A Genome-Wide Association Study Provides New Evidence That CACNA1C Gene is Associated With Diabetic Cataract

Citation for published version:
Chang, C, Zhang, K, Veluchamy, A, Hébert, HL, Looker, HC, Colhoun, HM, Palmer, CNA & Meng, W 2016, 'A Genome-Wide Association Study Provides New Evidence That CACNA1C Gene is Associated With Diabetic Cataract' Investigative Ophthalmology & Visual Science, vol. 57, no. 4, pp. 2246-50. DOI: 10.1167/iovs.16-19332

Digital Object Identifier (DOI):
10.1167/iovs.16-19332

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Investigative Ophthalmology & Visual Science

Publisher Rights Statement:
This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
A Genome-Wide Association Study Provides New Evidence That CACNA1C Gene is Associated With Diabetic Cataract

Cheng Chang, 1 Kaida Zhang, 1 Abiram Veluchamy, 1 Harry L. Hébert, 1 Helen C. Looker, 1 Helen M. Colhoun, 1 Colin N. A. Palmer, 2 and Weihua Meng 1

1 Division of Population Health Sciences, Ninewells Hospital and School of Medicine, University of Dundee, Dundee, United Kingdom
2 Centre for Pharmacogenetics and Pharmacogenomics, Ninewells Hospital and School of Medicine, University of Dundee, Dundee, United Kingdom

Correspondence: Weihua Meng, Division of Population Health Sciences, School of Medicine, University of Dundee, Dundee, UK, DD2 4BF; w.meng@dundee.ac.uk.

CC and KZ contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: February 12, 2016
Accepted: March 20, 2016

Cataract is defined as a loss of normal transparency of the crystalline lens in the eye due to opacification or optical dysfunction. 1 It affects light transmission and results in a deteriorated vision. 1 According to the visual impairment data reported by the World Health Organization in 2012, cataract is the leading cause of global blindness, accounting for 51% of the overall cases, far higher than blindness caused by glaucoma (8%) and by age-related macular degeneration (5%). 2 In addition to being a major ocular disorder, cataract also puts heavy economic burdens on health care systems. For example, the total direct medical cost of cataract was $6.8 billion in 2004 in the USA, representing 42% of the total direct medical cost of all visual disorders in that year. 3 Cataract is also associated with many ophthalmic and general health conditions such as myopia and a high rate of falling. 4, 5

Diabetic cataract is one of the major eye complications of diabetes. It was reported that cataract occurs two to five times more frequently in patients with diabetes compared with those with no diabetes. 6-8 The purpose of this study was to identify genetic contributors of diabetic cataract based on a genome-wide association approach using a well-defined Scottish diabetic cohort.

Methods. We adapted linked e-health records to define diabetic cataract. A diabetic cataract case in this study was defined as a type 2 diabetic patient who has ever been recorded in the linked e-health records to have cataracts in both eyes or who had previous cataract extraction surgeries in at least one eye. A control in this study was defined as a type 2 diabetic individual who has never been diagnosed as cataract in the linked e-health records and had no history of cataract surgeries. A standard genome-wide association approach was applied.

Results. Overall, we have 2341 diabetic cataract cases and 2878 controls in the genetics of diabetes audit and research in Tayside Scotland (GoDARTS) dataset. We found that the P value of rs2283290 in the CACNA1C gene was 8.81 × 10⁻¹⁰, which has reached genome-wide significance. We also identified that the blood calcium level was statistically different between diabetic cataract cases and controls.

Conclusions. We identified supporting evidence that CACNA1C gene is associated with diabetic cataract. The role of calcium in the cataractogenesis needs to be reevaluated in future studies.

Keywords: genome-wide association study, cataract, diabetes, genetics
Genome-Wide Association Study and Diabetic Cataract

METHODS

Participants
The genetics of diabetes audit and research in Tayside Scotland (GoDARTS) project was originally created to identify genetic factors for diabetes and its complications. All participants complete a lifestyle questionnaire, undergo a baseline clinical examination, and provide their biological samples (blood and urine). In addition, they allow researchers to use their health information and their biological samples for research purposes and give permissions to link their personal information anonymously to their national health service (NHS) medical records. The medical records include their prescribing history, general practice clinic visits, hospital admissions, and outpatient appointments. Furthermore, their personal information is anonymously linked with the Scottish Care Information-Diabetes Collaboration (SCI-DC) database, which is an electronic health record system designed to keep track of local diabetic patients and help health professionals to provide better care in Scotland. Written informed consent was obtained from all participants. Further information about the GoDARTS project is available in the public domain at http://diabetesgenetics.dundee.ac.uk/. The research followed the tenets of the Declaration of Helsinki. The ethics approval has been granted by Tayside Committee on Medical Research Ethics (REC reference 053/04).

