Aldose Reductase Polymorphisms, Fasting Blood Glucose, and Age-Related Cortical Cataract

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Purpose. To determine whether there is an association between polymorphisms of the *AKR1B1* gene and cortical cataract in the presence of hyperglycemia.

Methods. In the second cross section of the Blue Mountains Eye Study (BMES), 3508 participants (2334 at 5-year follow-up and 1174 newly recruited participants) were examined during 1997 to 2000. Cataract was graded from lens photographs using the Wisconsin Cataract Grading System. Fasting blood glucose (FBG) was measured. Continuous imputed dosages of minor alleles of 17 *AKR1B1* single nucleotide polymorphisms (SNPs) were assessed for associations with prevalent cortical cataract. Gene–environment interactions between SNPs and FBG were examined. Odds ratios (OR) and 95% confidence intervals (CI) for prevalent cortical cataract were estimated using logistic regression adjusting for age, sex, smoking, hypertension, education, and myopia. A P value of 0.005 was considered statistically significant after correction for 10 independent tests. Replication of significant associations found in the BMES sample was conducted in the Singapore Epidemiology of Eye Diseases (SEED) study (n = 10,033).

Results. No polymorphism was associated with prevalent cortical cataract. A significant interaction was observed between rs9640883 and FBG ($P_{\text{interaction}}$ = 0.004), with increased cortical cataract prevalence associated with rs9640883 minor allele dosage in those with FBG >6.0 mM (strata-specific OR 1.72, 95% CI 1.09–2.72). No similar association was found in participants with normal FBG (OR 0.85, 95% CI 0.69–1.04). This interaction was not evident in the SEED study.

Conclusions. The identified interaction between rs9640883 and FBG in relation to cortical cataract was not replicated but may warrant further investigation.

Keywords: cataract, epidemiology, genetic epidemiology

It has been estimated that diabetes affects more than 400 million adults worldwide.[1] Diabetes can damage many organs including the eye. Cataract is a frequent ocular complication among people with diabetes. The relationship between fasting blood glucose or diabetes and cortical cataract has been well documented in numerous population-based studies,[2–8] including the Blue Mountains Eye Study (BMES).[9–11] However, the underlying mechanisms of the association between diabetes and cataract development are not yet fully understood.

Growing evidence suggests the important role of both genetic factors and elevated blood glucose in the development of diabetic complications. A number of reports have documented associations between variation in the aldose reductase (*AKR1B1*) gene and diabetic retinopathy,[12–15] with specific *AKR1B1* gene polymorphisms associated with increased

diabetic retinopathy risk.[14,16] However, there have been limited reports on the association between the *AKR1B1* gene, glucose level, and cataract.[17]

The AKR1B1 enzyme has long been implicated in the development of diabetic ocular complications.[18,19] This rate-limiting enzyme is responsible for metabolizing glucose to sorbitol on the polyol pathway. It is possible that polymorphisms in the *AKR1B1* gene are associated with a modification effect that, in the presence of hyperglycemia, results in increased activity and faster conversion of glucose, leading to greater accumulation of sorbitol, lens swelling,[18] and eventual opacity.

In this study, we aimed to investigate the relationship between SNPs relevant to the *AKR1B1* gene, fasting glucose level, and cortical cataract in an older population cohort. Given that AKR1B1 enzyme activity varies depending on glucose level,[20–22] we hypothesized an association between the *AKR1B1* gene and cortical cataract in the presence of hyperglycemia. We planned to examine the association using both cross-sectional and longitudinal data in the BMES and replicate any significant associations in the Singapore Epidemiology of Eye Diseases (SEED) study population.

Methods

Study Populations

The BMES is a population-based study of common eye diseases in an older population aged 49 years and older, living in the Blue Mountains region of Sydney, Australia. In 1992 to 1994, baseline examinations (BMES 1) were conducted on 3654 participants (82.4% of eligible). Five-year follow-up examinations (BMES 2) were conducted during 1997 to 1999 on 2334 participants (75.1% of survivors) and 10-year follow-up examinations (BMES 3) conducted during 2002 to 2004 on 1952 participants (76.6% of survivors). During 1999 to 2000, 1174 newly eligible participants, who had moved to the area or were in the age range, were examined. The 2334 participants from the 5-year follow-up and the 1174 newly eligible participants comprise the cross-sectional 2 survey population (BMES C2).

