.660 – 122.036)
Average ROBO Protein level (range)	3.367 (1.235 – 6.393)	25.608 (13.238 – 48.937)

Table 2:

Homozygous Risk Allele Frequencies in the American Indian (AI) and Rancher (R) population with and without Hypertensive Retinopathy.

SNP	Alleles	Effect	AI Normal (n =23)	AI HTR (n=21)	R Normal (n=6)	R HTR (n=8)
ARMS2 rs10490924	G/T	T is Risk	9 (39%)	14 (67%)	2 (33%)	4 (50%)
ARMS2 rs10664316	delAT	- is Protective	5 (22%)	10 (48%)	0 (0%)	1 (13%)
CFH rs1061170	T/C	C is Risk	0 (0%)	0 (0%)	0 (0%)	4 (50%)
CFH rs12144939	G/T	T is Protective	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CFH rs16840422	C/T	T is Protective	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CFH rs2284664	C/T	T is Protective	2 (9%)	1 (5%)	2 (33%)	6 (75%)
CFH rs800292	G/A	A is Protective	2 (9%)	1 (5%)	2 (33%)	6 (75%)
CFHR2 rs12066959	C/T	C is Risk	1 (4%)	1 (5%)	3 (50%)	6 (75%)
HTRA rs11200638	G/A	A is Risk	0 (0%)	1 (5%)	1 (17%)	0 (0%)
HTRA rs1049331	C/T	T is Risk	0 (0%)	1 (5%)	1 (17%)	0 (0%)
HTRA rs2293870	G/T/C	T/C is Risk	1 (4%)	1 (5%)	3 (50%)	1 (13%)
HTRA rs2672598	T/C	C is Risk	3 (13%)	2 (10%)	4 (67%)	4 (50%)
ROBO1 rs1387665	C/T	T is Risk	0 (0%)	1 (5%)	1 (17%)	2 (25%)
ROBO1 rs4513416	G/A	A is Protective	1 (4%)	0 (0%)	2 (33%)	2 (25%)
ROBO1 rs7622444	C/T	C is Risk	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ROBO1 rs9809833	T/C	C is Risk	3 (13%)	1 (5%)	0 (0%)	1 (13%)
RORA rs12900948	A/G	G is Protective	1 (4%)	1 (5%)	0 (0%)	1 (13%)
RORA rs730754	A/G	G is Protective	1 (4%)	1 (5%)	1 (17%)	3 (38%)
RORA rs8034864	C/A	A is Risk	3 (13%)	2 (10%)	2 (33%)	4 (50%)

Table 3:

Hypertensive retinopathy association results in the total population.

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Age (Years)	Continuous	1.050	(1.006 – 1.096)	.025
ARMS2 rs10490924	T Allele	1.227	(.073 – 20.763)	.887
ARMS2 rs10664316	AT	2.333	(.592 – 9.202)	.226
BMI	Continuous	1.038	(.977 – 1.102)	.227
Overweight	25<=BMI<30	2.273	(.379 – 13.632)	.369
Obese	BMI >= 30	1.484	(.473 – 4.656)	.498
CFH rs1061170	C Allele	1.267	(.370 – 4.337)	.707
CFH rs12144939	C Allele	.480	(.102 – 2.256)	.353
CFH rs16840422	C Allele	.590	(.090 – 3.846)	.581
CFH rs2284664	C Allele	1.548	(.510 – 4.702)	.441
CFH rs800292	G Allele	1.662	(.552 – 4.999)	.366
CFHR2 rs12066959	G Allele	.955	(.322 – 2.834)	.933
Cholesterol	Continuous	1.010	(.993 – 1.027)	.250
DM	Dichotomous	2.667	(.775 – 9.172)	.120
DM I	Dichotomous	2.154	(.184 – 25.187)	.541
DM II	Dichotomous	2.500	(.657 – 9.514)	.179
# of DM Rx	Continuous	1.423	(.756 – 2.679)	.274
Yrs. With DM	Continuous	1.078	(.950 – 1.226)	.247
HBA1C	Continuous	1.235	(.865 – 1.765)	.245
HBP	Dichotomous	4.800	(1.305 – 17.658)	.018
Yrs. With HBP	Continuous	1.043	(.943 – 1.153)	.412
5+ years with HBP	Dichotomous	2.947	(.671 – 12.952)	.152
HDL	Continuous	.988	(.952 – 1.025)	.505
HTRA rs11200638	A Allele	.556	(.183 – 1.691)	.300
HTRA1 rs1049331	T Allele	.476	(.160 – 1.419)	.183
HTRA1 rs2293870	C or T Allele	.475	(.1600 – 1.406)	.179
HTRA1 rs2672598	C Allele	.681	(.230 – 2.013)	.487
ROBO1 rs1387665	T Allele	.593	(.182 – 1.934)	.368
ROBO1 rs4513416	G Allele	.711	(.230 – 2.192)	.552
ROBO1 rs7622444	C Allele	2.391	(.419 – 13.636)	.326
ROBO1 rs9809833	C Allele	1.600	(.446 – 5.742)	.471
ROBO1 Elisa	Continuous	1.038	(.978 – 1.102)	.224
RORA rs12900948	A Allele	1.231	(.417 – 3.640)	.707
RORA rs730754	A Allele	1.662	(.552 – 4.999)	.366
RORA rs8034864	A Allele	.727	(.234 – 2.256)	.581
RORA Elisa	Continuous	.990	(.975 – 1.005)	.173
Female Sex	Dichotomous	1.773	(.616 – 5.102)	.288

