

Mitochondrial Haplogroups Modify the Effect of Diabetes Duration and HbA_{1c} on Proliferative Diabetic Retinopathy Risk in Patients With Type 2 Diabetes

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Submitted: August 14, 2017

Accepted: November 20, 2017

Citation: Mitchell SL, Neiningger AC, Bruce CN, et al. Mitochondrial haplogroups modify the effect of diabetes duration and HbA_{1c} on proliferative diabetic retinopathy risk in patients with type 2 diabetes. *Invest Ophthalmol Vis Sci.* 2017;58:6481–6488. DOI: 10.1167/iovs.17-22804

PURPOSE. We previously demonstrated an association between European mitochondrial haplogroups and proliferative diabetic retinopathy (PDR). The purpose of this study was to determine how the relationship between these haplogroups and both diabetes duration and hyperglycemia, two major risk factors for diabetic retinopathy (DR), affect PDR prevalence.

METHODS. Our population consisted of patients with type 2 diabetes with ($n = 377$) and without ($n = 480$) DR. A Kruskal-Wallis test was used to compare diabetes duration and hemoglobin A1c (HbA_{1c}) among mitochondrial haplogroups. Logistic regressions were performed to investigate diabetes duration and HbA_{1c} as risk factors for PDR in the context of European mitochondrial haplogroups.

RESULTS. Neither diabetes duration nor HbA_{1c} differed among mitochondrial haplogroups. Among DR patients from haplogroup H, longer diabetes duration and increasing HbA_{1c} were significant risk factors for PDR ($P = 0.0001$ and $P = 0.011$, respectively). Neither diabetes duration nor HbA_{1c} was a significant risk factor for PDR in DR patients from haplogroup UK.

CONCLUSIONS. European mitochondrial haplogroups modify the effects of diabetes duration and HbA_{1c} on PDR risk in patients with type 2 diabetes. In our patient population, longer diabetes duration and higher HbA_{1c} increased PDR risk in patients from haplogroup H, but did not affect PDR risk in patients from haplogroup UK. This relationship has not been previously demonstrated and may explain, in part, why some patients with nonproliferative DR develop PDR and others do not, despite similar diabetes duration and glycemic control.

Keywords: diabetic retinopathy, mitochondria, genetics, proliferative diabetic retinopathy, mitochondrial haplogroup, HbA_{1c}

Diabetic retinopathy (DR), a common complication of both type 1 and type 2 diabetes, is a leading cause of visual impairment in working-age adults worldwide.¹ Approximately 35% of patients with diabetes will develop retinopathy, and of these, 20% will progress to the more severe proliferative diabetic retinopathy (PDR).² Early-stage DR, termed nonproliferative diabetic retinopathy (NPDR), is marked by retinal microaneurysms, blot hemorrhages, cotton wool spots, venous beading, and intraretinal microvascular abnormalities. Neovascularization of the retina or iris characterizes PDR. With the rising prevalence of diabetes,³ the incidence of DR is expected to increase markedly.

The strongest established risk factors for DR are diabetes duration and poor glycemic control, which is evaluated by plasma glycated hemoglobin (HbA_{1c}) levels.^{2,4} Factors affecting risk for PDR are less clear, but evidence indicates that diabetes duration and glycemic control, particularly early in the course of diabetes, may also play a role in PDR risk.^{4,5} However,

despite adequate glycemic control, a number of patients with NPDR do progress to PDR,⁶ and conversely, not all patients with poor glycemic control will develop PDR regardless of diabetes duration.⁴ Such clinical heterogeneity makes it difficult for clinicians to predict which patients will go on to develop PDR.

Mitochondrial haplogroups, defined by combinations of mitochondrial DNA (mtDNA) single nucleotide variants, are formed via the sequential accumulation of mutations through the maternal lineage. These haplogroups represent the major branch points in the phylogeny of mtDNA and are associated with continental ancestries. We previously identified an association between European mitochondrial haplogroups and DR severity.⁷ Among patients with either type 1 or type 2 diabetes, we have demonstrated that those from haplogroup H are at increased risk for PDR, while patients from haplogroup UK are at decreased risk for PDR.⁷ Furthermore, we have determined that, although mitochondrial haplogroups H and



UK are associated with DR severity, they are not associated with the presence of DR,⁸ suggesting mitochondrial haplogroups may impact ischemia and neovascularization, the hallmarks of PDR.