The GoDARTS project has recruited 9439 diabetic patients so far, among which 6927 were already genotyped by DNA chips. All GoDARTS participants’ health information was anonymously linked with their NHS and SCI-DC medical records from their enrollment until June 2011.

Definitions of Diabetic Cataract Cases and Controls
A diabetic cataract case in this study was defined as a type 2 diabetic patient who had ever been recorded in the linked e-health records as having cataracts in both eyes or who had previous cataract extraction surgeries in at least one eye. In the linked e-health records, there is no indication of the subtypes of cataract such as cortical cataract, nuclear cataract, and posterior subcapsular cataract as well as the severity of cataract. The diagnosis of cataract is mainly made by clinicians in the annual national retinal screen service.

A control in this study was defined as a type 2 diabetic individual who has never been diagnosed as cataract in the linked e-health records and had no history of cataract surgeries.

No other criteria (such as trauma and infection) were considered when defining cases and controls.

Genotyping and Quality Control
The project GoDARTS adopted two types of DNA chips to genotype its diabetic individuals. The Affymetrix SNP 6.0 chips (used on 3673 subjects; Affymetrix, Santa Clara, CA, USA) were funded by the Wellcome Trust Case Control Consortium 2 (WTCCC2) project15; the Illumina chips (used on 3254 subjects; OmniExpress BeadChip kit; Illumina, Inc., San Diego, CA, USA) were funded by the surrogate markers for micro- and macrovascular hard endpoints for innovative diabetes tools (SUMMIT) project.16 Genotype data quality controls were based on the standard protocols that were established for the WTCCC2 studies15 and the SUMMIT studies.16

Statistical Analysis
Software SHAPEIT and IMPUTE2 were used to impute non-directly genotyped single nucleotide polymorphisms (SNP) with reference files from the 1000 genomes phase I datasets (both directly genotyped SNPs and reference files were based on genome assembly National Center for Biotechnology Information b37).17,18 To filter out poorly imputed SNPs, a r2 < 0.3 is applied as it is the lower threshold value recommended by IMPUTE2 and we wanted to maximize the number of SNPs for further analysis.

The primary data manipulation software was PLINK and routine quality control steps were frequently applied during data analyses (e.g., removing SNPs with less than 95% genotyping call rate, SNPs with minor allele frequency less than 1%, SNPs that failed Hardy–Weinberg tests P < 0.000001 [based on control samples only], and removing individuals with more than 5% genotype data missing).19 Single nucleotide polymorphisms on sex chromosomes and mitochondrion were excluded. Multidimensional scaling analysis integrated in PLINK was used to detect population stratification. A lambda value was calculated to indicate the level of stratification. The lambda value should be very close to 1 indicating a minimum ancestry mixture. Samples with pi-hat > 0.125 were discarded due to relatedness. A logistic regression test with multiple covariates was applied to generate P values for SNP associations. A value of P < 5 × 10−8 is considered to be significant.

Other GWAS-related software used in our study were: SNPnexus for SNP functional annotation,20 HaploView for generating Manhattan plots and linkage disequilibrium (LD) blocks,21 and SNPEVG for generating corresponding quantile-quantile (q-q) plots to evaluate differences between cases and controls caused by potential confounders (different genotyping laboratories, different DNA extraction methods, etc.).22 Means of age, BMI, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and HbA1c were compared between cases and controls using independent t-test (SPSS 22; IBM Corp., Armonk, NY, USA). Sex difference was compared using a χ2 test. Blood calcium level was compared later using an independent t-test. The whole workflow was shown in Supplementary Figure S1.