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Smoking	Dichotomous	1.149	(.409 – 3.226)	.792
Poor Trigs	Dichotomous	3.000	(.950 – 9.475)	.061
VLDL	Continuous	1.027	(.986 – 1.070)	.201
Alcohol Consumption	Dichotomous	.588	(.190 – 1.825)	.358
Employment	Dichotomous	.300	(.070 – 1.281)	.104
Marital Status	Dichotomous	.659	(.233 – 1.859)	.431
Private Insurance	Dichotomous	1.000	(.319 – 3.135)	1.000
Education	Categorical	.868	(.475 – 1.588)	.647
LDL	Continuous	1.024	(.999 – 1.051)	.065
Vit. D exposure	Dichotomous	1.930	(.591 – 6.304)	.276

Table 4:

Hypertensive retinopathy association in American Indian population.

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Age (Years)	Continuous	1.026	(.984 – 1.070)	.225
ARMS2 rs10490924	T Allele	1.000	-	-
ARMS2 rs10664316	AT	1.810	(.297 – 11.027)	.520
BMI	Continuous	1.035	(.972 – 1.101)	.287
Overweight	25<=BMI<30	1.000	-	-
Obese	BMI >= 30	1.263	(.302 – 5.275)	.749
CFH rs1061170	C Allele	1.222	(.2195 – 6.807)	.819
CFH rs12144939	C Allele	.367	(.035 – 3.817)	.401
CFH rs16840422	C Allele	1.000	-	-
CFH rs2284664	C Allele	1.231	(.383 – 3.955)	.727
CFH rs800292	G Allele	1.100	(.341 – 3.551)	.873
CFHR2 rs12066959	G Allele	.664	(.179 – 2.460)	.540
Cholesterol	Continuous	1.012	(.995 – 1.030)	.181
DM	Dichotomous	1.583	(.490 – 5.117)	.443
DM I	Dichotomous	.877	(.133 – 5.783)	.892
DM II	Dichotomous	1.833	(.510 – 6.593)	.353
# of DM Rx	Continuous	.908	(.529 – 1.559)	.727
Yrs. With DM	Continuous	1.024	(.914 – 1.147)	.682
HBA1C	Continuous	1.101	(.805 – 1.505)	.547
HBP	Dichotomous	2.625	(.750 – 9.19)	.131
Yrs. With HBP	Continuous	1.030	(.936 – 1.133)	.550
5+ years with HBP	Dichotomous	3.00	(.655 – 13.747)	.157
HDL	Continuous	.989	(.952 – 1.028)	.574
HTRA rs11200638	A Allele	.500	(.149 – 1.676)	.216
HTRA1 rs1049331	T Allele	.429	(.130 – 1.410)	.163
HTRA1 rs2293870	C or T Allele	.429	(.130 – 1.410)	.163
HTRA1 rs2672598	C Allele	.410	(.125 – 1.348)	.142
ROBO1 rs1387665	T Allele	.941	(.217 – 4.074)	.935
ROBO1 rs4513416	G Allele	.418	(.107 – 1.632)	.210
ROBO1 rs7622444	C Allele	1.158	(.149 – 9.029)	.889
ROBO1 rs9809833	C Allele	.990	(.254 – 3.857)	.998
ROBO1 Elisa	Continuous	1.043	(.613 – 1.773)	.878
RORA rs12900948	A Allele	.931	(.288 – 3.011)	.905
RORA rs730754	A Allele	.931	(.288 – 3.011)	.905
RORA rs8034864	A Allele	.786	(.241 – 2.556)	.689
RORA Elisa	Continuous	.982	(.959 – 1.007)	.157
Female Sex	Dichotomous	1.583	(.490 – 5.117)	.443

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Smoking	Dichotomous	.903	(.280 – 2.914)	.864
Poor Trigs	Dichotomous	3.84	(1.037 – 14.213)	.044
VLDL	Continuous	1.027	(.984 – 1.072)	.216
Alcohol Consumption	Dichotomous	.583	(.148 – 2.294)	.440
Employment	Dichotomous	.638	(.125 – 3.256)	.588
Marital Status	Dichotomous	.992	(.303 – 3.242)	.989
Private Insurance	Dichotomous	1.371	(.343 – 5.488)	.655
Education	Categorical	1.048	(.505 – 2.176)	.899
LDL	Continuous	1.026	(.999 – 1.054)	.058
Vitamin D exposure	Dichotomous	1.063	(.258 – 4.374)	.933

Table 5:

Hypertensive retinopathy association in American Indian population with the condition.