The SEED study is a population-based study of common eye diseases in residents of Singapore among three major ethnic groups (Malays, Indians, and Chinese) aged 40 years and above. For each ethnic group, the baseline examinations were as follows: 3280 (78.7% of eligible) Malay participants recruited during 2004 to 2006, 3400 (75.6% of eligible) Indian participants recruited during 2007 to 2009, and 3353 (72.8% of eligible) Chinese participants recruited during 2009 to 2011.

Examination Procedures

For BMES, the same examination procedures were conducted for all examinations, and details of these procedures have been described in detail previously.[23] In SEED, the same examination procedures were conducted for all three ethnic groups and have also been described previously.[24,25] In brief, after pupil dilation, participants underwent detailed eye examination including lens photography. Questionnaires to collect demographic and medical information were administered by interviewers in English only for BMES and in participants' choice of English, Chinese, Malay, or Tamil for SEED. Both studies adhered to the tenets of the Declaration of Helsinki and were approved by the Human Ethics Committees of the University of Sydney (BMES), the Western Sydney Area Health Service (BMES), and the Singapore Eye Research Institute institutional review board (SEED). Written, informed consent was obtained from each participant.

Cataract Grading

The two studies employed the same lens photographic grader and cataract grading procedures, and details have been described previously.[23] Briefly, using the retroillumination (Neitz CT-R; Neitz Instruments, Tokyo, Japan, for BMES and Nidek EAS-1000; Nidek, Tokyo, Japan, for SEED) lens photographs taken during the examinations, presence of cortical cataract was assessed following the Wisconsin Cataract Grading System[26] in a masked manner. Total area of involvement was estimated using a grid laid over each photograph. Cortical cataract was defined if participants had cortical opacity $\geq$5% of the total lens area in at least one eye. Inter- and intragrader reliabilities for cataract grading were high.[27]

Incidence of cortical cataract was defined in BMES as the first appearance at follow-up of cortical cataract in those without cortical cataract in both eyes at baseline.

DNA Genotyping

For BMES, whole blood for DNA extraction was obtained from participants at the BMES C2 examination. DNA genotyping was performed at the Wellcome Trust Centre for Human Genetics (Sanger Institute, Cambridge, UK) using the Illumina Human 670-Quadv1 custom genotyping (Illumina, San Diego, CA, USA) array as part of the Wellcome Trust Case Consortium 2. Imputation was conducted for autosomes (chromosomes 1–22) using the 1000 Genomes (http://www.internationalgenome.org/; provided in the public domain by The European Bioinformatics Institute, Cambridgeshire, UK) reference data (Phase 1, v3, EUR reference panel). The imputed dosage data was generated using Minimac software, mapped to Build 37 (http://genome.sph.umich.edu/wiki/Minimac; provided in the public domain by University of Michigan, Ann Arbor, MI, USA). Continuous imputed dosage of minor alleles of 17 *AKR1B1* single nucleotide polymorphisms (SNPs), selected based on previous literature investigating genetic polymorphisms (tag SNPs) for diabetes, diabetic retinopathy, and nephropathy,[16,28] were used in the analyses.

In the SEED study, whole blood was also collected for DNA extraction at the baseline examination. DNA was genotyped using Illumina 610-Quad and Illumina OmniExpress. Genotyped SNP data were used for the replication analysis and coded as an additive model (0, 1, 2 indicates the number of risk alleles based on the BMES minor allele).

Blood Glucose Assessment

For BMES, fasting blood samples were collected at the study site and processed on the same day at the Institute of Clinical Pathology and Medical Research, Westmead Hospital. The hexokinase method was used for fasting blood glucose measurement.[29] For the prevalence analysis, fasting blood glucose level at BMES C2 was used, and for the incidence analysis, baseline fasting blood glucose level was used. Elevated fasting blood glucose level was defined as $>$ 6.0 mM.

In the SEED study, nonfasting blood samples were collected and processed on the same day at the National University Hospital Reference Laboratory (Singapore). Glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatography (Bio-Rad Variant II analyser; Bio-Rad Laboratories, Hercules, CA, USA) for the Malays, and by immunoassay with the Roche Cobas c501 (Roche Diagnostics, Indianapolis, IN, USA) for the Indian and Chinese cohorts. Elevated HbA1c was defined as $\geq$6.0%.

