Aldose Reductase Gene Polymorphisms and Diabetic Retinopathy Susceptibility

Sotoodeh Abhary, MBBS\(^1\)
Kathryn P. Burdon, PhD\(^1\)
Kate J. Laurie, BMedSci\(^1\)
Stacey Thorpe, RN\(^1\)
John Landers, PhD\(^4\)
Lucy Godd, MBBS\(^2\)
Stewart Lake, FRcophth\(^1\)
Nikolai Petrovsky, FRACP\(^3\)
Jamie E. Craig, FRANZCO\(^4\)

OBJECTIVE — Aldose reductase (ALR) is involved in diabetic microvascular damage via the polyol pathway. A recent meta-analysis found genetic variation in the ALR gene (AKR1B1) to be significantly associated with diabetic retinopathy (DR). We investigated the genetic association of AKR1B1 with DR.

RESEARCH DESIGN AND METHODS — The study enrolled 909 individuals with diabetes. Participants were genotyped for an AKR1B1 microsatellite and 14 tag single nucleotide polymorphisms, and ophthalmological assessment was performed.

RESULTS — A total of 514 individuals were found to have DR. rs9640883 was significantly associated with DR (\(P = 0.0005\)). However, AKR1B1 variation was not independently associated with DR development after adjusting for relevant clinical parameters. rs9640883 was associated with duration of diabetes (\(P = 0.002\)).

CONCLUSION — Many previous reports have failed to account for known risk factors for DR. The commonly reported association of AKR1B1 with DR may be due to an association of the gene with younger age at onset of diabetes.

Blood pressure, BMI, renal function tests, serum cholesterol, and A1C levels were obtained. Hypertension was defined as blood pressure \(\geq 140/90\) mmHg or use of antihypertensive medication at recruitment. Hypercholesterolemia was defined as total cholesterol of \(>5.5\) mmol/l or use of lipid-lowering medication. Albuminuria was defined as urine albumin \(\geq 30\) mg/day.

The AKR1B1 (CA)n microsatellite was PCR amplified using fluorescently labeled primers published by Ko et al. (6) in 883 individuals (263 with type 1 and 620 with type 2 diabetes) and genotyped by electrophoresis on an ABI PRISM 3100 (Applied Biosystems).

Using the tagger program implemented in Haploview 4.0, 14 tag single nucleotide polymorphisms (SNPs) across the AKR1B1 gene, including the promoter region, were selected and genotyped in 909 individuals (271 with type 1 and 638 with type 2 diabetes) using iPLEX Gold chemistry on an autoflex mass spectrometer (Sequenom, San Diego, CA).

The \(\chi^2\) test for categorical and univariate binary logistic regression for continuous clinical covariates with DR were calculated in SPSS (version 15.0; SPSS, Chicago, IL). Allelic and genotypic associations of the (CA)n microsatellite were calculated using the \(\chi^2\) test and multivariate analysis with the binary logistic regression controlling for associated variables. Testing for association of all SNPs and haplotypes with DR was undertaken with the \(\chi^2\) test for univariate analysis and binary logistic for multivariate analysis in PLINK (version 1.06) (7) and also CLUMPHAP (8) when microsatellites were incorporated into haplotype analyses. Bonferroni correction was applied to microsatellite and haplotype analyses. Multiple testing of individual SNPs was adjusted for using Nyholt’s SNP spectral decomposition method (9), modified by Li and Ji (10), which estimated 10 independent tests.

RESULTS — A total of 514 participants had DR, of which 311 had NPDR (95 with type 1 and 216 with type 2 diabetes), 188 had PDR (71 with type 1 and 117 with type 2 diabetes), and 150 had...
CONCLUSIONS — There have been numerous studies assessing polymorphisms of the AKR1B1 gene and DR susceptibility, with (CA)n microsatellite and rs759853 most commonly studied. A recent meta-analysis found the z +2 allele in type 1 diabetes, and z −2 allele in any type of diabetes conferred protection from and risk for DR, respectively. The C allele of rs759853 conferred risk for DR in type 1 diabetes (4).