Building upon our previous work, we identified and genotyped additional patients, expanding our cohort of DR cases and controls for the current study. While our previous studies have included patients with either type 1 or type 2 diabetes,^{7,8} the current study focused on patients with type 2 diabetes because the effect of mitochondrial haplogroups on DR severity was stronger in these patients.⁷ Here, we investigated the relationship between mitochondrial haplogroups and both diabetes duration and HbA_{1c} to determine how this relationship influences PDR prevalence. Differences in diabetes duration and HbA_{1c} levels among the haplogroups could underlie the observed association, such that patients from haplogroup H had longer diabetes duration and/or higher HbA_{1c}, thereby increasing their risk for PDR. Alternatively, diabetes duration and HbA_{1c} levels may not differ among the haplogroups, but the effect of these risk factors may be of greater consequence in patients from haplogroup H. We tested these hypotheses to characterize the relationship between European mitochondrial haplogroups and both diabetes duration and HbA_{1c} on PDR risk in patients with type 2 diabetes.

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 from all participants enrolled at the Vanderbilt Eye Institute (VEI). Additionally, we accessed data from BioVU, the Vanderbilt University Medical Center biobank, which contains DNA samples linked to de-identified electronic medical records (EMRs). Before study initiation, all BioVU projects are reviewed by the Vanderbilt University Human Research Protection Program and are classified as nonhuman subjects research.

VEI Patients

From the retina clinic of the VEI, we previously recruited Caucasian patients (age ≥ 18 years) who had been diagnosed with type 2 diabetes by their primary care physician or endocrinologist and were taking at least one diabetes medication.^{7,8} All patients were evaluated for presence of DR via a comprehensive dilated ophthalmologic examination by a fellowship-trained retina specialist. Patients with type 2 diabetes and no evidence of DR were classified as diabetic controls ($n = 81$), and those with evidence of DR ($n = 64$) were graded on severity, either NPDR or PDR, as previously described.^{7,8} Briefly, NPDR was determined by presence of blot hemorrhages, microaneurysms, cotton wool spots, or intraretinal microvascular abnormalities, as well as the absence of signs or history of retinal neovascularization. Diagnosis of PDR was based on presence of iris or retinal neovascularization, or evidence of laser photocoagulation treatment for PDR.

At the time of study enrollment, VEI patients underwent venipuncture to provide a blood sample. Using a 21- or 23-gauge butterfly needle, approximately 3 mL blood was drawn from each study participant and immediately transferred to a 4-mL K₂ EDTA blood collection tube. These blood samples were

delivered to the Vanderbilt Technologies for Advanced Genetics (VANTAGE) Center for DNA isolation and storage.

BioVU Patients

The Vanderbilt University Medical Center biorepository, BioVU, contains more than 200,000 DNA samples extracted from blood remaining after routine clinical testing. These DNA samples are linked to a de-identified version of the EMR called the Synthetic Derivative (SD).⁹ In BioVU, we previously identified a cohort of diabetic patients with DR and a diabetic control group consisting of patients with diabetes and no evidence of DR.⁸ Briefly, we identified all Caucasian individuals with existing genome-wide variant data whose records contained International Classification of Diseases-9 (ICD-9) codes for type 2 diabetes (250.00, 250.02, 250.50, or 250.52) with and without DR ICD-9 codes (362.0–362.07), as well as Current Procedural Terminology (CPT) codes for ophthalmology exams (92004 or 92014).

Under the supervision of a fellowship-trained retina specialist, manual review of SD charts was performed to verify diabetes type and DR severity for each patient. A diagnosis of type 2 diabetes required the presence of a corresponding ICD-9 code and an internal medicine or endocrinology visit on the same day. To confirm absence of DR in diabetic control patients, we required the presence of at least one dilated ophthalmology exam with no evidence of DR. If multiple dilated ophthalmology exams were available, then all such exams were reviewed to confirm absence of DR. To provide further verification of the absence of DR in these patients, the SD charts were searched for the terms “retinopathy,” “DR,” “NPDR,” and “PDR.”

A DR diagnosis required the presence of both a DR code and an ophthalmology CPT code. Severity of DR was determined by presence of an ICD-9 code for NPDR (362.04–362.06) or PDR (362.02) occurring on the same day as an ophthalmology CPT code. For ICD-9 code 362.01 (“diabetic retinopathy not otherwise specified”) or for patients without NPDR or PDR codes on the same day as an ophthalmology clinic visit, the SD charts were reviewed in detail to determine the severity of DR. If severity could not be determined with these methods, the patient was excluded from the study.

Applying the same methodology for case-control determination described above, we identified an additional 170 patients with type 2 diabetes in BioVU who did not have existing genome-wide variant data. For the current study, this resulted in a total of 399 diabetic controls and 313 patients with type 2 diabetes and DR from BioVU. To ensure independence from the VEI cohort, the medical record numbers of the patients recruited through the VEI were supplied to BioVU administrators before SD data set creation, and overlapping individuals were excluded from the BioVU cohort.