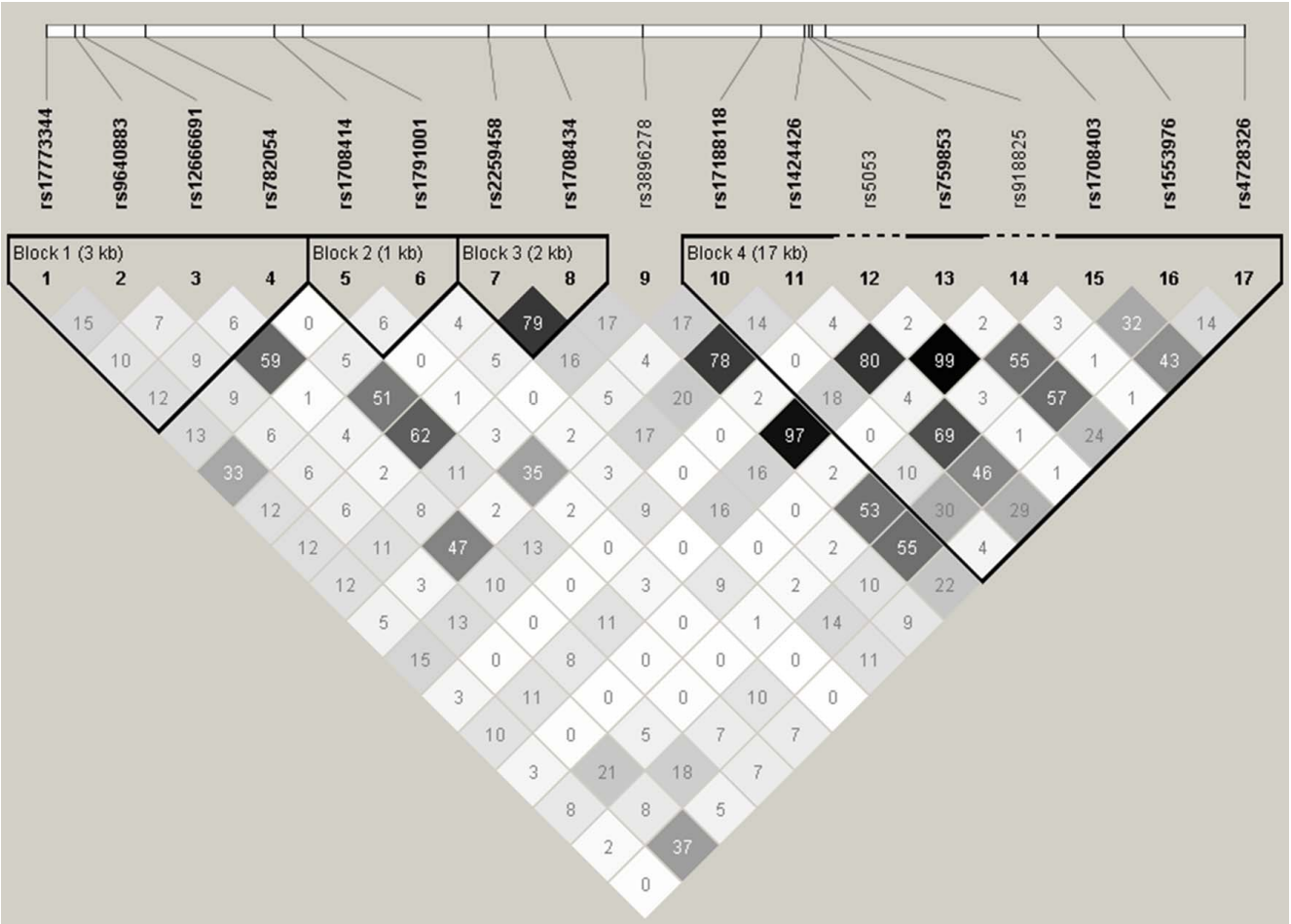

FIGURE. Linkage disequilibrium (LD) plot of the 17 *AKR1B1* SNPs. The LD plot was generated with data from the Blue Mountains Eye Study population using Haploview software. *Darker shades of gray* indicate higher LD correlation coefficient (r^2) values. A pairwise r^2 value is represented in each *diamond*.

