

Mitochondrial Haplogroups Affect Severity But Not Prevalence of Diabetic Retinopathy

Jana A. Bregman,¹ David J. Herren,¹ Christopher B. Estopinal,¹ Isaac M. Chocron,¹ Paula A. Harlow,¹ Cassandra Warden,¹ Milam A. Brantley Jr,¹ and David C. Samuels²

¹Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, Tennessee, United States

²Vanderbilt Genetics Institute and Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee, United States

Correspondence: Milam A. Brantley Jr, Vanderbilt Eye Institute, Vanderbilt University Medical Center, 2311 Pierce Avenue, Nashville, TN 37232-8808, USA; milam.brantley@vanderbilt.edu.

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PURPOSE. We previously reported European mitochondrial haplogroup H to be a risk factor for and haplogroup UK to be protective against proliferative diabetic retinopathy (PDR) among Caucasian patients with diabetic retinopathy (DR). The purpose of this study was to determine whether these haplogroups are also associated with the risk of having DR among Caucasian patients with diabetes.

METHODS. Deidentified medical records for 637 Caucasian patients with diabetes (223 with DR) were obtained from BioVU, Vanderbilt University's electronic, deidentified DNA databank. An additional 197 Caucasian patients with diabetes (98 with DR) were enrolled from the Vanderbilt Eye Institute (VEI). We tested for an association between European mitochondrial haplogroups and DR status.

RESULTS. The percentage of diabetes patients with DR did not differ across the haplogroups ($P = 0.32$). The percentage of patients with nonproliferative DR (NPDR; $P = 0.0084$) and with PDR ($P = 0.027$) significantly differed across the haplogroups. In logistic regressions adjusting for sex, age, diabetes type, duration of diabetes, and hemoglobin A1c, neither haplogroup H nor haplogroup UK had a significant effect on DR compared with diabetic controls. Haplogroup UK was a significant risk factor (OR = 1.72 [1.13-2.59], $P = 0.010$) for NPDR compared with diabetic controls in the unadjusted analysis, but not in the adjusted analysis (OR = 1.29 [0.79-2.10], $P = 0.20$).

CONCLUSIONS. Mitochondrial haplogroups H and UK were associated with severity, but not presence, of DR. These data argue that the effect of these haplogroups is related to ischemia and neovascularization, the defining features of PDR.

Keywords: diabetes, diabetic retinopathy, genetics, mitochondrial haplogroup, mitochondrial genetics, mitochondrial DNA

Diabetic retinopathy (DR) is the leading cause of preventable cases of blindness in U.S. working-age adults.¹ It was estimated in 2012 that 9.3% of the U.S. population, or approximately 29 million people, had diabetes.² Given recent increases in diabetes incidence, it is predicted that diabetes prevalence will reach as high as 25% to 28% by 2050.³ It is projected that this will result in 16 million Americans older than 40 with DR and 3.4 million with vision-threatening DR by 2050.⁴ Research to better understand the pathophysiology of DR is thus needed to help prevent and manage vision loss associated with DR.

Mitochondrial dysfunction has been implicated in DR pathogenesis, most likely due to the susceptibility of mitochondrial DNA to oxidative stress-induced damage.^{5,6} Mitochondrial haplogroups are specific patterns of point mutations in mitochondrial DNA that are thought to lead to subtle variation in mitochondrial function.⁷ Oxidative and other stress conditions may accentuate these subtle variations, contributing to the potential association of mitochondrial haplogroups and DR.⁸⁻¹¹ We recently reported a link between European mitochondrial haplogroups H and UK and DR severity in a cohort of DR patients from BioVU, Vanderbilt University

Medical Center's deidentified DNA databank, and from the Vanderbilt Eye Institute (VEI).¹⁰ In that study, we demonstrated that DR patients from haplogroup H were more likely to have proliferative DR (PDR), whereas DR patients from haplogroup UK were less likely to have PDR.