Total Study Population

The combined study population from the VEI and BioVU included 857 patients consisting of 480 diabetic controls and 377 patients with DR. Compared to our previous study,⁸ this study included an additional 170 patients. Of these, 5 diabetic controls and 165 DR cases were added from BioVU, nearly doubling the number of DR cases for this study. Demographic and clinical variables were obtained from the EMR or the SD for the VEI and BioVU subjects, respectively. For each patient the HbA_{1c} reported is the median of all values in the EMR or SD. Age is reported as the age at last clinical record. Diabetes duration was determined by calculating the difference between the age at diagnosis (obtained by manual review of

TABLE 1. Study Population Characteristics

Characteristic	Diabetic Controls (<i>n</i> = 480)	Diabetic Retinopathy (<i>n</i> = 377)	<i>P</i> Value	
Age, y (SD)	68.1 (12.5)	66.0 (11.3)	0.010	
Sex, % female	50.0	40.9	0.0087	
Diabetes duration, y, median (IQR)	8 (5-13)	19 (14-26)	$<2.2 \times 10^{-16}$	
HbA _{1c} , %, median (IQR)	6.8 (6.3-7.4)	7.7 (6.9-8.6)	$<2.2 \times 10^{-16}$	
		NPDR (<i>n</i> = 229)	PDR (<i>n</i> = 148)	<i>P</i> Value
Age, y (SD)		67.4 (11.1)	63.7 (11.3)	0.0018
Sex, % female		41.5	39.9	0.83
Diabetes duration, y, median (IQR)		18 (12-24)	21 (15-29)	5.4×10^{-5}
HbA _{1c} , %, median (IQR)		7.6 (6.8-8.4)	7.9 (7.0-9.1)	0.0053

Comparisons between groups were performed by using a two-tailed Fisher's exact test for sex and two-tailed *t*-test for age. The Mann-Whitney *U* test was used to compare diabetes duration and HbA_{1c} between groups. IQR, interquartile range; SD, standard deviation.

the charts) and the age at last clinical record. All SD reviews were performed blinded to the genetic data.

Genotyping and Mitochondrial Haplogroup Determination

Existing genotype data were available for those BioVU samples previously identified.^{7,8} These samples have been genotyped on the Illumina 660W, Illumina 1M, or the Illumina Infinium HumanExome BeadChip (San Diego, CA, USA), and genotype data have been deposited in BioVU for use in additional research projects.¹⁰ The Illumina 660W and 1M genotyping arrays contain 138 single nucleotide polymorphisms (SNPs) from the mitochondrial genome, while the Exome chip contains 219 mtDNA SNPs.

The BioVU samples newly identified for this study did not have available genotype data. These samples along with the patient samples recruited through the VEI were genotyped for a pool of 22 mtDNA SNPs selected to distinguish European mitochondrial haplogroups.¹¹ Genotyping of these samples was performed by VANTAGE using the MassArray System (Agena Bioscience, San Diego, CA, USA). Haplogrep software was used to determine the mitochondrial haplogroups for all samples.^{11,12}

Statistics

Tests comparing demographic variables by DR presence and severity were performed by using two-tailed Fisher's exact test for sex and two-tailed *t*-test for age. Median diabetes duration and median HbA_{1c} were compared by using the Mann-Whitney *U* test. We then stratified our study population by mitochondrial haplogroup. Analysis focused on haplogroups H and UK, the most common European-descent haplogroups, which in Caucasians occur at frequencies of ~45% and ~25%, respectively.¹³ Haplogroups occurring less frequently (J, T, I, W, X) were combined into a single group, "Other," for analyses. We performed Kruskal-Wallis tests to determine if the median diabetes duration or median HbA_{1c} differed among haplogroups for all DR patients as well as among haplogroups for NPDR and PDR patients.

Next, we investigated the relationship between specific European mitochondrial haplogroups and the DR risk factors, diabetes duration and HbA_{1c}. Given that complications of diabetes, including retinopathy, increase for patients with HbA_{1c} > 8%,^{6,14} we dichotomized HbA_{1c} to compare patients with a history of poor glycemic control (HbA_{1c} > 8%) to patients with better glycemic control (HbA_{1c} < 8%). Logistic regression was used to model the relationship, allowing us to adjust for multiple confounding variables. We performed a

series of logistic regressions with PDR as the outcome, each one testing for an interaction between mitochondrial haplogroup H or UK and diabetes duration or HbA_{1c}. Regressions were adjusted for age, sex, diabetes duration, and HbA_{1c} > 8%. We then stratified by mitochondrial haplogroup and performed logistic regression analyses with DR or PDR as the outcome. All of the stratified logistic regression models were also adjusted for age, sex, diabetes duration, and HbA_{1c} > 8%. For each set of regressions, a Bonferroni correction for multiple testing was applied, based on the number of tests performed.

RESULTS

The study population consisted of patients with type 2 diabetes with DR (*n* = 377) and without evidence of DR (diabetic controls, *n* = 480) (Table 1). When compared to diabetic controls, DR patients were slightly younger, and had a lower proportion of females, longer median diabetes duration, and a higher median HbA_{1c}. Compared to NPDR patients (*n* = 229), PDR (*n* = 148) patients were younger, but had a longer median diabetes duration and a higher median HbA_{1c}. To determine if diabetes duration and HbA_{1c} levels differed among haplogroups H, UK, and Other in our cohort, we compared median diabetes duration and median HbA_{1c}. There were no significant differences in median diabetes duration or median HbA_{1c} among haplogroups for all DR patients or for NPDR or PDR patients (Fig. 1).