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Age (Years)	Continuous	.894	(.792 – 1.009)	.069
ARMS2 rs10490924	T Allele	1.000	-	-
ARMS2 rs10664316	AT	3.800	(.424 – 34.077)	.233
BMI	Continuous	1.107	(.965 – 1.269)	.146
Overweight	25<=BMI<30	1.000	-	-
Obese	BMI >= 30	5.333	(.834 – 34.092)	.077
CFH rs1061170	C Allele	.067	(.009 – .515)	.009
CFH rs12144939	C Allele	.125	(.009 – 1.671)	.116
CFH rs16840422	C Allele	1.000	-	-
CFH rs2284664	C Allele	1.000	-	-
CFH rs800292	G Allele	1.000	-	-
CFHR2 rs12066959	G Allele	1.000	-	-
Cholesterol	Continuous	1.010	(.983 – 1.038)	.486
DM	Dichotomous	4.500	(.457 – 44.287)	.197
DM I	Dichotomous	1.000	-	-
DM II	Dichotomous	3.000	(.300 – 30.019)	.350
# of DM Rx	Continuous	1.187	(.447 – 3.152)	.730
Yrs. With DM	Continuous	1.270	(.875 – 1.843)	.209
HBA1C	Continuous	1.187	(.633 – 2.225)	.593
HBP	Dichotomous	.75	(.122 – 4.623)	.757
Yrs. With HBP	Continuous	1.072	(.851 – 1.352)	.554
5+ Years with HBP	Dichotomous	1.000	-	-
HDL	Continuous	.986	(.927 – 1.05)	.646
HTRA rs11200638	A Allele	.667	(.116 – 3.838)	.650
HTRA1 rs1049331	T Allele	.667	(.116 – 3.838)	.650
HTRA1 rs2293870	C or T Allele	.375	(.065 – 2.159)	.272
HTRA1 rs2672598	C Allele	.246	(.038 – 1.583)	.140
ROBO1 rs1387665	T Allele	.314	(.049 – 1.998)	.220
ROBO1 rs4513416	G Allele	.094	(.013 – .674)	.019
ROBO1 rs7622444	C Allele	.140	(.017 – 1.134)	.065
ROBO1 rs9809833	C Allele	.417	(.069 – 2.527)	.341
ROBO1 Elisa	Continuous	5.322	-	-
RORA rs12900948	A Allele	.440	(.069 – 2.798)	.384
RORA rs730754	A Allele	1.000	-	-
RORA rs8034864	A Allele	.183	(.019 – 1.799)	.145
RORA Elisa	Continuous	.963	(.926 – 1.002)	.060
Female Sex	Dichotomous	.450	(.085 – 2.395)	.349

Risk Factor	Risk	Odds Ratio	95% CI	p-value
Smoking	Dichotomous	2.708	(.504 – 14.541)	.245
Poor Trigs	Dichotomous	1.280	(.187 – 8.756)	.801
VLDL	Continuous	1.013	(.942 – 1.091)	.721
Alcohol Consumption	Dichotomous	14.000	(1.371 – 142.887)	.026
Employment	Dichotomous	.832	(.283 – 2.449)	.739
Marital Status	Dichotomous	.909	(.178 – 4.636)	.909
Private Insurance	Dichotomous	.806	(.336 – 1.934)	.630
Education	Categorical	.832	(.283 – 2.449)	.739
LDL	Continuous	1.011	(.980 – 1.044)	.486
Vit. D exposure	Dichotomous	.240	(.043 – 1.335)	.103

Table 6:

Multivariate analysis of the Total population and the HTR population.

	Total Population	
Risk factor	Odds ratio (95% CI)	p-value
Age	1.034 (.988–1.083)	.145
High blood pressure	3.667 (.951–14.141)	.059
	American Indian Population	
Risk factor	Odds ratio (95% CI)	p-value
LDL	1.025 (.994–1.056)	.114
Poor Trigs	4.228 (.998–17.912)	.050
	HTR Population	
Risk factor	Odds ratio (95% CI)	p-value
Alcohol Consumption	14.947 (.578–386.389)	.103
CFH rs1061170	.039 (.002 – .841)	.038
ROBO1 rs4513416	.104 (.005–2.332)	.154

Table 7:

Allele Frequencies of American Indians and Ranchers in Normal Subjects.