Given the results of our previous work, it is possible that haplogroup H is simply associated with worsening complications of diabetes, whereas haplogroup UK is protective against such complications. If this is the case, we might expect a similar association of these haplogroups to the presence of DR, in addition to the effect on DR severity. The purpose of the current study was to determine whether haplogroups H and UK are also associated with risk and protection from DR, and more specifically nonproliferative DR (NPDR), among patients with diabetes.

MATERIALS AND METHODS

Ethics Statement

The clinical case-control study was approved by the Vanderbilt University Human Research Protection Program. Research



adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with Health Insurance Portability and Accountability Act regulations. Written informed consent was obtained before study enrollment for all patients enrolled at the VEI. All BioVU projects are reviewed by the Vanderbilt University Human Research Protection Program and are classified as nonhuman subjects' research before study initiation. DNA was extracted from discarded blood samples drawn for routine medical care at Vanderbilt outpatient clinics. The electronic medical records were deidentified following provisions of Title 45, Code of Federal Regulations, part 46, regarding protection of human subjects. A more extensive description of the ethical oversight of BioVU has been published.¹²

BioVU Patients

BioVU is the Vanderbilt University Medical Center's repository of DNA extracted from discarded blood collected during routine clinical testing and linked to a deidentified copy of the electronic medical record (the Synthetic Derivative [SD]).^{12,13} For deidentification purposes, all dates in each SD record are consistently shifted backward by a random amount up to 364 days. The SD has search functions for International Classification of Diseases, Ninth Revision (ICD-9) and Current Procedural Terminology (CPT) codes to identify patients of interest for research studies.

We previously identified a cohort of DR patients from BioVU ($n = 291$) to examine the relationship between mitochondrial haplogroups and severity of DR.¹⁰ A subset of these DR patients with complete covariate data is included in the current study (BioVU DR cohort, $n = 223$). The methodology used to identify these DR patients is detailed in Estopinal et al.,¹⁰ and is briefly summarized here. We first identified all Caucasian individuals in the SD with available genome-wide variant data who had ICD-9 codes for both diabetes and DR. We manually reviewed the SD charts of these patients to confirm the diagnoses. Diabetes diagnosis was confirmed by presence of a diabetes ICD-9 code and an internal medicine or endocrinology visit on the same day. Diagnosis of DR was confirmed by presence of a DR code and an ophthalmology CPT code. To determine severity of DR (NPDR or PDR), we identified DR ICD-9 codes occurring on the same day as an ophthalmology CPT code. If DR severity could not be definitively determined from the codes, clinic notes and letters from the SD were reviewed in detail to assign severity status.

Age for each patient was reported as the age at most recent patient encounter or at death. Diabetes type (type 1 or type 2) was determined from endocrinology notes when available. If endocrinology notes were not available, diabetes type was determined by reviewing problem lists and internal medicine notes. Hemoglobin A1c (HgbA1c) for each patient was reported as the median of all values in the SD. Age at diagnosis was determined by identifying specific references in SD notes to the patient's initial diabetes diagnosis. Duration of diabetes was calculated as the difference between the age at diagnosis and the age at the most recent patient encounter or at death. All assessments of the SD were performed blinded to the genetic data.

For the current study, we identified a new group of BioVU patients who had diabetes and did not have DR (BioVU Diabetic control cohort, $n = 414$). We required each of these patients to have a diabetes diagnosis as well as a minimum of one dilated ophthalmology examination without evidence of DR and the absence of DR on all dilated ophthalmology examinations. We first identified all Caucasian individuals in

the SD with available genome-wide variant data who had ICD-9 codes for diabetes (250.0, 250.00–250.03) but did not have a code for DR (362.0–362.07). A total of 891 individuals met these criteria, and their SD charts were manually reviewed under the supervision of a fellowship-trained retina specialist (MAB) to confirm the diagnosis. Diabetes diagnosis was confirmed by presence of a diabetes ICD-9 code and an internal medicine or endocrinology visit on the same day, and patients with inconsistent or questionable documentation of diabetes in clinic notes or letters from the SD were excluded. Proof of a dilated eye examination (as evidence that the patient had been examined for DR) was confirmed by the presence of a high-level ophthalmology CPT code (92004 or 92014). The absence of DR was confirmed for these patients by searching for the terms “retinopathy,” “DR,” “NPDR,” and “PDR” in the SD chart and ensuring that these diagnoses were not present. Demographic and clinical characteristics for these patients were obtained as described above for the BioVU DR cohort.

