Original Paper

Ophthalmic Research

Ophthalmic Res 2011;46:1–8
DOI: 10.1159/000317057

Received: February 18, 2010
Accepted after revision: June 9, 2010
Published online: November 19, 2010

Variants of the Adenosine A_{2A} Receptor Gene Are Protective against Proliferative Diabetic Retinopathy in Patients with Type 1 Diabetes

Bashira A. Charles[a–c] Yvette P. Conley[c] Guanjie Chen[a] Rachel G. Miller[c]
Janice S. Dorman[c] Michael B. Gorin[d, e] Robert E. Ferrell[c] Susan M. Sereika[c]
Charles N. Rotimi[a] Trevor J. Orchard[c]

[a]Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, and [b]National Institute of Nursing Research, National Institutes of Health, Bethesda, Md., [c]University of Pittsburgh, Pittsburgh, Pa., [d]Jules Stein Eye Institute, and [e]University of California Los Angeles, Los Angeles, Calif., USA

Key Words
Diabetes · Diabetic retinopathy · Single nucleotide polymorphism · Adenosine receptor

Abstract
Aims: The adenosine A_{2A} receptor ($ADORA_{2A}$) may ameliorate deleterious physiologic effects associated with tissue injury in individuals with diabetes. We explored associations between variants of the $ADORA_{2A}$ gene and proliferative diabetic retinopathy (PDR) in a cohort of patients with type 1 diabetes (T1D). ***Methods:*** The participants were from the Pittsburgh Epidemiology of Diabetes Complications prospective study of childhood-onset T1D. Stereoscopic photographs of the retinal fundus taken at baseline, then biennially, for 10 years were used to define PDR according to the modified Airlie House system. Two tagging single nucleotide polymorphisms (tSNPs; rs2236624-C/T and rs4822489-G/T) in the $ADORA_{2A}$ gene were selected using the HapMap (haplotype map) reference database. ***Results:*** A significant association was observed between SNP rs2236624 and PDR in the recessive genetic model. Participants homozygous for the T allele displayed a decreased risk of developing prevalent PDR (odds ratio, OR = 0.36; p = 0.04) and incident PDR (hazard ratio = 0.156; p = 0.009), and for all cases of PDR combined (OR = 0.23; p = 0.001). The protective effect of T allele homozygosity remained after adjusting for covariates. Similarly, for SNP rs4822489, an association between PDR and T allele homozygosity was observed following covariate adjustment (OR = 0.55; 95% CI: 0.31–0.92; p = 0.04). ***Conclusion:*** Genetic variants of $ADORA_{2A}$ offer statistically significant protection against PDR development in patients with T1D.

Introduction

Diabetic retinopathy (DR) is the cause of up to 24,000 incident cases of blindness in the USA each year, and is the most prevalent cause of blindness between the second and seventh decades of life [1]. Most individuals with diabetes eventually experience some form of DR during the course of their lives; however, a greater proportion of patients with type 1 diabetes (T1D) develop proliferative DR

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ore

Bashira A. Charles, PhD, Center for Research on Genomics and Global Health
National Human Genome Research Institute
Building 12A, Room 4047, 12 South Drive, MSC 5635
Bethesda, MD 20892-5635 (USA)
Tel. +1 301 435 0137, Fax +1 301 451 5426, E-Mail charlesba@mail.nih.gov

(PDR) than do patients with T2D, with reported estimates as high as 50% compared to 10%, respectively [2, 3].

The presence of DR and/or severity of DR have been shown to be partially heritable [4–6]. The Family Investigation of Nephropathy and Diabetes-Eye study consisting of European Americans, African Americans and Mexican Americans estimated heritability for PDR to be about 25% [7]. Observed ethnic differences in susceptibility to DR also provide some support for the potential role of genetic factors in the etiology of DR [4–10].

Adenosine is a powerful physiologic mediator that modulates cellular damage and the resulting tissue injury caused by biologic stressors. In the presence of oxidative stress (OS), hydrogen peroxide is formed in the cytosol, rendering vascular endothelial cells more permeable [11], and adenosine appears to ameliorate this process. The effects of adenosine are directed by adenosine receptors (AR), with the adenosine A_1 receptor having a proinflammatory response to tissue injury, while the adenosine A_2 receptor ($ADORA_{2A}$) restricts inflammation and guards tissues from further damage [12, 13]. In rats, the AR exhibit variability in function and a long-term response to ischemic brain injury [14]. Other animal studies have provided evidence that $ADORA_{2A}$ protects the kidneys and the heart from ischemic reperfusion injury by reducing the generation of reactive oxygen species, thereby limiting mitochondrial damage and guarding against apoptosis [15–17].