Given that neither diabetes duration nor HbA_{1c} was significantly different among mitochondrial haplogroups, we next assessed whether haplogroups modified the effects of these risk factors on PDR. We performed logistic regressions testing for interaction effects between mitochondrial haplogroups H or UK and diabetes duration or HbA_{1c}. These regressions were adjusted for age, sex, diabetes duration, and HbA_{1c} > 8% (Supplementary Table S1). After correcting for multiple tests, we identified a significant interaction effect between mitochondrial haplogroup H and HbA_{1c} (odds ratio [OR] = 3.60 [1.47-8.97]; *P* = 0.0055).

As mitochondrial haplogroups may modify the effects of diabetes duration and HbA_{1c} on PDR risk, we next performed stratified logistic regression analyses. All DR patients were stratified by mitochondrial haplogroup, and separate logistic regressions for haplogroups H, UK, and Other were performed with PDR as the outcome, controlling for age, sex, diabetes duration, and HbA_{1c} > 8% (Table 2). Diabetes duration was a significant risk factor for PDR in patients from haplogroups H (OR = 1.08 [1.04-1.12], *P* = 1.5×10^{-4}) and Other (OR = 1.08 [1.03-1.14], *P* = 0.0023), but not in patients from haplogroup UK (OR = 1.02 [0.96-1.08], *P* = 0.55). HbA_{1c} > 8% was a

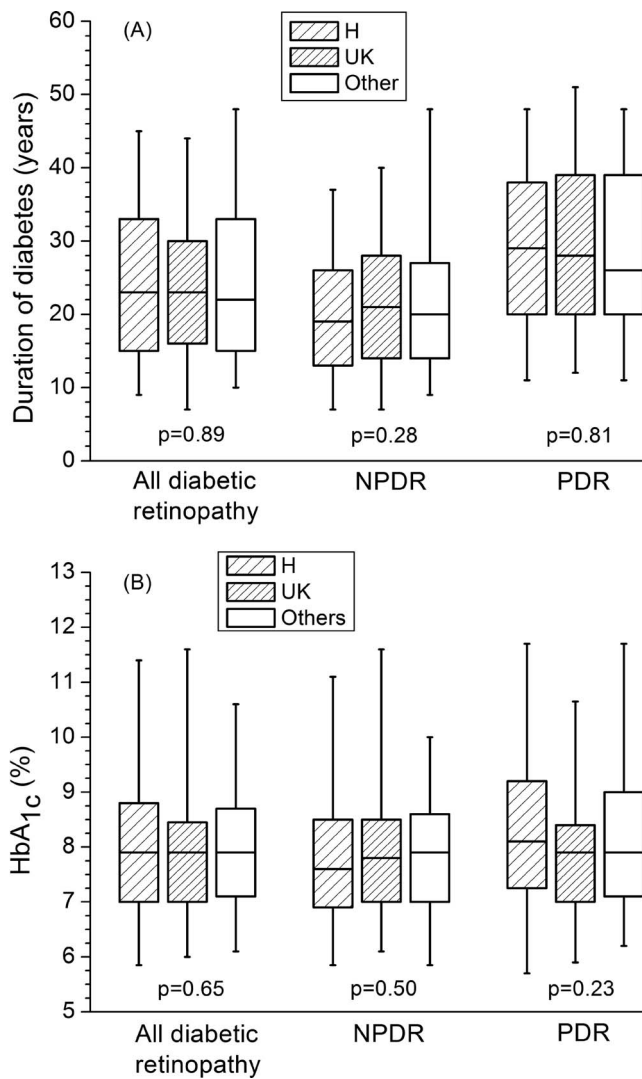


FIGURE 1. Diabetes duration and HbA_{1c} do not differ by mitochondrial haplogroup. (A) Box plots showing diabetes duration (median and interquartile range [IQR]) by haplogroup in all DR patients, NPDR patients, and PDR patients. (B) Box plots showing HbA_{1c} (median and IQR) by haplogroup in all DR patients, NPDR patients, and PDR patients. Comparisons of diabetes duration and HbA_{1c} among haplogroups were made with the Kruskal-Wallis test.

significant risk factor for PDR in patients from haplogroup H (OR = 2.35 [1.22–4.60], $P = 0.011$), but not in those from haplogroups UK (OR = 1.19 [0.43–3.30], $P = 0.74$) or Other (OR = 0.43 [0.17–1.06], $P = 0.075$). For comparison, we also performed these stratified logistic regressions in all patients with type 2 diabetes, with DR as the outcome (Supplementary Table S2). Diabetes duration was a significant risk factor for DR in patients from all three haplogroups, while HbA_{1c} was a significant risk factor for DR in patients from mitochondrial haplogroups H and UK.