Vanderbilt Eye Institute Cohorts

We previously enrolled a cohort of adult DR patients from the Retina Division of the VEI ($n = 101$) to examine the relationship between mitochondrial haplogroups and severity of DR.¹⁰ A subset of these DR patients with complete covariate data is included in the current study (VEI DR cohort, $n = 98$). All patients were required to have a diagnosis of diabetes made by their primary care provider or endocrinologist and to be taking at least one diabetes medication (insulin or an oral medication). Patients were diagnosed with DR based on a comprehensive dilated ophthalmologic examination by a fellowship-trained retina specialist, and each patient was classified as having either NPDR or PDR. Nonproliferative DR was diagnosed based on the presence of blot hemorrhages, microaneurysms, cotton-wool spots, or intraretinal microvascular abnormalities, and the absence of signs or history of retinal neovascularization. Proliferative DR was diagnosed based on presence of iris or retinal neovascularization, or evidence of treatment for PDR with laser photocoagulation. Retinopathy status was documented by high-resolution color fundus photography.

For the current study, we enrolled from the Retina Division of the VEI a new group of patients who had diabetes and did not have DR (VEI diabetic control cohort, $n = 99$). Patients were required to have a diagnosis of diabetes made by their primary care provider or endocrinologist and to be taking at least one diabetes medication. Absence of DR was confirmed by a comprehensive dilated ophthalmology examination by a fellowship-trained retinal specialist and fundus photography when indicated.

For all VEI patients, medical history was obtained from the electronic medical record (EMR). The HgbA1c value reported is the median of all HgbA1c values in the EMR. Enrollment exclusion criteria for the VEI cohort included the presence of non-DR, glaucoma, active uveitis or ocular infection, or ocular surgery within 60 days before enrollment.

At the time of study enrollment, all VEI patients underwent venipuncture to provide a blood sample and responded to a standardized set of questions regarding disease history. Blood was collected from study participants using a 21- or 23-gauge butterfly needle. For each participant, approximately 8 mL of blood was drawn into a 10-mL K₂ EDTA blood collection tube. These samples were delivered to the Vanderbilt Technologies for Advanced Genetics (VANTAGE) Center for DNA isolation and storage.

TABLE 1. Demographics

Characteristic	Diabetic Controls	Any DR	NPDR	PDR	Diabetic Controls vs. DR	Diabetic Controls vs. NPDR	NPDR vs. PDR
<i>n</i>	513	321	154	167	NA	NA	NA
Female, <i>n</i> (%)	257 (50)	137 (43)	71 (46)	66 (40)	0.04	0.44	0.28
Median age, y [IQR]	68 [58-77]	62 [52-72]	66 [58-76]	57 [49-67]	<0.0001	0.38	<0.0001
Median diabetes duration, y [IQR]	9 [6-14]	24 [16-33]	20 [13-26]	29 [20-38]	<0.0001	<0.0001	<0.0001
Type 1 diabetes, <i>n</i> (%)	38 (7)	109 (34)	36 (23)	73 (44)	<0.0001	<0.0001	0.00020
Median HgbA1c, % [IQR]	6.9 [6.4-7.6]	7.9 [7.1-8.7]	7.6 [6.9-8.5]	8.2 [7.3-9.0]	<0.0001	<0.0001	0.0022