Growing biological evidence suggests that reactive oxygen species and OS caused by long-term exposure to hyperglycemia may be responsible for some of the tissue damage associated with microvascular complications of diabetes [18–20]. There is evidence in bovine retinal endothelial cells that adenosine may play a role in the upregulation of expression of a gene involved in glucose transport (GLUT1) [21]. Other investigators using electroretinogram measurements were able to demonstrate that adenosine caused vasodilatation, an increase in the rod-driven b-wave, an increase in the cone-driven b-wave, followed by a decrease in the cone-driven b-wave that was mediated by $ADORA_{2A}$ in the cat retina [22]. Others found that adenosine or its agonist decreased the blood flow and induced apoptosis in rat or rabbit retinae [23–25]. Furthermore, it has been demonstrated that OS is evident in the retinae of individuals with diabetes – a phenomenon that is likely to contribute to the development of DR [26]. $ADORA_{2A}$ also appears to ameliorate the effects of hypoxia, inflammation, vasodilatation, intraocular hyperglycemia and the speed of glucose metabolism out of the cell [13, 21, 27]. Both human and animal studies provide evidence that adenosine [28] or its receptors [29] play a role in angiogenesis [30, 31]. Specifically, $ADORA_{2A}$ [32] has been shown to increase the proliferation of human retinal endothelial cells.

Based upon the multiple roles that adenosine and the $ADORA_{2A}$ gene play in ameliorating the effects of biologic stressors in humans and animals, it is reasonable to consider $ADORA_{2A}$ as a plausible candidate gene for increased susceptibility to or protection from the development and progression of PDR. We therefore selected tag single nucleotide polymorphisms (tSNPs) from the European ancestry (CEU) HapMap (haplotype map) data that were representative of the entire $ADORA_{2A}$ gene, as described below in the Methods section. We evaluated the association between tSNPs and PDR in a well-characterized sample of patients with T1D.

Research Design and Methods

Study Population

The participants in this study were from the Pittsburgh Epidemiology of Diabetes Complications (EDC) prospective study of childhood-onset (<17 years) T1D. All participants were listed in the Children's Hospital of Pittsburgh diabetes registry. Potential participants received a letter inviting them to have a physical examination and to complete several questionnaires. There were 979 eligible participants, 658 of whom (67.2%) participated in the entire EDC evaluation process [33, 34]. The current study, Genetic Basis of Diabetic Retinopathy, consists of the 496 EDC participants for whom DNA was available (75.4%).

This study adhered to the tenets of the Declaration of Helsinki, and was approved by the institutional review board at the University of Pittsburgh. Written informed consent was obtained from the participants prior to baseline data collection. The data collection methodologies for this population have been published previously [34–36]. Data collection relevant to the Genetic Basis of Diabetic Retinopathy included baseline and biennial stereoscopic retinal examinations, measurement of blood pressure (systolic blood pressure, diastolic blood pressure), hypertension (HTN), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glycosylated hemoglobin (GHb), body mass index (BMI), and documentation of ever-smoker status [34, 35].

Determination of PDR

Dilated eye examinations and stereoscopic images of the retinal fundus were obtained for participants at baseline (1986–1988), then biennially, for those without PDR over the course of 10 years, and again at 18 years. ETDRS (Early Treatment Diabetic Retinopathy Study) fundus fields 1, 2 and 4 [37] were taken using the Zeiss Fundus camera (Carl Zeiss, Oberkochen, Germany), and diagnosis and severity grading were based on the assessment of these images by the Fundus Photography Reading Center at the University of Wisconsin using the Arlie House sys-

tem. Three images were used instead of the standard 7 images since it has been previously validated that this gives acceptable results with good sensitivity and reliability [33, 38]. PDR was defined as either grade ≥60 in one eye or grade <60 but with panretinal chorioretinal scars consistent with laser therapy, according to the modified Airlie House system. Baseline PDR was defined as the presence of PDR at the initial evaluation. Incident PDR was defined as PDR first diagnosed at a subsequent biennial follow-up time point.

Genotyping

Based on the European ancestry (CEU) HapMap (NCBI build 35) data (39), we identified 2 tSNPs for the $ADORA_{2A}$ gene. The 2 tSNPs selected for analyses in our study (rs2236624-C/T and rs4822489-G/T) were the only SNPs in the CEU population that met the selection criteria of minor allele frequency (MAF) of ≥0.2. The tSNPs were genotyped using TaqMan allele discrimination assays (Applied Biosystems, Foster City, Calif., USA) with the ABI Prism® 7000 Sequence Detection System, and the genotype assignments were determined by 2.0 ABI software (Applied Biosystems). The cycling conditions provided by Applied Biosystems yielded poor genotype cluster separation, and an extended protocol was used and resulted in improved separation and genotype discrimination. Conditions were 2 min of activation at 50°C, denaturing at 95°C, then 50 cycles of 15 s of denaturing at 95°C, followed by annealing and extension at 58°C for 1.5 min.