To visualize the relationship between diabetes duration and DR prevalence, we divided patients into four groups by using 10-year increments of diabetes duration (<10 years, 10–19 years, 20–29 years, and ≥ 30 years), and plotted the proportion of patients with DR for each group. As expected, when assessed in all patients with type 2 diabetes, the probability of DR increased with increasing diabetes duration. Among those patients with type 2 diabetes ≥ 30 -year duration, 82% had DR (Fig. 2A). When patients were stratified by mitochondrial

TABLE 2. Adjusted Logistic Regressions in DR Patients Stratified by Haplogroup

Haplogroup	Variable	Odds Ratio (95% CI)	P Value
H ($n = 405$)			
	Age, y	0.96 (0.93–0.99)	0.017
	Female sex	1.17 (0.60–2.29)	0.64
	Diabetes duration, y	1.08 (1.04–1.12)	0.00015
	HbA _{1c} > 8%	2.35 (1.22–4.60)	0.011
UK ($n = 184$)			
	Age, y	0.96 (0.92–1.01)	0.13
	Female sex	0.56 (0.18–1.63)	0.30
	Diabetes duration, y	1.02 (0.96–1.08)	0.55
	HbA _{1c} > 8%	1.19 (0.43–3.30)	0.74
Other ($n = 268$)			
	Age, y	0.96 (0.92–0.996)	0.035
	Female sex	0.86 (0.36–2.02)	0.73
	Diabetes duration, y	1.08 (1.03–1.14)	0.0023
	HbA _{1c} > 8%	0.43 (0.17–1.06)	0.075

Logistic regressions were performed with PDR as the outcome, adjusting for age, sex, diabetes duration, and HbA_{1c} > 8%. Logistic regressions were performed with PDR as the outcome, adjusting for age, sex, diabetes duration, and HbA_{1c} > 8%. Bold values indicate significance after applying a Bonferroni correction for multiple testing ($P < 0.017$).

haplogroup, a similar relationship of higher DR prevalence with longer diabetes duration was observed for all haplogroups (Fig. 2B).

We next plotted the relationship between diabetes duration and PDR prevalence in only the DR patients. Among all DR patients, the prevalence of PDR increased concomitantly with diabetes duration (Fig. 2C). Of the DR patients with diabetes duration ≥ 30 years, 64% were affected by PDR. However, when the DR patients were stratified by haplogroup (Fig. 2D) there was a substantial difference among the haplogroups in the relationship between diabetes duration and PDR. Haplogroups H and Other exhibited an overall increase in PDR prevalence with increasing diabetes duration. Of the patients from haplogroups H and Other with diabetes duration ≥ 30 years, 80% and 61%, respectively, had PDR. In stark contrast, the probability of PDR for UK patients remained relatively constant as diabetes duration increased, with only 20% of haplogroup UK DR patients with diabetes duration ≥ 30 years having PDR (Fig. 2D).

To visualize the relationship between HbA_{1c} levels and DR, we divided patients into four groups by using set HbA_{1c} cutoffs (<6.5%, 6.5%–7.49%, 7.5%–8.49%, and $\geq 8.5\%$). These cutoffs were determined from glycemic control where <6.5% is considered in the normal to prediabetes range.¹⁵ Increments of 1% HbA_{1c} were added per division, as large-scale studies have shown that for each additional 1% elevation in HbA_{1c} the risk for diabetic complications increases considerably.^{16–20} We plotted the proportion of patients with DR for each HbA_{1c} group. As expected, the prevalence of DR increased as HbA_{1c} levels increased, with 73% of patients from the highest HbA_{1c} group affected by DR (Fig. 3A). When patients were stratified by haplogroup, a similar relationship between DR prevalence and increasing HbA_{1c} (Fig. 3B) was observed for all haplogroups.

We then plotted the proportion of DR patients with PDR in each HbA_{1c} group. For all DR patients the prevalence of PDR increased with rising HbA_{1c} (Fig. 3C). However, stratifying DR patients by mitochondrial haplogroup revealed striking differences in the relationship between HbA_{1c} and PDR. Compared to DR patients as a whole, DR patients from haplogroup H