Genotyping and Mitochondrial Haplogroup Determination

BioVU subjects had been previously genotyped on the Illumina 660W, Illumina 1M, or the Illumina Infinium Human-Exome BeadChip (Illumina, San Diego, CA, USA), and the genotyping data had been deposited in BioVU for use in additional research projects.¹⁴ The Illumina 660W and 1M genotyping chips contain 138 single-nucleotide polymorphisms (SNPs) from the mitochondrial genome, whereas the Exome chip contains 219 mitochondrial DNA (mtDNA) SNPs. A detailed list of the mtDNA SNPs used for genotyping in this project is provided in the Supplementary Materials. For each individual, a variant list was defined based on the differences from the standard mtDNA reference, the revised Cambridge Reference Sequence.¹⁵ This variant list was used to determine the mitochondrial haplogroup of each BioVU subject using HaploGrep.^{16,17}

For the VEI cohort, a pool of 22 mtDNA SNPs was designed to identify the standard European mitochondrial haplogroups. These SNPs are listed in the Supplementary Materials. DNA samples from the VEI cohort were genotyped using the MassARRAY System (Agena Bioscience, San Diego, CA, USA) in the VANTAGE Center. As with the BioVU cohort, a variant list for each subject was then generated and HaploGrep was used to identify the mitochondrial haplogroup of each subject.

Statistics

Logistic regressions with DR status as the outcome variables were performed both without adjustment and adjusting for sex, age, diabetes type (type 1 versus type 2), diabetes duration, and HgbA1c level. Statistics were calculated in R (<https://www.r-project.org/>, in the public domain). Error bars on proportions were calculated from the sampling error $\pm 2 \text{ SQRT}(p[1-p]/N)$, where N is the sample size. Multiple testing correction for four tested haplogroups was carried out by Bonferroni correction of the significance threshold of the P value to $P < 0.05/4 = 0.0125$.

RESULTS

Patient demographics are summarized in Table 1. Patients were classified as either diabetic controls (those with diabetes but without retinopathy) or those with diabetic retinopathy. The latter group was then split into those patients with DR but no indication of PDR (classed as NPDR), and those with PDR at any time in their medical record. The percentage of females in the three cohorts (diabetic controls, NPDR, and PDR) was similar. The median age of the PDR group was approximately 10 years younger than the other groups, primarily due to the larger proportion of type 1 diabetes patients in that group. As expected, the median duration of diabetes and the median HgbA1c increased as the severity of retinopathy increased, and these differences were highly significant.

For our primary analysis, we evaluated the two largest European mitochondrial haplogroups H and UK and grouped all smaller haplogroups into the category "other." Most of our subjects (68%) were in either haplogroup H or UK. The proportion of diabetic patients with NPDR, with PDR, and with any DR (NPDR + PDR) was determined for each of these haplogroups (Fig. 1). The percentage of diabetic patients who had any DR (NPDR + PDR) was not significantly different across the haplogroups ($P = 0.32$; Fig. 1A). The percentage of patients with NPDR did significantly differ across the haplogroups ($P = 0.0084$), with haplogroup UK having the largest proportion of NPDR patients (Fig. 1B). The percentage of patients with PDR also significantly differed across the