Statistical Analysis

All analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). Prevalence of cortical cataract from BMES C2 examinations was the cross-sectional study outcome. Logistic regression models were constructed for cross-sectional analysis, adjusting for age, sex, smoking status, hypertension, education (trade certificate or higher qualification), and myopia (<−1.0 diopter for BMES, <−0.5 diopter for SEED). Ten-year incidence of cortical cataract from the BMES cohort was the longitudinal study outcome. Discrete logistic regression models (using PROC PHREG) were used for longitudinal analysis, adjusting for baseline age, sex, smoking status, hypertension, education level, and myopia. The discrete logistic regression model is a type of survival model in which event times are treated as being genuinely discrete in truth[30] and was used to capture cataract incidence at the 5- and 10-year follow-up time points. Haploview software (version 4.2; Broad Institute, Cambridge, MA, USA) was used to visualize the linkage disequilibrium (LD) structure among the selected SNPs. Correction for multiple testing was based on the number of effectively independent tests accounting for the LD structure among SNPs of the *AKR1B1* gene (Fig.), as determined using the SNPSpDLite[31] (https://neurogenetics.qimrberghofer.edu.au/SNPSpDlite/; provided in the public domain by QIMR GenEpi Laboratories, Brisbane, Australia) and the estimates proposed by Li and Ji.[32] A Bonferroni-adjusted P value of 0.005 was considered statistically significant, accounting for 10 independent tests. No further multiplicity adjustment for the incident and prevalent models was conducted as the two models assessed the same association with concordance in outcome and study variables.

Replication of significant findings from the BMES study was conducted in the SEED cross-sectional cohort using the same logistic regression models as were used in the BMES cross-sectional analysis. Findings from each ethnic group are presented separately as well as combined for all three groups. Quanto (Version 1.2.4; University of Southern California, Los Angeles, CA, USA) was used to conduct a post hoc power calculation for genetic interaction.

Results

Of the 3508 participants from the BMES C2 population, 2534 (72.2%) had genotype data available. Of those participants, 2223 (87.7%) had gradable photos and 2439 (96.3%) had fasting blood glucose. In total, 2181 (62.2%) participants with gradable photos, fasting blood glucose, and genotype data available were included in the cross-sectional analysis. Compared to those included, participants who were not included were slightly older (mean age 67.4 vs. 66.2, $P = 0.0011$) and more likely to have myopia (21.3% vs. 14.7%, $P < 0.0001$) (Table 1).

Table 1. Comparison of the Blue Mountains Eye Study (BMES) Cross Section 2 ($N = 3508$) and Singapore Epidemiology of Eye Diseases Study (SEED, $N = 10,033$) Characteristics for Those Participants That Were Included and Not Included in the Cross-Sectional Analysis

Characteristics	Included in Prevalence Analyses %	Not Included in Prevalence Analyses* %	*P*
BMES	$N = 2181$	$N = 1327$	
Age: mean (SD)	**66.2 (8.8)**	**67.4 (10.5)**	**0.0011**
Female	57.3	56.4	0.6326
Current smokers	9.4	11.3	0.0736
Hypertension	50.6	51.3	0.6853
Diabetes	9.9	10.0	0.9315
Education	64.5	62.8	0.3260
Myopia	**14.7**	**21.3**	**<0.0001**
Fasting blood glucose level, mM, mean (SD)	5.4 (1.6)	5.5 (1.6)	0.3716
SEED	$N = 6745$	$N = 3288$	
Age, mean (SD)	**57.8 (9.8)**	**61.0 (11.2)**	**<0.0001**
Female	**49.6**	**53.0**	**0.0014**
Current smokers	**16.6**	**14.8**	**0.0170**
Hypertension	**59.8**	**65.4**	**<0.0001**
Diabetes	**22.1**	**31.0**	**<0.0001**
Education	**40.4**	**37.5**	**0.0054**
Myopia	**35.5**	**43.2**	**<0.0001**
HbA1c %, mean (SD)	**6.3 (1.3)**	**6.4 (1.4)**	**0.0002**

P values based on *t*-test for means and χ^2 for frequencies. Bolded values are statistically significant ($P < 0.05$).
* Reasons for exclusion from analysis include missing demographic or genetic data and ungradable photographs.