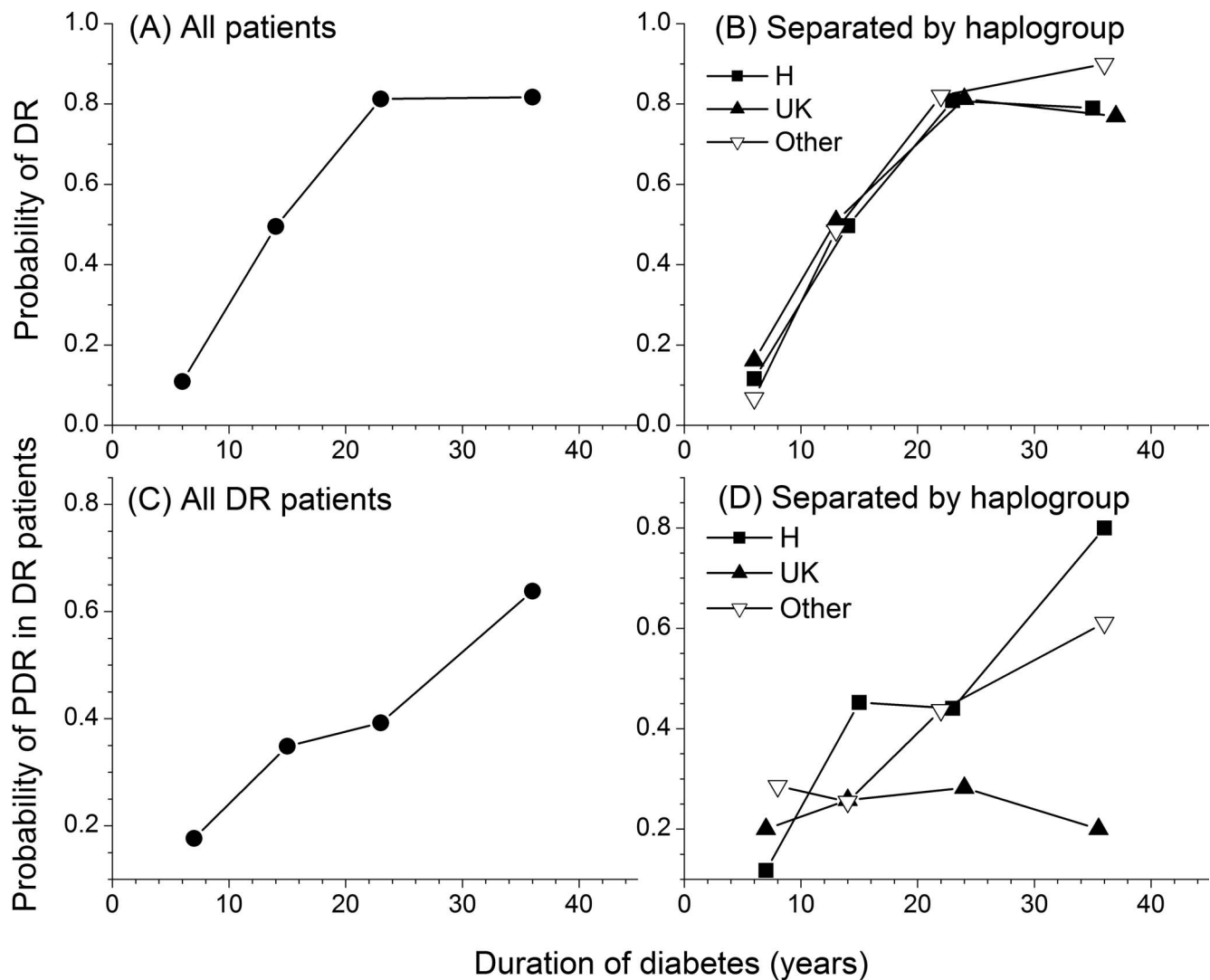


FIGURE 2. Prevalence of DR and PDR with increasing diabetes duration. (A) Proportion of patients with DR among all type 2 diabetic patients. (B) Proportion of patients with DR among all type 2 diabetic patients, separated by haplogroup. (C) Proportion of type 2 diabetic patients with PDR among those with DR. (D) Proportion of type 2 diabetic patients with PDR among those with DR, separated by haplogroup.

demonstrated a much greater rise in PDR prevalence with increasing HbA_{1c} (Fig. 3D). Conversely, there was a minimal increase in PDR prevalence with rising HbA_{1c} for DR patients from haplogroups UK or Other (Fig. 3D). Among those DR patients from Haplogroup H in the highest HbA_{1c} group, 65% had PDR. In contrast, among DR patients with comparable HbA_{1c} levels from haplogroups UK and Other, only 32% and 33%, respectively, were affected by PDR.

DISCUSSION

A number of studies have established diabetes duration and HbA_{1c} as risk factors for DR; however, fewer studies have focused on risk factors for PDR.² To characterize the role mitochondrial haplogroups play in DR severity, we investigated the relationship between European mitochondrial haplogroups and both diabetes duration and HbA_{1c} as PDR risk factors. In our cohort, diabetes duration and HbA_{1c} did not differ among the haplogroups. However, the effect of diabetes duration and HbA_{1c} on PDR prevalence among DR patients differed significantly by haplogroup, indicating that mitochondrial

haplogroups strongly modify the effect of diabetes duration and HbA_{1c} on PDR risk.