Statistical Analysis

The data were analyzed using SAS version 9.1.3 (SAS Institute Inc., Cary, N.C., USA). Selected covariates (age, BMI, diabetes duration, GHb, HDL, LDL, total cholesterol and triglycerides) found to be significant in the univariate analysis were included in the multivariate analyses. We conducted 3 separate analyses evaluating the association of selected tSNPs with incident PDR, prevalent PDR and all PDR cases combined. Using the more conservative Bonferroni adjustment, we set the levels of significance to be 0.025 and 0.05 for highly and marginally significant p values, respectively. As far as we know this is the first study investigating the potential role of $ADORA_{2A}$ in the pathophysiology of PDR.

Haploview was used to construct linkage disequilibrium plots. Genetic association analyses of PDR using additive, dominant and recessive genetic models were carried out using Plink version 1.06 [40]. The stepwise Cox proportional hazard backward regression method (significance level of 0.10), as implemented in SAS, was used to identify tSNPs associated with incident PDR while adjusting for significant covariates.

Results

A total of 161 participants (32%) developed incident PDR during the follow-up period (table 1a). There was no significant difference in duration of diabetes in those that developed incident PDR compared to those that did not (p = 0.77). In contrast, participants who developed incident PDR had a higher rate of HTN and high-

Table 1. Patient characteristics

a Patients who developed incident PDR compared to patients who were PDR free at the end of follow-up

	Incident PDR (n = 161)	No PDR at end of follow-up (n = 183)	p
Age, years	25.8 ± 6.9	24.8 ± 8.1	0.1992
Duration, years	17.0 ± 6.3	16.3 ± 7.1	0.7675
Male/female, n	79/82	92/91	0.9132
GHb, %	10.7 ± 1.9	9.9 ± 1.7	<0.0001
Prevalence of HTN, n	15 (9.3)	7 (3.8)	0.0435
Total cholesterol, mg/dl	190 ± 40.0	173.3 ± 32.5	<0.0001
HDL cholesterol, mg/dl	54.7 ± 12.1	55.3 ± 12.0	0.6155
LDL cholesterol, mg/dl	114.3 ± 31.4	102.2 ± 26.6	0.0004
Triglycerides, mg/dl	106.6 ± 87.0	81.1 ± 50.1	0.0012
BMI	23.7 ± 3.0	22.9 ± 3.4	0.0466
Ever smoker, n	46 (28.8)	61 (34.7)	0.1602

b Patients who had PDR at baseline compared to patients who were PDR free at baseline

	Prevalent PDR (n = 142)	No PDR at baseline (n = 183)	p
Age, years	37.2 ± 5.8	24.8 ± 8.1	<0.0001
Duration, years	25.1 ± 6.1	16.3 ± 7.1	<0.0001
Male/female, n	79/63	92/91	0.4562
GHb, %	10.3 ± 1.8	9.9 ± 1.7	0.0020
Prevalence of HTN, %	35.9	3.8	<0.0001
Total cholesterol, mg/dl	204.9 ± 45.3	173.3 ± 32.5	0.0001
HDL cholesterol, mg/dl	51.7 ± 11.3	55.3 ± 12.0	0.0058
LDL cholesterol, mg/dl	128.9 ± 38.0	102.2 ± 26.6	<0.0001
Triglycerides, mg/dl	120.8 ± 71.2	81.1 ± 50.1	<0.0001
BMI	24.2 ± 3.3	22.9 ± 3.4	0.0051
Ever smoker, n	66 (47.5)	61 (34.7)	0.9456

c All patients who developed PDR (incident and prevalent) compared to patients who were PDR free at the end of follow-up

	Any PDR (n = 306)	No PDR (n = 185)	p
Age, years	29.0 ± 7.3	24.7 ± 7.9	<0.375
Duration, years	20.8 ± 7.8	16.2 ± 7.0	<0.009
Male/female, n	159/147	94/91	0.456
GHb, %	10.5 ± 1.8	9.9 ± 1.7	<0.001
Prevalence of HTN, n	68 (90.7)	7 (9.3)	<0.001
Total cholesterol, mg/dl	204.9 ± 45.3	173.6 ± 32.5	<0.001
HDL cholesterol, mg/dl	53.4 ± 12.1	55.2 ± 11.9	0.145
LDL cholesterol, mg/dl	120.9 ± 35.2	102.5 ± 26.7	<0.001
Triglycerides, mg/dl	113.2 ± 79.5	81.7 ± 50.0	<0.001
BMI	23.9 ± 3.1	22.9 ± 3.4	0.016
Ever smoker, n	113 (64.6)	62 (35.4)	0.434

Values denote means ± SD unless otherwise specified.
Figures in parentheses are percentages.