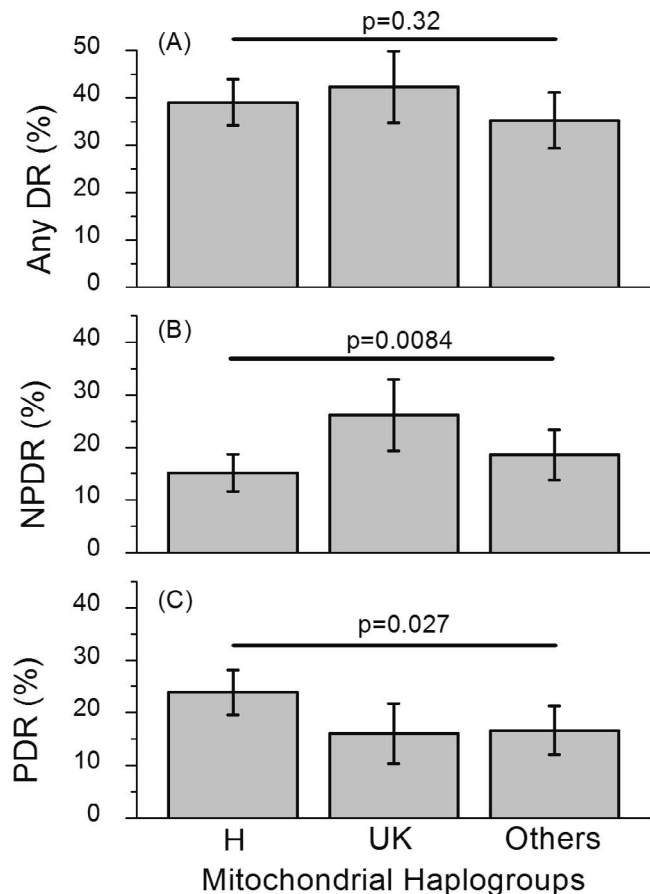


FIGURE 1. Distribution of the DR disease states across the major European mitochondrial haplogroups H and UK. (A) Proportion of patients in each haplogroup with any diabetic retinopathy (NPDR and PDR combined). (B) Proportion with NPDR (H = 61/402, UK = 44/168, other = 49/264). (C) Proportion with PDR (H = 96/402, UK = 27/168, other = 44/264). P values are from χ^2 tests.

TABLE 2. Unadjusted Logistic Regressions

Variable	Diabetic Controls vs. DR	P	Diabetic Controls vs. NPDR	P	NPDR vs. PDR	P
Haplogroup H, OR [95% CI]	1.05 [0.79–1.38]	0.75	0.72 [0.50–1.04]	0.075	2.06 [1.32–3.22]	0.0014
Haplogroup UK, OR [95% CI]	1.22 [0.86–1.72]	0.26	1.72 [1.13–2.59]	0.010	0.48 [0.28–0.83]	0.0075

haplogroups ($P = 0.027$), with haplogroup H having the highest proportion of PDR patients (Fig. 1C).

To determine if key demographic and clinical covariates affect the relationship between mitochondrial haplogroups and DR status, we performed logistic regressions for the effect of haplogroups H and UK on DR status. We first carried out simple regressions without adjustments (Table 2). We then performed regressions adjusting for sex, age, diabetes type, duration of diabetes, and HgbA1c (Tables 3, 4). Figure 2 compares the results of the unadjusted and adjusted regressions evaluating the effect of haplogroup H and of haplogroup UK on diabetic controls versus all DR, diabetic controls versus NPDR, and NPDR versus PDR.

Haplogroup H was not significant in the comparison of all DR patients with diabetic controls in either the unadjusted or adjusted regression (Fig. 2A), indicating that in our cohort, haplogroup H was not a significant factor for prevalence of DR among diabetic individuals. Comparison of NPDR patients with diabetic controls showed a trend toward a protective effect for haplogroup H, which was not greatly changed by adjusting for covariates (Fig. 2A, unadjusted odds ratio [OR] [95% confidence interval (CI)] 0.72 [0.50–1.04], $P = 0.075$; adjusted OR 0.75 [0.50–1.15], $P = 0.20$). Finally, comparing PDR patients with NPDR patients (Fig. 2A), haplogroup H was a significant risk factor for PDR in the unadjusted analysis (OR 2.06 [1.32–3.22], $P = 0.0014$) and had a slightly stronger effect in the adjusted regression (OR 2.29 [1.40–3.80] $P = 0.0011$).