This study examined the (CA)n microsatellite and 14 tag SNPs. Although AKR1B1 variation was associated with DR, once established risk factors including diabetes duration and A1C were considered, no association remained. This suggests that particular SNPs may be associated with the clinical covariates rather than having a direct association with DR. We found the DR-associated SNP rs9640883 to also be associated with duration of diabetes. ALR reduces toxic aldehydes generated by reactive oxygen species to inactive alcohols. Decreased availability of the cofactor NADPH could induce or exacerbate intracellular oxidative stress (1). Chronic hyperglycemia and oxidative stress can result in permanent irreversible damage to pancreatic β-cells (11). Subsequent deterioration of β-cell function and increased disease severity results, with animal studies providing support for this hypothesis (12,13). Variation in ALR activity may affect the extent of oxidative stress, and genetic variation in AKR1B1 may account for altered ALR activity. The association observed between rs9640883 and DR, and those previously reported for this gene, may reflect the effect this gene has on age of onset of diabetes and therefore on diabetes duration, in turn influencing DR risk (14,15).

The majority of previous studies examining the relationship between AKR1B1 and DR have not undertaken multivariate analysis to consider known risk factors for DR. They may be influenced by the same confounding effect of duration of diabetes observed in this study.

Acknowledgments — This research was supported by a grant from the Ophthalmic Research Institute of Australia. K.P.B. is a Peter Doherty Fellow of the National Health and Medical Research Council of Australia (NHMRC). J.E.C. is an NHMRC Practitioner Fellow.

No potential conflicts of interest relevant to this article were reported.

The authors thank participating patients and their ophthalmologists, the research nurses, laboratory assistants, and statistician Dr. Thu-Lan Kelly for her statistical advice and expertise.

References

1. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–820
2. Das Evcimen N, King GL. The role of protein kinase C activation and the vascular complications of diabetes. Pharmacol Res 2007;55:498–510
3. Anil Kumar P, Bhanuprakash Reddy G. Focus on molecules: aldose reductase. Exp Eye Res 2007;85:739–740
4. Abhary S, Hewitt AW, Burdon KP, Craig JE. A systematic meta-analysis of genetic association studies for diabetic retinopathy. Diabetes 2009;58:2137–2147

5. Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991;98:786–806

6. Ko BC, Lam KS, Wat NM, Chung SS. An (A-C)n dinucleotide repeat polymorphic marker at the 5’ end of the aldose reductase gene is associated with early-onset diabetic retinopathy in NIDDM patients. Diabetes 1995;44:727–732

7. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575

8. Knight J, Curtis D, Sham PC. CLUMP-HAP: a simple tool for performing haplotype-based association analysis. Genet Epidemiol 2008;32:539–545

9. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–769

10. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 2005;95:221–227

11. Robertson R, Zhou H, Zhang T, Harmon JS. Chronic oxidative stress as a mechanism for glucose toxicity of the beta cell in type 2 diabetes. Cell Biochem Biophys 2007;48:139–146

12. Kaneto H, Kajimoto Y, Miyagawa J, Matsuo T, Fujitani Y, Umayahara Y, Hanafu T, Matsuzawa Y, Yamagaki Y, Hori M. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta cells against glucose toxicity. Diabetes 1999;48:2398–2406

13. Hamaoka R, Fujii J, Miyagawa J, Takahashi M, Kishimoto M, Moriwaki M, Yamamoto K, Kajimoto Y, Yamazaki Y, Hanafusa T, Matsuzawa Y, Taniguchi N. Overexpression of the aldose reductase gene induces apoptosis in pancreatic beta cells by causing a redox imbalance. J Biochem 1999;126:41–47

14. Jerneld B, Algvere P. Relationship of duration and onset of diabetes to prevalence of diabetic retinopathy. Am J Ophthalmol 1986;102:431–437

15. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol 1984;102:527–532