Of the 3654 participants from BMES baseline survey, 651 (17.8%) had cortical cataract or had cataract surgery at the time and were excluded from the longitudinal analysis, leaving 3003 participants at risk of incident cortical cataract. Of the 3003 at risk, 1305 (43.4%) participants who had follow-up data, gradable photos, fasting blood glucose, and genotype data available were included. Compared to those included, participants who were not included were younger (mean age 62.2 vs. 66.0, $P < 0.0001$), more likely to be current smokers (17.9 vs. 12.7, $P = 0.0001$), and more likely to have hypertension (47.1 vs. 38.6, $P < 0.0001$), diabetes (8.7 vs. 5.2, $P = 0.0002$), and lower mean fasting blood glucose level (5.1 vs. 5.4 mM, $P < 0.0001$) (Table 2).

Of the 10033 participants of the SEED study, 7439 (74.1%) had SNP data available. Of those participants, 6796 (91.4%) had gradable photos and 7386 (99.3%) had HbA1c. In all, 6745 (67.2%) participants with gradable photos, HbA1c, and SNP data available were included in the replication analysis. Those who were not included were slightly older, more likely to be female, and more likely to have hypertension, diabetes, myopia, and slightly higher mean HbA1c but less likely to be current smokers or have higher education, compared to those included in the replication analysis (Table 1).

Cross-Sectional Associations of *AKR1B1* Gene With Prevalent Cortical Cataract in the BMES

In the BMES C2, 23.5% ($n = 522/2223$ with gradable photos) had prevalent cortical cataract. Participants with elevated fasting blood glucose (>6.0 mM) were more likely to have prevalent cortical cataract (odds ratio [OR], 1.38, 95% confidence interval [CI], 1.03–1.86) compared to those with normal fasting blood glucose (≤6.0 mM), after adjusting for age, sex, smoking status, hypertension, education level, and myopia. None of the 17 relevant SNPs investigated were directly associated with prevalent cortical cataract (Table 3). However, an interaction between the SNP rs9640883 (minor allele 'A' frequency 0.25) and fasting blood glucose was detected ($P = 0.0040$) (Table 4). Stratification by fasting blood glucose level showed a significant association between this SNP and cortical cataract prevalence among those with elevated fasting blood glucose (per minor allele increase, multivariate adjusted OR 1.72, 95% CI 1.09–2.72, $P = 0.0189$), while no similar association was found among participants with normal fasting blood glucose (≤6.0 mM: OR 0.85, 95% CI 0.69–1.04, $P = 0.11$).

Table 2. Comparison of Baseline Characteristics of Blue Mountains Eye Study Participants at Risk of Cortical Cataract Between Those Included and Not Included in the Longitudinal Analysis

Characteristics	At Risk and Included in Incidence Analyses, $N = 1305$ (%)	At Risk but Not Included in Incidence Analyses*, $N = 1698$ (%)	*P*
Age: mean (SD)	**66.0 (9.6)**	**62.6 (7.8)**	**<0.0001**
Female	55.9	54.8	0.52
Current smokers	**12.7**	**17.9**	**0.0001**
Hypertension	**38.6**	**47.1**	**<0.0001**
Diabetes	**5.2**	**8.7**	**0.0002**
Education†	**62.9**	**57.1**	**0.002**
Myopia	14.6	16.6	0.13
Fasting blood glucose level, mM: mean (SD)	**5.4 (1.8)**	**5.1 (1.1)**	**<0.0001**

Those not at risk ($N = 651$) due to presence of cortical cataract or cataract surgery at BMES 1 (baseline) were not included in this table. Bolded values are statistically significant ($P < 0.05$)
* Reasons for exclusion from analysis include missing demographic or genetic data and ungradable photographs.
† Defined as trade certificate or higher qualification.