Haplogroup UK also was not significant in the comparison of all DR patients with diabetic controls in either the unadjusted or adjusted regressions (Fig. 2B). Haplogroup UK was a significant risk factor (OR 1.72 [1.13–2.59], $P = 0.010$) for NPDR compared with diabetic controls in the unadjusted analysis. However, in the adjusted analysis, the effect of UK was greatly weakened and was no longer significant (OR 1.29 [0.79–2.10], $P = 0.31$; Fig. 2B). Note that this loss of significance occurs primarily due to the decrease in the OR once the covariates were added to the regression, and not simply due to a loss of statistical power from the additional covariates. This indicates that the effect of haplogroup UK on NPDR is confounded by one or more of the covariates (sex, age, diabetes type, duration of diabetes, and HgbA1c). In contrast, for the PDR versus NPDR comparison, haplogroup UK had a significant protective effect on PDR compared with NPDR in the unadjusted analysis (OR 0.48 [0.28–0.83], $P = 0.0075$), and this protective effect was slightly strengthened in the adjusted analysis (OR 0.38 [0.21–0.70], $P = 0.0018$; Fig. 2B).

As a secondary analysis, we tested the association of the two next most common European haplogroups, J and T, with DR.

Ten percent (83/834) of our study subjects were from haplogroup J and 12% (101/834) were from haplogroup T. Logistic regression adjusted for the same covariates as above found no significant associations for either J or T with either DR severity or prevalence (Supplementary Tables S1, S2). However, the power to test these lower-frequency haplogroups is limited, and the CIs on the measured ORs are large. As an additional secondary analysis, we compared diabetic controls and patients with PDR by logistic regression, adjusting for sex, age, diabetes duration, diabetes type, and HgbA1c levels as in Tables 3 and 4. In these comparisons, neither haplogroup H (OR 1.01 [0.60–1.70], $P = 0.96$) nor haplogroup UK (OR 0.82 [0.4–1.6], $P = 0.58$) was significant (Supplementary Tables S3, S4).

DISCUSSION

We previously reported that DR patients from haplogroup H are more likely to have PDR, and DR patients from haplogroup UK are less likely to have PDR.¹⁰ We wanted to determine whether haplogroups H and UK are also associated with risk of and protection from DR in patients with diabetes. The present study showed no association of haplogroup H or haplogroup UK with the presence of DR among patients with diabetes, indicating that these haplogroups affect severity, but not presence of DR. Together, these findings demonstrate that haplogroups H and UK are associated specifically with PDR in this cohort. This suggests that their risk and protective effects are not simply related to mechanisms of diabetes progression, but may be fundamentally related to the ischemia and neovascularization of PDR.

To look more closely at the relationship between mitochondrial haplogroups and early DR, we asked specifically whether haplogroups H and UK influenced the presence of NPDR compared with diabetic controls. Haplogroup H showed no association with NPDR in either the unadjusted or adjusted analyses. In contrast, haplogroup UK was positively associated with NPDR in the unadjusted analysis, but the effect size decreased and lost significance after adjusting for sex, age, type of diabetes, duration of diabetes, and HgbA1c. This finding suggests that haplogroup UK's effect on DR status is related to one or more of these covariates. This degree of shift in effect size with covariate adjustment was not seen for any of the other comparisons.

The association of mitochondrial haplogroups with DR was first studied by Kofler et al.,⁸ who reported a weak association of haplogroup T with the presence of DR in patients with type 2 diabetes ($P = 0.046$). That study was small, including only

TABLE 3. Adjusted Logistic Regressions on Haplogroup H

Variable	Diabetic Controls vs. DR	P	Diabetic Controls vs. NPDR	P	NPDR vs. PDR	P
Haplogroup H, OR [95% CI]	0.89 [0.62–1.29]	0.55	0.75 [0.50–1.15]	0.20	2.29 [1.40–3.80]	0.0011
Sex, OR [95% CI]	0.65 [0.45–0.95]	0.025	0.82 [0.53–1.24]	0.35	0.55 [0.33–0.91]	0.021
Age, OR [95% CI]	1.00 [0.98–1.01]	0.69	1.01 [0.99–1.03]	0.20	0.95 [0.93–0.98]	0.00017
Diabetes duration, OR [95% CI]	1.16 [1.13–1.19]	<2E-16	1.12 [1.09–1.15]	<2E-16	1.08 [1.05–1.11]	1.9E-7
Diabetes type, OR [95% CI]	1.01 [0.48–2.10]	0.98	0.60 [0.25–1.42]	0.25	2.03 [0.92–4.60]	0.085
HgbA1c, OR [95% CI]	1.68 [1.43–1.97]	2.6E-10	1.62 [1.35–1.96]	3.9E-7	1.20 [1.00–1.45]	0.058