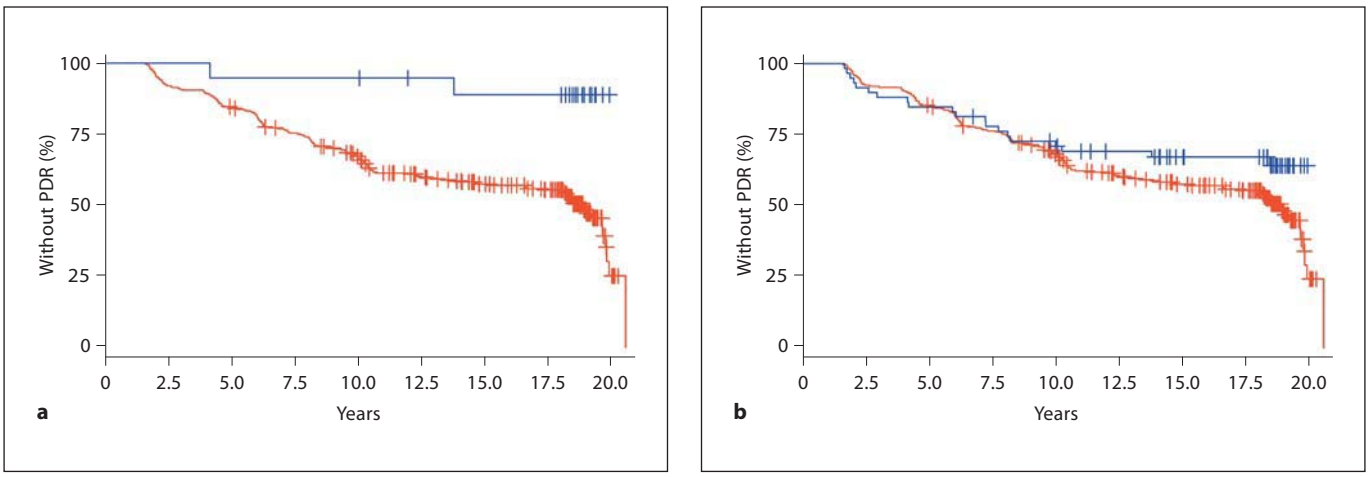

Fig. 1. a Kaplan-Meier curve showing follow-up time for development of PDR in 2.5-year increments by rs2236624 genotype. Blue line: T allele. Red line: C allele. **b** Kaplan-Meier curve showing follow-up time for development of PDR in 2.5-year increments by rs4822489 genotype. Blue line: T allele. Red line: G allele.

Table 2. Association of ADORA SNPs with all cases of PDR under 3 genetic models

SNP (reference allele)	OR	95% CI	p
rs2236624 (T)			
Recessive adjusted	0.18	0.06–0.59	0.004
Dominant adjusted	1.24	0.78–1.95	0.363
Additive adjusted	0.93	0.64–1.34	0.690
rs4822489 (T)			
Recessive adjusted	0.55	0.31–0.97	0.040
Dominant adjusted	0.97	0.62–1.49	0.873
Additive adjusted	0.84	0.62–1.13	0.240

Adjusted for duration of T1D, LDL, hypertension and GHb.

er mean levels of GHb, total cholesterol, LDL, triglycerides and BMI (table 1a). These findings suggest that those who did not develop PDR at the end of the follow-up period had better glycemic control and consequentially developed significantly fewer risk factors for cardiovascular disease than those who developed incident PDR.

There were significant differences between participants with prevalent PDR and those who had not developed PDR at the time of their baseline evaluation (table 1b). Those with prevalent PDR tended to be older, had a longer duration of T1D and higher levels of GHb; they also had higher levels of other cardiovascular disease risk factors including total cholesterol, LDL, triglycerides and lower levels of HDL. In addition, they were heavier and had a higher prevalence of HTN. The characteristics of those who developed incident or prevalent PDR during the follow-up period versus those who did not develop PDR during the follow-up period are displayed in table 1c and are consistent with the finding reported above.

The rs2236624 tSNP is in Hardy-Weinberg equilibrium ($p > 0.05$), while the rs4822489 tSNP is not ($p = 0.05$) and shows some signs of heterozygote advantage: GG = 195, GT = 212 and TT = 84. The minor allele frequency for rs2236624 is 0.21, and for rs4822489 it is 0.39, and the r^2 between the SNPs is 0.36. In the recessive genetic model, comparing all participants with PDR (i.e. prevalent plus incident cases), carriers homozygous for any T allele of SNP rs2236624 had a significant protective odds ratio (OR) of 0.23 compared to carriers of any C allele (table 2). Interestingly, the protective effect of the homozygote T allele improved (OR of 0.23 vs. 0.18) following adjustment for important covariates (i.e. duration of diabetes, GHb, HTN status and LDL cholesterol (table 2). Age, gender, BMI, ever-smoker status, HDL cholesterol and triglycerides dropped out of the model. Similarly, carriers homozygous for the T allele of SNP rs4822489 had a protective OR of 0.65 compared to carriers of any G allele, and the observed protective effect of the T allele improved after adjusting for covariates (OR = 0.55) (table 2).

Modeling Prevalent and Incident PDR and Variants of the $ADORA_{2A}$ Separately

Among prevalent cases and in a recessive model, carriers homozygous for the T allele (rs2236624) had a protective OR of 0.36 compared to carriers of any C allele (p = 0.04; 95% CI: 0.128–0.991). The observed protective effect was enhanced with covariate adjustment. The additive and dominant models did not reveal any association with prevalent PDR in the univariate or multivariate models for PDR (data not shown). SNP rs4822489 was not associated with prevalent PDR in the additive, dominant or recessive models (data not shown).