Table 3. Association Between Aldose Reductase SNPs and Cortical Cataract in the Blue Mountains Eye Study Sample

AKR1B1 SNP	Position*	Function	Major Allele	Minor Allele	MAF	Prevalent Cortical Cataract OR (95 % CI)†	*P*	Incident Cortical Cataract HR (95% CI)‡	*P*
rs17773344	7:134115551	Intron	G	C	0.32	1.21 (1.04–1.42)	0.02	1.12 (0.94–1.35)	0.22
rs9640883	7:134116633	Intron	G	A	0.25	0.96 (0.80–1.14)	0.62	1.03 (0.84–1.26)	0.79
rs12666691	7:134116954	Intron	C	G	0.18	1.02 (0.84–1.24)	0.82	0.94 (0.75–1.19)	0.61
rs782054	7:134119228	Unknown	A	G	0.22	0.83 (0.68–1.00)	0.05	0.86 (0.69–1.08)	0.20
rs1708414	7:134124005	Unknown	T	C	0.23	1.01 (0.85–1.21)	0.89	1.00 (0.81–1.22)	0.97
rs1791001	7:134125056	Unknown	G	C	0.18	1.04 (0.85–1.27)	0.70	0.98 (0.77–1.24)	0.85
rs2259458	7:134131955	Intron	G	T	0.30	0.81 (0.69–0.96)	0.02	0.88 (0.72–1.08)	0.22
rs1708434	7:134134077	Intron	G	A	0.26	0.84 (0.71–1.01)	0.06	0.89 (0.72–1.10)	0.27
rs3896278	7:134137705	Intron	C	T	0.40	1.13 (0.97–1.33)	0.13	0.96 (0.79–1.17)	0.69
rs17188118	7:134142068	Intron	A	C	0.11	1.10 (0.85–1.43)	0.46	0.89 (0.65–1.22)	0.47
rs1424426	7:134143690	Intron	T	C	0.45	1.11 (0.94–1.30)	0.22	0.95 (0.79–1.15)	0.59
rs5053	7:134143863	5′ UTR	G	C	0.35	1.04 (0.68–1.57)	0.87	1.21 (0.75–1.93)	0.43
rs759853	7:134143958	5′ UTR	G	A	0.40	1.13 (0.96–1.32)	0.14	0.95 (0.78–1.15)	0.58
rs918825	7:134144485	Upstream 2 kb	T	C	0.03	1.01 (0.66–1.55)	0.95	1.18 (0.73–1.89)	0.51
rs1708403	7:134152364	Unknown	A	G	0.46	0.92 (0.78–1.08)	0.29	1.06 (0.88–1.28)	0.56
rs1553976	7:134155533	Unknown	C	T	0.28	1.05 (0.89–1.25)	0.55	0.98 (0.80–1.21)	0.86
rs4728326	7:134159995	Unknown	G	A	0.27	1.02 (0.86–1.21)	0.83	1.01 (0.82–1.24)	0.92

$P < 0.005$ considered statistically significant after multiple testing correction for 10 independent tests, accounting for LD structure among 17 selected SNPs on the *AKR1B1* gene. 5′ UTR, five prime untranslated region; MAF, minor allele frequency.

* Position is base-pair location in NCBI build 37 (GRCh37).

† Adjusted for age, sex, smoking status, hypertension, diabetes, education, and myopia at cross section 2.

‡ Adjusted for age, sex, smoking status, hypertension, diabetes, education, and myopia at baseline.

Longitudinal Associations of *AKR1B1* Gene With Incident Cortical Cataract in the BMES

Of the BMES 1 participants at risk of cortical cataract, 22.7% (n = 316/1388 with gradable photos) had incident cortical cataract at the 5- or 10-year visit. Participants with elevated fasting blood glucose (>6.0 mM) at baseline had a 2-fold greater risk of incident cortical cataract (hazard ratio [HR], 2.01, 95% CI 1.31–3.10) compared to those with normal fasting blood glucose (≤6.0 mM), after adjusting for age, sex, smoking status, hypertension, education level, and myopia. None of the 17 relevant SNPs investigated were directly associated with incident cortical cataract (Table 3), nor were any significant interactions between these SNPs and fasting blood glucose detected (Table 4).