RESULTS
In this general diabetic cohort, we identified 2501 type 2 diabetic patients with cataract and 3052 controls with no cataract according to the linked e-health records after removing type 1 diabetic patients and patients with no genetic data. Then, after filtering out related samples and outlier samples detected by population stratification analyses, we had 2341 diabetic cataract cases (males = 1249, females = 1092) and 2878 controls (males = 1655, females = 1223). The prevalence of diabetic cataract in our cohort was 44.9%. We compared the means of sex, age, BMI, cholesterol, triglycerides, HDL, LDL, HbA1c between cases and controls. There were statistical differences in sex, age, BMI, LDL, HbA1c between cases and controls while there was no statistical difference in cholesterol and triglycerides (Table 1).

Affymetrix SNP6.0 chips contain 704,847 directly genotyped and quality-controlled SNPs and Illumina OmniExpress chips contain 601,394 directly genotyped and quality-controlled SNPs. Altogether, 6,398,685 genotyped and imputed SNPs were available for further analysis, after routine quality control of genotyping and imputation. The lambda value, which indicates the level of population stratification, was 1.06 and therefore no further adjustments for population stratification was needed. We then performed logistic regression tests
on all SNPs, with age, sex, BMI, HDL, LDL, and HbA1c as covariates. We identified rs2283290 in the CACNA1C gene with a $P$ value of $8.81 \times 10^{-10}$ and an odds ratio (OR) of 0.72 (A allele, 95% confidence interval: 0.66–0.80; Fig.). We calculated the correlation between rs2283290 and 10 upstream and 10 downstream SNPs using PLINK and found that it was not in LD ($R^2 > 0.8$) with any of its nearby SNPs (Supplementary Fig. S2). We also downloaded the linkage information of this SNP from HapMap and found the SNP was not in LD with its nearby SNPs as well (Supplementary Fig. S3). The plot q-q of the association results was shown in the Supplementary Figure S4.

We extracted the baseline blood calcium values of cases and controls from the linked e-health records (only 4222 individuals have those values recorded). We found that there was a statistical difference of the blood calcium levels between diabetic cataract cases and controls in both men and women ($P = 0.001$, Table 2).

**DISCUSSION**

We performed a GWAS on diabetic cataract using a Scottish diabetic cohort based on phenotype information from linked e-health records and genetic information from DNA chips. We found that CACNA1C gene may be involved with diabetic cataract.

All diabetic patients in Scotland are invited to have retinal screening annually. During the screening, clinicians determine whether patients have cataracts or not, along with the diagnosis of diabetic retinopathy. However, in the case of a diagnosis of a cataract, the specific subtype of the cataract or the severity of the cataract is not reported. In fact, cataract appears more often in a mixed format—a combination of nuclear cataract, cortical cataract or posterior subcapsular cataract—than a single entity in clinical settings. It was reported that around one in three cataracts are a mixed type in a diabetic population. Therefore, the phenotype used in our study is “any cataract,” including mixed cataracts and any subtypes of cataracts. The prevalence of diabetic cataract in our cohort is 44.9%, which is matched with the prevalence of 47.9% for diabetic cataract in an Indian diabetic population. In principle, using a specific subtype of cataracts will have a higher ability to identify relative genes for genetic studies while in reality, these advantages normally are offset by reduced sample size and correspondingly reduced study power. In this study, using the CaTS power calculator, we had 80% power based on 2341 cases and 2878 controls, assuming a minor disease allele frequency of 0.15, a genotypic relative risk of 1.21, a prevalence of diabetic cataract in the diabetic population of 0.45, and the significance level of $5 \times 10^{-8}$. We identified that the smallest $P$ value was $8.81 \times 10^{-10}$ with an OR of 0.72 for rs2283290 in the CACNA1C gene, which reached GWAS significance ($P < 5 \times 10^{-8}$). It was a sporadic SNP with no supporting SNPs in the plot. The linkage disequilibrium analysis of the cohort revealed that this SNP had small $R^2$ values (indicating levels of LD) with its nearby SNPs (10 upstream and 10 downstream SNPs). This finding was matched with the HapMap Caucasian dataset, which also shows that rs2283290 and its nearby SNPs are not in LD, indicating that the significance is not caused by a genotyping error (Supplementary Fig. S3). The closest SNP in LD with rs2283290 is rs2239032 (10 kb away, $R^2 = 0.97$) in the HapMap.