There were 162 incident cases of PDR during the 20-year follow-up period. The Cox proportional hazards regression showed that carriers homozygous for the T allele of SNP rs2236624 were less likely to progress to PDR compared to carriers homozygous for the C allele, resulting in a protective hazard ratio (HR) of 0.156 (95% CI: 0.040–0.63; p = 0.009); covariate adjustment significantly improved the observed protective effect from a HR of 0.156 to 0.090 (95% CI: 0.01–0.64; p = 0.016). Kaplan-Meier analysis showed that only 11% of those homozygous for the T allele progressed to PDR during follow-up compared to 48.9% of carriers of any copy of the C allele (fig. 1a). The two-group log rank χ^2 test showed that the observed difference in time free of PDR (i.e. time to event) was significantly different (p = 0.0027) between carriers homozygous for the T allele and carriers of any copy of the C allele.

Similar, though not as dramatic, results were observed for rs4822489: carriers homozygous for the T allele of SNP rs4822489 were less likely than carriers of any G allele to progress to PDR during follow-up (HR = 0.67; 95% CI: 0.42–1.07; p = 0.093). Thirty-four percent of those homozygous for the T allele progressed to PDR compared to 49% among carriers of any G allele. The two-group log rank χ^2 test revealed marginal significance (p = 0.091) (fig. 1b).

Haplotypes were generated from the 2 SNPs with the following frequencies: TC 594 (61.36%), GC 172 (17.77%), TG 1 (0.10%) and GG 201 (20.86%). The TG haplotype was excluded from the analysis as there was only 1 individual with this haplotype. None of the haplotypes were associated with PDR (data not shown).

Discussion

The analyses of this prospective study of persons with T1D demonstrate that variants of the $ADORA_{2A}$ gene confer significant protection against the development of PDR in persons with T1D. At baseline, the OR of developing PDR was only 0.23 for carriers of 2 copies of the T allele compared to carriers of any copy of the C allele. Remarkably, only 10% of the carriers of 2 copies of the T allele developed incident PDR compared to 50% for carriers of any copy of the C allele. As far as we know, this is the first study to implicate the $ADORA_{2A}$ gene in PDR in individuals with childhood-onset T1D.

The reason for this decreased association for carriers homozygous for the T allele with PDR is not clear and warrants further investigation. While they appear to be protected against development of PDR, we note that the number of participants homozygous for the T allele was small (n = 24 for rs2236624), and only 2 of the 24 developed PDR during follow-up. For rs4822489, 84 were homozygotes for the protective allele, of which 20 progressed to PDR during follow-up. However, the biologic functions of the $ADORA_{2A}$ gene, specifically its role in angiogenesis and protection against biologic stressors associated with diabetes, suggest that the OR and HR associated with these data are unlikely to be due solely to small sample sizes of participants homozygous for the T allele.

The $ADORA_{2A}$ gene is part of the G-protein-coupled receptor super family. It is expressed in the basal ganglia, blood vessels, platelets and other tissues in the body. It encompasses 1 intron and 2 exons [41–44]. SNP rs2236624 is in linkage disequilibrium with 15 currently known SNPs. Four of these SNPs are within exons but are synonymous coding SNPs, 3 are intronic, 6 lie in the 3′>untranslated region, 1 is a nonsynonymous SNP and another is a frame shift polymorphism [45].

At this stage of the investigation, the reasons for the differences in the phenotypes associated with the 2 tSNPs in our study are not clear and require further exploration. Investigators have reported that $ADORA_{2A}$ receptor activation may decrease vascular endothelial growth factor (VEGF) and endothelial cell production in rodent pheochromocytoma cells [46]. Similar modulation of VEGF may occur in humans and, thus, the possibility of influencing the development and/or the progression of PDR.

Myriad physiological processes may be affected by adenosine and its receptors in vivo since it is found in the vasculature of all organs and is a byproduct of adenosine triphosphate metabolism. Variation in $ADORA_{2A}$ expression and the ability of the receptor to bind with adenosine may have an impact on its ability to attenuate the negative effects of OS and other biologic homeostatic processes in ways that have yet to be elucidated. To date ad-

enosine has been linked to retinal VEGF production [32, 47] and regulation of angiogenesis via VEGF production [32, 48]. It is also suspected to play a role in interleukin-8, mast cell and insulin-like-growth-factor-induced angiogenesis [49].

$ADORA_{2A}$ stimulation has been linked to psychomotor depression, sleep induction, immune suppression and vasodilatation, while its suppression has been linked to HTN, aggression and inflammation, alleviation of symptoms of ethanol withdrawal and amelioration of neurotoxicity [44]. There is evidence that $ADORA_{2A}$ activation decreases the expression of VEGF, a primary mitogen associated with the development of DR [46]. Experimental models have also shown that the $ADORA_{2A}$ receptor plays a role in glucose transport, vasodilatation, hypoxia, and inflammation, resolution of inflammation and prevention of apoptosis [13, 16, 21, 50, 51].