It is well established that longer diabetes duration is associated with increased risk of DR, and evidence suggests that longer diabetes duration also increases risk for PDR.² Here, we demonstrated in DR patients from haplogroups H and Other that diabetes duration is associated with PDR prevalence. This effect is considerably stronger in DR patients from haplogroup H. Conversely, the proportion of patients with PDR among DR patients from haplogroup UK was essentially unchanged regardless of diabetes duration. These results suggest diabetes duration exerts a powerful effect on risk for PDR in DR patients from haplogroup H, but is not a risk factor for PDR in DR patients from mitochondrial haplogroup UK.

Glycemic control, the strongest modifiable risk factor for DR, has been implicated in risk for PDR.^{4,21,22} Among all DR patients, we observed increased prevalence of PDR with rising HbA_{1c}. However, when we examined HbA_{1c} levels in the context of mitochondrial haplogroups, the effect of higher HbA_{1c} on PDR prevalence was observed only in patients from haplogroup H. There was virtually no effect of increasing HbA_{1c} on PDR risk in DR patients from haplogroups UK or

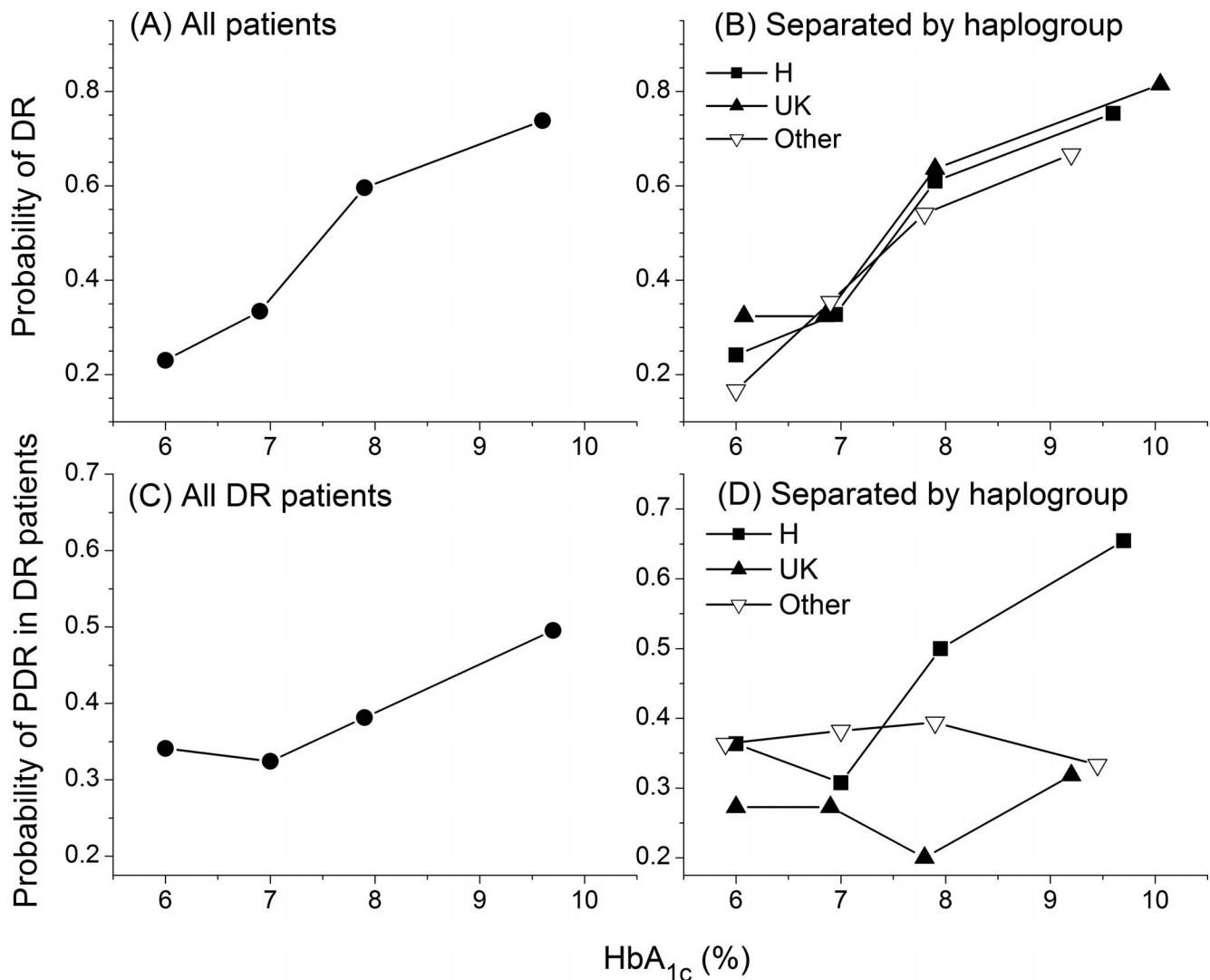


FIGURE 3. Prevalence of DR and PDR with increasing HbA_{1c} levels. **(A)** Proportion of patients with DR among type 2 diabetic patients. **(B)** Proportion of patients with DR among type 2 diabetic patients, separated by haplogroup. **(C)** Proportion of type 2 diabetic patients with PDR among DR patients. **(D)** Proportion of type 2 diabetic patients with PDR among DR patients, separated by haplogroup.