![Figure](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/Journals/IOVS/935164/ on 07/29/2016)
dataset, but the $R^2$ score is 0.70 between the two SNPs in our GWAS dataset. This can be due to the population difference since we used a diabetic population while HapMap used a general Caucasian population. The imputation score of this SNP in our dataset is 0.970 (imputed in Illumina chips only since the SNP is directly genotyped in Illumina chips), indicating a good imputation result. The CACNA1C gene encodes a protein called the alpha-1 subunit of a voltage-dependent, dependent L-type calcium channel (also known as CaV1.2). The calcium channel CaV1.2 is expressed and distributed in the epithelium and cortical fiber cells in the mouse lens, especially in the short arms of the hexagonal lens fibers. The channel CaV1.2 influences different cellular responses as it relates to the regulation of intracellular calcium. Inhibition of the L-type calcium channel with felodipine or nifedipine has been reported to induce progressive cortical cataract formation and be associated with decreased lens weight in ex vivo mouse lenses. In addition, L-type calcium channel inhibitors have been reported to delay formation of diabetic cataract in rodents. Proliferation of human lens epithelial cells is also inhibited by blocking of calcium channels. Researchers have confirmed there was a more than a 23-fold increase in total lens calcium with cataract. Calcium homeostasis has been confirmed to be associated with diabetic cataract in a Scottish diabetic cohort. This can be due to the population difference to identify diabetic cataract in an elder diabetic population. We also extracted our results of the SNPs which were reported by other GWAS on cataract such as Lin et al. (diabetic cataract), Liao et al. (age-related cataract), and Ritchie et al. (age-related cataract: Supplementary Table S1). The statistical P values of these SNPs in our study were not small enough to merit further investigation.

In conclusion, we identified that CACNA1C gene is associated with diabetic cataract in a Scottish diabetic cohort using a GWAS approach. We also reported that the blood calcium level is significantly different between diabetic cataract cases and controls indicating the potential role of calcium in the cataractogenesis.

Acknowledgments

The authors of this manuscript would like to thank all the participants recruited in the GoDARTS. We thank the Health Informatics Centre in the School of Medicine, University of Dundee, for their help in data linkage.

Supported by the GoDARTS project that was jointly supported by DIABETES UK and The Wellcome Trust, and by Tenovus small grant 2015 T15/40.

Disclosure: C. Chang, None; K. Zhang, None; A. Veluchamy, None; H.L. Hebert, None; H.C. Looker, None; H.M. Colhoun, None; C.N.A Palmer, None; W. Meng, None

References

1. Patel DK, Prasad SK, Kumar R, Hemalatha S. Cataract: a major secondary complication of diabetes, its epidemiology and an overview on major medicinal plants screened for anticataract activity. Asian Pac J Trop Dis. 2011;1:323–329.
2. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol. 2012;96:614–618.
3. Rein DB, Zhang P, Wirth KE, et al. The economic burden of major adult visual disorders in the United States. Arch Ophthalmol. 2006;124:1754–1760.
4. Sach TH, Foss AJ, Gregson RM, et al. Falls and health status in elderly women following first eye cataract surgery: an economic evaluation conducted alongside a randomized controlled trial. Br J Ophthalmol. 2007;91:1675–1679.
5. Chang MA, Congdon NG, Bykhovskaya I, Munoz B, West SK. The association between myopia and various subtypes of lens opacity: SEE (Salisbury Eye Evaluation) project. Ophthalmology. 2005;112:1395–1401.
6. Klein BE, Klein R, Wang Q, Moss SE. Older-onset diabetes and lens opacities. The Beaver Dam Eye Study. Ophthalmic Epidemiol. 1995;2:49–55.
7. Klein BE, Klein R, Moss SE. Incidence of cataract surgery in the Wisconsin Epidemiologic Study of Diabetic Retinopathy. Am J Ophthalmol. 1995;119:295–300.
8. Kim SJ, Kim SJ. Prevalence and risk factors for cataracts in persons with type 2 diabetes mellitus. Korean J Ophthalmol. 2006;20:201–204.