This study evaluated only the $ADORA_{2A}$ gene, which is one of 4 members of the adenosine receptor family. $ADORA_1$ plays a significant role in the proinflammatory responses of the cell, while $ADORA_{2A}$ and $ADORA_{2B}$ play significant roles in limiting the cell response to inflammation, thereby producing an antiinflammatory response. $ADORA_3$ plays a role in the cell's response to ischemia [13]. The potential role of the remaining 3 genes of the adenosine receptor family in PDR susceptibility deserves further investigation.

The majority (98%) of the participants enrolled in this study of T1D were of European ancestry. As a result, the generalizability of these findings awaits confirmation in other ancestral population groups. The fact that the mean diabetes duration was 19 years at the time of entry into the study limited our ability to explore the natural history of DR in reference to specific genotypes. However, because 85% of the cohort had some form of DR at the time of entry into the study, we were able to explore the effect of genetic variation on the progression to PDR. It would be beneficial to explore the pathophysiologic role of the $ADORA_{2A}$ gene in patients with T2D.

Replication of our observed associations in other cohorts, fully evaluating this segment of the gene for underlying variability, as well as functional characterization of this variability regarding receptor function or mRNA stability are potential areas of further investigation. Based on the HapMap populations, there is a high frequency of the T allele in populations of Asian ancestry (29.8% in Han Chinese from Beijing China, CHB, and 22.1% in Japanese from Tokyo, JPT) and Western and Northern European ancestry (CEU; 23.5%). In contrast, it is far less common in the HapMap Yoruba sample from Ibadan, Nigeria (YRI; 0.9%). These observations suggest that a more comprehensive documentation of the global distribution of the genetic variation of the $ADORA_{2A}$ gene may contribute to the understanding of how this gene influences differential susceptibility to visual complications of diabetes, particularly the growing evidence that DR is less prevalent in West African individuals with diabetes than in Caucasians [52].

In summary, findings from this study should spark interest in all of the AR as potential modifiers in diabetes complications. If replicated, this association of $ADORA_{2A}$ with PDR could lead to additional studies of $ADORA_{2A}$ and its role in humans with DR and other microvascular complications of diabetes. Illustratively, the potential therapeutic effects of adenosine in ischemic retinal diseases have been suggested [22].

Acknowledgments

We would like to thank/acknowledge Dr. Adebowale Adeyemo, Deputy Director, of the Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health (NIH), for his contributions to the manuscript. This research was conducted at the University of Pittsburgh and supported by grants NIH/National Institute of Nursing Research 1F31NR008970-01A2 'Genetic Basis of Diabetic Retinopathy', Charles B. (PI), American Nurses Foundation 'Genes Implicated in Time to Onset and Severity of Diabetic Retinopathy', Charles B. (PI), and NIH/National Institute of Diabetes and Digestive and Kidney Diseases R01 DK034818-21 'Epidemiology of Diabetes Complications, Phase II', Orchard (PI). This research was also supported in part by the Intramural Research Program of the National Human Genome Research Institute, NIH, in the Center for Research in Genomics and Global Health (Z01HG200362).

References

1 American Diabetes Association: All about diabetes; in American Diabetes Association (ed): All About Diabetes. American Diabetes Association, 2008.
2 Frank R: Diabetic retinopathy. N Engl J Med 2004;350:48–58.
3 Klein R, Klein BEK, Moss SE, Davis MD, DeMets DL: The Wisconsin epidemiologic study of diabetic retinopathy. 3. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol 1984;102:527–532.
4 Hallman M, Huber C, Gonzalez V, Klein BE, Klein R, Hanis C: Familial aggregation of severity of diabetic retinopathy in Mexican Americans from Starr County, Texas. Diabetes Care 2005;28:1163–1168.