Table 4. Interaction Terms Between *AKR1B1* SNPs and Fasting Blood Glucose in the Blue Mountains Eye Study Sample

AKR1B1 SNP	Prevalent Cortical Cataract Interaction Term*	Incident Cortical Cataract Interaction Term†
rs17773344	0.03	0.66
rs9640883	**0.0040**	0.46
rs12666691	0.30	0.96
rs782054	0.45	0.59
rs1708414	0.94	0.73
rs1791001	0.82	0.14
rs2259458	0.92	0.99
rs1708434	0.91	0.76
rs3896278	0.34	0.95
rs17188118	0.92	0.36
rs1424426	0.82	0.94
rs5053	0.25	0.49
rs759853	0.42	0.99
rs918825	0.29	0.65
rs1708403	0.70	0.45
rs1553976	0.59	0.51
rs4728326	0.77	0.65

Bolded value $P < 0.005$ considered statistically significant after multiple testing correction for 10 independent tests, accounting for LD structure among 17 selected SNPs on the *AKR1B1* gene.

* Adjusted for age, sex, smoking status, hypertension, education level, and myopia at cross section 2.

† Adjusted for age, sex, smoking status, hypertension, education, and myopia at baseline.

Replication of Interaction Between rs9640883 and HbA1c on Cortical Cataract Prevalence in the SEED Population

In the SEED, 26.8% (n = 1810/6745) had prevalent cortical cataract. Elevated HbA1c levels (≥6.0%) were associated with increased cortical cataract prevalence (combined SEED: 1.45, 95% CI 1.30–1.63), after adjusting for age, sex, smoking status, hypertension, education level, and myopia. We sought to replicate the significant interaction observed in the BMES (between SNP rs9640883 and elevated fasting blood glucose, >6.0 mM) in the SEED population using available HbA1c measures. The minor allele 'A' frequencies of SNP rs9640883 in the Malay, Indian, and Chinese samples were 0.63, 0.26, and 0.60, respectively. There was no direct association between the SNP rs9640883 and prevalent cortical cataract (Malays P = 0.60, Indians $P = 0.63$, Chinese $P = 0.18$, SEED $P = 0.44$). No interaction was observed between rs9640883 minor allele and elevated HbA1c (≥6.0%) in any of the three ethnic groups (Malays $P = 0.23$, Indians $P = 0.33$, Chinese $P = 0.56$) or in the combined SEED population ($P = 0.85$).

Discussion

In an older Australian population, we observed increased prevalence and incidence of cortical cataract among participants with elevated fasting blood glucose (>6.0 mM). No association was found between *AKR1B1* SNPs and prevalent cortical cataract. However, we observed effect modification by fasting blood glucose on the association between SNP

rs9640883 and cortical cataract prevalence, with the genetic association evident only among those with elevated fasting blood glucose. Unfortunately, we were unable to replicate this interaction in the SEED population, where elevated HbA1c was used due to unavailability of fasting glucose.

The association between elevated fasting blood glucose and cortical cataract is consistent with previous reports from this[10,11] and other cohorts in the literature.[2–8] Our findings provide evidence supporting the key role of elevated blood glucose in the development of cortical cataract. The proposed mechanisms for cortical cataract development in the presence of elevated blood glucose include glycosylation of lens proteins leading to oxidation and subsequent opacity,[33] accumulation of sorbitol through aldose reductase conversion of glucose,[34,35] and formation of reactive oxygen species.[35]

Although we did not find direct association between the *AKR1B1* SNPs and cortical cataract in the BMES population, we observed an association between the SNP rs9640883 and cortical cataract in the presence of hyperglycemia. Only among participants with elevated fasting blood glucose, there was an increased odds of cortical cataract with each minor allele increase. A previous study[17] reported a significant interaction between another allele of *AKR1B1*, the z-4 allele, and poor glycemic control in relation to the odds of cataract. Our finding from the BMES is the first to show an effect modification of blood glucose on the association between the rs9640883 SNP and cortical cataract. Interestingly, the same SNP, rs9640883, has previously been associated with diabetes duration and diabetic retinopathy in a diabetic population[16] and was considered as part of the *AKR1B1* gene. However, according to the database of SNPs (dbSNP), currently this SNP is part of a gene with unknown function, *LOC105375519*, located ~10 kb away from the *AKR1B1* gene. This SNP is classed as noncoding intronic variant, according to Genome Variation Server (http://gvs.gs.washington.edu/GVS144/; provided in the public domain by Nation Heart, Lung, and Blood Institute, Bethesda, MD, USA), which can affect disease through a number of mechanisms that may result in structural and functional changes and gene expression.[36] There are also enhancers that may be situated away (between 10 and 100 kb) from the target gene but still have the ability to affect gene function.[36] Although there appears to be no current evidence that the rs9640883 SNP (or any SNPs from *LOC105375519*) influences expression of AKR1B1, there have been suggestions that the SNP is associated with diabetes duration and diabetic retinopathy.[16]