TABLE 4. Adjusted Logistic Regressions on Haplogroup UK

Variable	Diabetic Controls vs. DR	P	Diabetic Controls vs. NPDR	P	NPDR vs. PDR	P
Haplogroup UK, OR [95% CI]	1.08 [0.68-1.70]	0.75	1.29 [0.79-2.10]	0.31	0.38 [0.21-0.70]	0.0018
Sex, OR [95% CI]	0.65 [0.45-0.95]	0.027	0.83 [0.54-1.27]	0.39	0.56 [0.33-0.92]	0.025
Age, OR [95% CI]	1.00 [0.98-1.01]	0.68	1.01 [0.99-1.03]	0.23	0.95 [0.92-0.97]	9.1e-5
Diabetes duration, OR [95% CI]	1.16 [1.13-1.18]	<2E-16	1.11 [1.09-1.14]	2.6E-16	1.08 [1.05-1.11]	1.3E-7
Diabetes type, OR [95% CI]	1.01 [0.48-2.11]	0.97	0.62 [0.26-1.48]	0.29	2.23 [1.00-5.14]	0.053
HgbA1c, OR [95% CI]	1.67 [1.43-1.97]	3.2E-10	1.61 [1.34-1.95]	5.5E-7	1.21 [1.00-1.45]	0.052

149 DR cases and 78 diabetic controls without retinopathy. Our larger study found no significant association of DR with haplogroup T. In their study, Kofler et al.⁸ found no association of haplogroup T with PDR. Achilli et al.⁹ later reported a strong positive association of haplogroup H with DR in patients with type 2 diabetes (OR 2.0 [1.3-3.1]). In contrast, we found no

association between haplogroup H and DR. However, it is notable that Achilli et al.⁹ separated the H and HV clades instead of combining them as we did. If their H and HV data had been combined, the effect of that combined group would have been much weaker (OR 1.53 [1.02-2.30], $P=0.039$), and there would have been more overlap between their CIs and ours (Table 2). Additionally, it was not clear whether the analyses in Achilli et al.⁹ were adjusted for clinical and demographic covariates. A recent study of patients with type 2 diabetes by Martikainen et al.¹¹ demonstrated a protective effect of haplogroup U against vascular complications, including DR, ischemic heart disease, ischemic stroke or transient ischemic attack, peripheral artery occlusive disease, and nephropathy. These vascular complications were analyzed together, and we cannot directly compare their results with our data. It is possible that haplogroup UK provides a broad protective effect against vascular complications of diabetes, and that we are detecting this in our study as a protection against PDR.

The strengths of this study include the use of large cohorts of patients with DR and diabetic patients without retinopathy. We have demonstrated the feasibility of using BioVU, a medical center-wide deidentified database, to identify not only patients with well-classified DR, but also patients with a diagnosis of diabetes and no signs of retinopathy on ocular examination. As in our previous work,¹⁰ manual review of the SD charts provided the most precise possible phenotyping.

The study is limited by the constraints inherent in any deidentified database search. Codes from ICD-9 and CPT codes are used for billing purposes and are often not sufficient to fully characterize clinical phenotypes. Clinic notes and other pertinent information are not available on all patients. The common presence of vague DR codes (e.g., 362.01, diabetic retinopathy not otherwise specified) in the SD did not allow us to fully characterize diabetes phenotypes in all patients meeting initial screening criteria. Sample size constraints led us to group the less common mitochondrial haplogroups into a single "other" group for the primary analyses, and a larger cohort would be necessary to allow detailed investigation of these haplogroups as well. Finally, our analysis included some diabetic controls ($n=88$, 17%) with duration of diabetes less than 5 years. Adding the requirements that all diabetic controls have duration of diabetes ≥ 5 years did not affect the results of the analyses (Supplementary Tables S5, S6).