5 Rema M, Saravanan R, Deepa R, Mohan V: Familial clustering of diabetic retinopathy in South Indian type 2 diabetic patients. Diabet Med 2002;19:910–916.
6 Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. N Engl J Med 2000;342:381–389.
7 Arar NH, Freedman BI, Adler SG, Iyengar SK, Chew EY, Davis MD, Satko SG, Bowden DW: Heritability of severity of diabetic retinopathy: the FIND-Eye study. Invest Ophthalmol Vis Sci 2008;49:3839–3845.
8 Diabetes Control and Complications Trial Research Group: Clustering of long-term complications in families with diabetes in the Diabetes Control and Complications Trial. Diabetes 1997;46:1829–1839.
9 Harris M, Klein R, Cowie C, Rowland M, Byrd-Holt D: Is the risk of diabetic retinopathy greater in non-Hispanic blacks and Mexican Americans than in non-Hispanic whites with type 2 diabetes? A US population study. Diabetes Care 1998;21:1230–1235.
10 Tudor S, Hamman R, Baron A, Johnson D, Shetterly S: Incidence and progression of diabetic retinopathy in Hispanics and non-Hispanic whites with type 2 diabetes. San Luis Valley Diabetes Study, Colorado. Diabetes Care 1998;21:53–61.
11 Hecquet CM, Ahmmed GU, Vogel SM, Malik AB: Role of TRPM2 channel in mediating H_2O_2-induced Ca^{2+} entry and endothelial hyperpermeability. Circ Res 2008;102:347–355.
12 Salmon JE, Brogle N, Brownlie C, Edberg JC, Kimberly RP, Chen B-X, Erlanger BF: Human mononuclear phagocytes express adenosine a_1-receptors. J Immunol 1993;151:2775–2785.
13 McKusick VA: Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore, Johns Hopkins University Press, 2006.
14 Trincavelli ML, Melani A, Guidi S, Cuboni S, Cipriani S, Pedata F, Mattini C: Regulation of A_{2A} adenosine receptor expression and functioning following permanent focal ischemia in rat brain. J Neurochem 2008;104:479–490.
15 Xu Z, Park S-S, Mueller RA, Bagnell RC, Patterson C, Boysen PG: Adenosine produces nitric oxide and prevents mitochondrial oxidant damage in rat cardiomyocytes. Cardiovasc Res 2005;65:803–812.
16 Xu Z, Mueller RA, Park S-S, Boysen PG, Cohen MV, Downey JM: Cardioprotection with adenosine A_2 receptor activation at reperfusion. J Cardiovasc Pharmacol 2005;46:794–802.
17 Yonehana T, Gemba M: Ameliorative effect of adenosine on hypoxia-reoxygenation injury in LLC-PK_1, a porcine kidney cell line. Jpn J Pharmacol 1999;80:163–167.
18 Kostolanska J, Jakus V, Barak L: HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well-controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2009;22:433–442.
19 Kostolanska J, Jakus V, Barak L: Monitoring of early and advanced glycation in relation to the occurrence of microvascular complications in children and adolescents with type 1 diabetes mellitus. Physiol Res 2009;58:553–561.
20 Negre-Salvayre A, Salvayre R, Augé N, Pamplona R, Portero-Otín M: Hyperglycemia and glycation in diabetic complications. Antioxid Redox Signal 2009;11:3071–3109.
21 Takagi H, Kinh GL, Aiello LP: Hypoxia upregulates glucose transport activity through and adenosine-mediated increase of GLUT1 expression in retinal capillary endothelial cells. Diabetes 1998;47:1480–1488.
22 Macaluso C, Frishman LJ, Frueh B, Kaelin-Lang A, Onoe S, Niemeyer G: Multiple effects of adenosine in the arterially perfused mammalian eye: possible mechanisms for the neuroprotective function of adenosine in the retina. Doc Ophthalmol 2003;106:51–59.
23 Sugiyama T, Oku H, Komori A, Ikeda T: Effect of P2X7 receptor activation on the retinal blood velocity of diabetic rabbits. Arch Ophthalmol 2006;124:1143–1149.
24 Sugiyama T, Kobayashi M, Kawamura H, Li Q, Puro DG: Enhancement of P2X7-induced pore formation and apoptosis: an early effect of diabetes on the retinal microvasculature. Invest Ophthalmol Vis Sci 2004;45:1026–1032.
25 Liao SD, Puro DG: NAD+-induced vasotoxicity in the pericyte-containing microvasculature of the rat retina: effect of diabetes. Invest Ophthalmol Vis Sci 2006;47:5032–5038.
26 Pan H-Z, Zhang H, Chang D, Li H, Sui H: The change of oxidative stress products in diabetes mellitus and diabetic retinopathy. Br J Ophthalmol 2008;92:548–551.
27 Iwamoto T, Umenmura S, Toya Y, Uchibori T, Kogi K, Takgi N, Ishii M: Identification of adenosine A_2 receptor-cAMP system in human aortic endothelial cells. Biochem Biophys Res Commun 1994;199:905–910.
28 Adair TH, Montani JP, Strick DM, Guyton AC: Vascular development in chick embryos: a possible role for adenosine. Am J Physiol 1989;256:H240–H246.
29 Feoktistov I, Goldstein AE, Ryzhov S, Zeng D, Belardinelli L, Voyno-Yasenetskaya T, Biaggioni I: Differential expression of adenosine receptors in human endothelial cells: role of A_{2B} receptors in angiogenic factor regulation. Circ Res 2002;90:531–538.
30 Sapieha P, Hamel D, Shao Z, Rivera JC, Zaniolo K, Joyal JS, Chemtob S: Proliferative retinopathies: angiogenesis that blinds. Int J Biochem Cell Biol 2010;42:5–12.
31 Sapieha P, Sirinyan M, Hamel D, Zaniolo K, Joyal J-S, Cho J-H, Honoré J-C, Kermorvant-Duchemin E, Varma DR, Tremblay S, Leduc M, Rihakova L, Hardy P, Klein WH, Mu X, Mamer O, Lachapelle P, di Polo A, Beauséjour C, Andelfinger G, Mitchell G, Sennlaub F, Chemtob S: The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. Nat Med 2008;14:1067–1076.
32 Grant MB, Tarnuzzer RW, Caballero S, Ozeck MJ, Davis MI, Spoerri PE, Feoktistov I, Biaggioni I, Shryock JC, Belardinelli L: Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells. Circ Res 1999;85:699–706.
33 Kostraba JN, Klein R, Dorman JS, Becker DJ, Drash AL, Maser RE, Orchard TJ: The epidemiology of diabetes complications study. Am J Epidemiol 1991;133:381–391.
34 Costacou T, Chang Y, Ferrell RE, Orchard TJ: Identifying genetic susceptibilities to diabetes-related complications among individuals at low risk of complications: an application of tree-structured survival analysis. Am J Epidemiol 2006;164:862–872.
35 Lloyd CE, Klein R, Maser RE, Kuller LH, Becker DJ, Orchard TJ: The progression of retinopathy over 2 years: the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study. J Diabetes Complications 1995;9:140–148.
36 Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH: Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes 1990;39:1116–1124.
37 Diabetic Retinopathy Study Research Group: A modification of the Arlie House classification of diabetic retinopathy: Diabetic Retinopathy Study report No. 7. Invest Ophthalmol Vis Sci 1981;21:210–226.
38 Moss SE, Meuer S, Klein R, et al: Are seven standard photographic fields necessary for classification of diabetic retinopathy? Invest Ophthalmol Vis Sci 1989;30:823–828.
39 International HapMap Consortium: The International HapMap Project. Nature 2003;426:789–796.
40 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira M, Bender D, Maller J, Sklar P, de Bakker P, Daly M, Sham PC: PLINK: a toolset for whole-genome association and population-based linkage analysis. Am J Hum Genet 2007;81:559–575.
41 Maglott D, Ostell J, Pruitt KD, Tatusova T: Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res 2007;35:D26–D31.