We can only speculate on the mechanisms that may be involved in the interaction between *AKR1B1* gene and elevated fasting blood glucose linking to risk of cortical cataract. The *AKR1B1* gene encodes the enzyme involved in metabolism of glucose to sorbitol on the polyol pathway. It has been speculated that the accumulation of sorbitol is involved in cataractogenesis.[34,35] However, it has also been argued that the levels of sorbitol in human diabetic lenses are too low to elicit such changes to the lens.[37] Previously it has been suggested that, in the presence of hyperglycemia, the enzymatic properties of the aldose reductase enzyme is affected.[22] Under euglycemic conditions, it has been estimated that only 3% of glucose is converted to sorbitol, whereas in hyperglycemic conditions approximately 30% of glucose is converted.[20,21] The slow process of metabolizing sorbitol to fructose could lead to accumulation of sorbitol, which results in lens swelling and eventual opacity[18] in the cortical region.[38]

The lack of interaction between the rs9640883 SNP and elevated HbA1c in the replication population (SEED) may be due to differing ancestry from the BMES population, which principally comprises individuals of European ancestry. There were also differing minor allele frequencies observed among the study samples. A post hoc assessment of the power to detect an interaction in this replication sample was conducted. Using Quanto, we calculated that the SEED sample had 71% power to detect an interaction using the following parameters: 1810 cortical cases and 2.5 controls per case (for a total sample of 6335), with allele frequency of 0.26 (the smallest A allele frequency for the three ethnicities), elevated HbA1c prevalence of 46.75% for the SEED population, baseline population risk of cortical cataract 26.8% in SEED, environmental effect of OR 1.45 for elevated HbA1c in SEED, genetic effect of rs9640883 of OR 1.01 in SEED, and gene–environment interaction of OR 1.72 (from BMES). However, when interaction effect sizes (β coefficients) and standard errors between BMES and SEED samples were compared, it was observed that while the SEED study offered reasonable power to detect the effect observed in the BMES, in the SEED sample the effect was much closer to the null.

Although recommended cutoffs for impaired glucose tolerance were used for both elevated fasting blood glucose (WHO $>$ 6.0 mM)[39] and HbA1c (International Expert Committee $\geq$ 6.0%),[40] there may be some misclassification in the environmental variable used, which may explain the lack of agreement between the study samples. However, we were unable to find alternative replication populations with comparable age structure and cataract assessments with genetic and exposure data (fasting blood glucose) available to confirm the interaction found in the BMES population at this time. We cannot exclude the possibility that the interaction finding from the BMES was by chance only, although there may be a biologically plausible mechanism for the interaction observed, and the interaction was seen in a previous study involving diabetics.[16]

Strengths of our study include the population-based samples, the use of standardized examination methods for data collection, and masked cataract grading procedures for both the BMES and the SEED, with reported high reproducibility.[27] Given the number of tests conducted, we used a corrected significance level of $P = 0.005$ to take into account the multiple comparisons based on the LD structure of SNPs[31,32] among the *AKR1B1* gene. Limitations include the possible existence of unknown confounding factors that we have not accounted for in the models, although we have minimized this by adjusting for a number of known confounding factors. For the longitudinal analysis, only 43% of our BMES cohort had data available, which may be a reason for the lack of longitudinal association observed. Also, for the longitudinal analysis, those that were not included in the analysis were more likely to have risk factors associated with cortical cataract, which may also explain the lack of association for the incidence analysis. Lastly, we have taken a simplified view to determine whether associations exist between fasting blood glucose, *AKR1B1* gene, and cataract. As cataract is a complex disease, it is plausible that multiple processes are involved that contribute to the development of cortical cataract.

In an older Australian population, we confirmed previous reports of an association between elevated fasting blood glucose and cortical cataract. The finding from BMES suggesting a possible interaction between rs9640883 and fasting blood glucose on increased odds of cortical cataract, although consistent with our hypothesis, could not be replicated in Singaporeans (Asian samples). Further investigation in better-designed replication studies with the capacity for functional analyses may be warranted.

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