Other, even in those patients with the highest HbA_{1c} levels. Thus, in our patient population, the effect of increasing HbA_{1c} on PDR prevalence is driven entirely by DR patients from haplogroup H.

Retinal photoreceptors require high levels of energy to function, and to meet this demand there is a high concentration of mitochondria in these cells. Mitochondrial dysfunction has been linked to eye disease, including degenerative retinal diseases.^{23,24} Mitochondrial haplogroups have been identified as risk modifiers for multiple eye diseases, and haplogroup-related differences in mitochondrial function are hypothesized to underlie this variation in disease risk.^{25–29} Differences in mitochondrial function between haplogroups H and UK could contribute to the observed variation in the effect of diabetes duration and HbA_{1c} on PDR risk. Studies have reported higher oxidative phosphorylation capacity in haplogroup H compared with haplogroup U/UK.^{30,31} As a consequence of diabetes, levels of reactive oxygen species (ROS) and oxidative stress increase,^{24,32} which has been linked to pericyte loss and formation of acellular capillaries in the retina, resulting in hypoxia and ischemia, ultimately leading to neovascularization.³² It is plausible that haplogroup H produces more ROS in

response to high glucose and/or has less capacity to handle increased oxidative stress, giving rise to greater retinal vascular damage. With longer diabetes duration, the effects of increased oxidative stress are likely to be amplified. Thus, patients from haplogroup H may be more vulnerable to the effects of longer diabetes duration and poor glycemic control.

Determining the impact of specific mtDNA variants on common disease is a challenging task.³³ Five mtDNA variants distinguish haplogroup H from the other major European haplogroups.³⁴ Two of these variants are synonymous (T7028C in MT-CO1 and A11719G in MT-ND4) and unlikely to affect mitochondrial function. The nonsynonymous variant T14766C results in an isoleucine to threonine change in the MT-CYB protein. This variant occurs independently several times in the human mtDNA phylogenetic tree and its predicted pathogenicity is nominal.³⁵ The remaining two variants, G2706A and G73A, are located in the 16S rRNA gene and the noncoding D-loop, respectively.³⁶ It is possible these variants could affect the translation of mitochondrial proteins or replication of mtDNA, thereby affecting overall capacity for mitochondrial function.

Challenges inherent in leveraging data from EMRs for research studies include variability in the density of data for each patient and accuracy of phenotyping. This study was limited by variability in the number of HbA_{1c} measures available for each patient. To address this, we used the median of all HbA_{1c} laboratory measures in each patient record, as this best reflected overall glycemic control. A major strength of this study was the rigorous phenotyping performed in both the clinically ascertained VEI patients and the patients from BioVU. Through systematic manual review we identified patients with type 2 diabetes with and without DR. We also determined DR severity as either NPDR or PDR, distinguishing our approach from previous studies.

This study characterized the relationship between European mitochondrial haplogroups and diabetes duration and HbA_{1c} as risk factors for PDR. The results demonstrated that the effect of diabetes duration and HbA_{1c} on PDR risk differs strongly by haplogroup. Diabetes duration and HbA_{1c} exert powerful effects on PDR risk in patients from haplogroup H, which represents nearly half of those from European-descent populations. Collectively, these results revealed mitochondrial genetics as an important determinant of the clinical heterogeneity observed in PDR risk among patients with type 2 diabetes. This relationship has not been previously demonstrated and may explain, in part, why some patients develop PDR and others do not, despite similar diabetes duration and glycemic control.

Acknowledgments

Supported by National Institutes of Health (NIH) Grants R01EY22618 (MAB), T32GM007347, T35DK007383, the International Retinal Research Foundation (MAB), and CTSA Award No. UL1TR000445 from the National Center for Advancing Translational Sciences. Genome-wide genotyping was funded by NIH grants RC2GM092618 from NIGMS/OD and U01HG004603 from NHGRI/NIGMS.

Disclosure: **S.L. Mitchell**, None; **A.C. Neininger**, None; **C.N. Bruce**, None; **I.M. Chocron**, None; **J.A. Bregman**, None; **C.B. Estopinal**, None; **A. Muhammad**, None; **A.C. Umfress**, None; **K.L. Jarrell**, None; **C. Warden**, None; **P.A. Harlow**, None; **M. Wellons**, None; **D.C. Samuels**, None; **M.A. Brantley Jr**, None

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