42 Hubbard TJP, Aken BL, Beal K, Ballester B, Caccamo M, Chen Y, Clarke L, Coates G, Cunningham F, Cutts T, Down T, Dyer SC, et al: Ensembl 2007. Nucleic Acids Res 2007; 35:D610–D617.
43 Le F, Townsend-Nicholson A, Baker E, Sutherland GR, Schofield PR: Characterization and chromosomal localization of the human A_{2A} adenosine receptor gene: $ADORA_{2A}$. Biochem Biophys Res Commun 1996;223:461–467.
44 Yu L, Firth MC, Suzuki Y, Peterfreund RA, Gearan T, Sugano S, Schwarzschild MA, Weng Z, Fink SJ, Chen J-F: Characterization of genomic organization of the adenosine A_{2A} receptor gene by molecular and bioinformatics analysis. Brain Res 2004;1000: 156–173.
45 Sherry S, Ward M, Kholodov M, Baker J, Phan L, Smigielski E, Sirotkin K: dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 2001;29:308–311.
46 Olah ME, Roudabush FL: Down-regulation of vascular endothelial growth factor expression after A_{2A} adenosine receptor activation in PC12 pheochromocytoma cells. J Pharmacol Exp Ther 2000;293:779–787.
47 Lutty G, Matthews M, Merges C, McLeod D: Adenosine stimulates canine retinal microvascular endothelial cell migration and tube formation. Curr Eye Res 1998;17:594–607.
48 Leibovich S, Chen J, Pinhal-Enfield G, Belem P, Elson G, Rosania A, et al: Synergistic up-regulation of vascular endothelial growth factor expression in murine macrophages by adenosine A_2 receptor agonists and endotoxin. Am J Pathol 2002;160:2231–2244.
49 Valls MD, Cronstein BN, Montesinos MC: Adenosine receptor agonists for promotion of dermal wound healing. Biochem Pharmacol 2009;77:1117–1124.
50 Frobert O, Hanink G, Simonsen U, Gravholt CH, Levin M, Deussen A: Adenosine concentration in the porcine coronary artery wall and A_{2A} receptor involvement in hypoxia-induced vasodilation. J Physiol 2006;570: 375–384.
51 Lee H, Emala C: Systemic adenosine given after ischemia protects renal function via A_{2A} adenosine receptor activation. Am J Kidney Dis 2001;38:610–618.
52 Rotimi C, Danile H, Zhou J, Obisesan A, Chen G, Chen Y, Amoah A, Opoku V, Acheampong J, Agyenim-Boateng K, Eghan BA Jr, Oli J, Okafor G, Ofoegbu E, Osotimehin B, Abbiyesuku F, Johnson T, Fasanmade O, Doumatey A, Aje T, Collins F, Dunston G: Prevalence and determinants of diabetic retinopathy and cataract in West African type 2 diabetes patients. Ethn Dis 2003;13:S100–S107.