SNP	Retinal Disease Effect	Alleles	American Indian	Rancher	Odds Ratio (95% CI)	p-value
ARMS2 rs10490924	T is Risk	G/T	1.00	1.000	-	-
ARMS2 rs10664316	- is Protective	-AT	.800	.800	1.000 (.086–11.588)	1.000
CFH rs1061170	C is Risk	T/C	.200	.400	.375 (.046 – 3.056)	.360
CFH rs12144939	T is Protective	G/T	.150	.600	.118 (.013–1.03)	.053
CFH rs16840422	T is Protective	C/T	.095	.400	.158 (.016–1.586)	.117
CFH rs2284664	T is Protective	C/T	.524	1.000	-	-
CFH rs800292	A is Protective	G/A	.524	.600	.733 (.101–5.330)	.759
CFHR2 rs12066959	G is Risk	A/G	.350	1.000	-	-
HTRA rs11200638	A is Risk	G/A	.400	.474	1.350 (.182–10.006)	.769
HTRA1 rs1049331	T is Risk	C/T	.524	.400	1.650 (.227–11.993)	.621
HTRA1 rs2293870	T/C is Risk	G/T/C	.524	.600	.733 (.101–5.330)	.759
HTRA1 rs2672598	C is Risk	T/C	.600	.600	1.000(.135–7.392)	1.000
ROBO1 rs1387665	T is Risk	C/T	.400	.300	.643 (.085–4.889)	.669
ROBO1 rs4513416	A is Protective	G/A	.450	.400	1.227 (.167–9.017)	.840
ROBO1 rs7622444	C is Risk	T/C	.158	.000	-	-
ROBO1 rs9809833	C is Risk	T/C	.200	.200	1.000 (.086–11.588)	1.000
RORA rs12900948	G is Protective	A/G	.600	.400	2.250 (.304–16.632)	.427
RORA rs730754	G is Protective	A/G	.600	.400	2.250 (.304–16.632)	.427
RORA rs8034864	A is Risk	C/A	.600	.800	.375(.035–3.999)	.417

Table 8:

Characteristics of normal subjects within different populations.

Characteristic	Timor	NESC	Ranchers	Greek	Korean	American Indian
n	533	198	6	213	384	23
Males (% total)	268 (50.1%)	87 (43.9%)	4 (66.7%)	100 (46.9%)	194 (50.5%)	19 (65.5%)
Age (range)	55.12 (40–94)	75.78 (48–95)	44.18(21–61)	73.78 (48–95)	68.46 (50–87)	41.75 (22–78)

Table 9:

Allele Frequencies of normal population that was genotyped.

SNP	Retinal Disease Effect	Alleles	Timor	NESC	Ranchers	Greek	Korean	American Indian
ARMS2 rs10490924	T is Risk	G/T	.336	.311	1.000	.237	.398	1.00
ARMS2 rs10664316	- is Protective	-AT	.331	.396	.800	.415	.358	.800
CFH rs1061170	C is Risk	T/C	.067	.429	.400	.373	.069	.200
CFH rs12144939	T is Protective	G/T	.146	.170	.600	.213	.053	.150
CFH rs16840422	T is Protective	C/T	.155	.131	.400	.110	.083	.095
CFH rs2284664	T is Protective	C/T	.613	.197	1.000	.207	.364	.524
CFH rs800292	A is Protective	G/A	.688	.205	.600	.220	.397	.524
CFHR2 rs12066959	G is Risk	A/G	-	-	1.000	-	-	.350
HTRA rs11200638	A is Risk	G/A	.635	.303	1.000	.226	.405	.400
HTRA1 rs1049331	T is Risk	C/T	.358	.306	.400	.231	.407	.524
HTRA1 rs2293870	T/C is Risk	G/T/C	.356	.397	.600	.390	.407	.524
HTRA1 rs2672598	C is Risk	T/C	.523	.492	.600	.534	.711	.600
ROBO1 rs1387665	T is Risk	C/T	.553	.497	.300	.493	.380	.400
ROBO1 rs4513416	A is Protective	G/A	.346	.413	.400	.407	.468	.450
ROBO1 rs7622444	C is Risk	T/C	-	-	.000	-	-	.158
ROBO1 rs9809833	C is Risk	T/C	.178	.124	.200	.172	.308	.200
RORA rs12900948	G is Protective	A/G	.413	.410	.400	.502	.580	.600
RORA rs730754	G is Protective	A/G	.420	.397	.400	.468	.618	.600
RORA rs8034864	A is Risk	C/A	.417	.209	.800	.290	.587	.600