In this study, we found that, although mitochondrial haplogroup H is a risk factor for PDR and haplogroup UK is protective against PDR among DR patients, there was no association of haplogroup H or UK with the presence of DR or of NPDR when compared with diabetic controls.

The fact that the significant association of haplogroup UK with NPDR is greatly weakened and not significant after adjustment for clinically relevant covariates indicates that the effect of UK on NPDR acts through these covariates. The association of haplogroups H and UK with DR severity but not with DR prevalence argues that the effect of these haplogroups

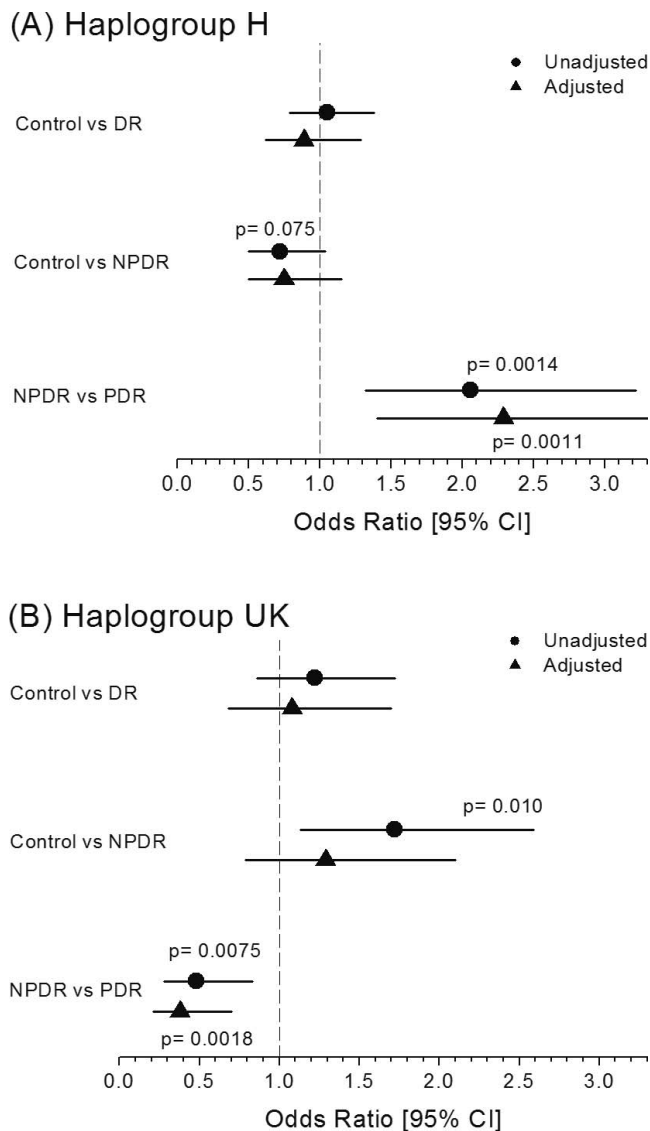


FIGURE 2. Results for logistic regression of haplogroup on DR state. Odds ratios and 95% CIs are shown for the effect of the haplogroup in the regression for simple unadjusted regression (circles) and regressions adjusted for sex, diabetes type, diabetes duration, and HgbA1c level (triangles). (A) Odds ratios for haplogroup H as a variable (subjects in haplogroup H = 1, subjects not in haplogroup H = 0). (B) Odds ratios for haplogroup UK as a variable.

is related to ischemia and neovascularization, the defining features of PDR.

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