| Dataset | Blood Calcium in Controls, mmol/L | Blood Calcium in Cases, mmol/L | $P$ |
|---------|-----------------------------------|--------------------------------|-----|
| Overall (samples, $n$) | 2.330 ± 0.140 (2015) | 2.344 ± 0.154 (2207) | 0.001 |
| Males only (samples, $n$) | 2.319 ± 0.137 (1049) | 2.351 ± 0.153 (1251) | 0.03 |
| Females only (samples, $n$) | 2.342 ± 0.143 (966) | 2.360 ± 0.367 (976) | 0.005 |

The blood calcium levels were presented as mean ± standard deviation.
9. Raman R, Pal SS, Adams JS, Rani PK, Vaitheeswaran K, Sharma T. Prevalence and risk factors for cataract in diabetes. Sankara Nethralaya diabetic retinopathy epidemiology and molecular genetics study, report no 17. *Invest Ophtalmol Vis Sci* 2010; 51:6253–6261.

10. Olson RJ, Mamalis N, Werner L, Apple DJ. Cataract treatment in the beginning of the 21st century. *Am J Ophtalmol*. 2003; 136:146–154.

11. Liao J, Su X, Chen P, et al. Meta-analysis of genome-wide association studies in multietnic Asians identifies two loci for age-related nuclear cataract. *Hum Mol Genet*. 2014;23:6119–6128.

12. Huang B, He W. Molecular characteristics of inherited congenital cataracts. *Eur J Med Genet*. 2010;53:347–357.

13. Meng W, Deshmukh HA, van Zuydam NR, et al. A genome-wide association study suggests an association of Chr8p21.3 (GFRA2) with diabetic neuropathic pain. *Eur J Pain*. 2015;19:392–399.

14. Lin HJ, Huang YC, Lin JM, et al. Novel susceptibility genes associated with diabetic cataract in a Taiwanese population. *Ophtalmic Genet*. 2013;34:35–42.

15. Zhou K, Bellenguez C, Spencer CA, et al. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat Genet*. 2011;43:117–120.

16. Fagerholm E, Ahlvist E, Forsblom C, et al. SNP in the genome-wide association study hotspot on chromosome 9p21 confers susceptibility to diabetic nephropathy in type 1 diabetes. *Diabetologia*. 2012;55:2386–2393.

17. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. 2009;5:e1000529.

18. Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods*. 2012;9:179–181.

19. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.

20. Dayem Ullah AZ, Lemoine NR, Chelala C. A practical guide for the functional annotation of genetic variations using SNPnexus. *Brief Bioinform*. 2013;14:437–447.

21. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265.

22. Wang S, Dvorkin D, Da Y. SNPEVG: a graphical tool for GWAS graphing with mouse clicks. *BMC Bioinformatics*. 2012;13:319.

23. Nirmalan PK, Robin AL, Katz J, et al. Risk factors for age related cataract in a rural population of southern India: the Aravind Comprehensive Eye Study. *Br J Ophtalmol*. 2004;88:989–994.

24. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet*. 2006;38:209–213.

25. Maddala R, Nagendra T, de Ridder GG, Schey KL, Rao PV. L-type calcium channels play a critical role in maintaining lens transparency by regulating phosphorylation of aquaporin-0 and myosin light chain and expression of connexins. *PLoS One*. 2013;8:e64676.

26. Catterall WA. Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol*. 2011;3:a003947.

27. Ettl A, Daxer A, Göttinger W, Schmid E. Inhibition of experimental diabetic cataract by topical administration of RS-verapamil hydrochloride. *Br J Ophtalmol*. 2004;88:44–47.

28. Meissner A, Noack T. Proliferation of human lens epithelial cells (HLE-B3) is inhibited by blocking of voltage-gated calcium channels. *Pflugers Arch*. 2008;457:47–59.

29. Tang D, Borchman D, Yappert MC, Vrensen GF, Rasi V. Influence of age, diabetes, and cataract on calcium, lipid-calcium, and protein-calcium relationships in human lenses. *Invest Ophtalmol Vis Sci*. 2003;44:2059–2066.

30. Cekic O, Bardak Y. Lenticular calcium, magnesium, and iron levels in diabetic rats and verapamil effect. *Ophtalmic Res*. 1998;30:107–112.

31. Hightower KR, Riley MV, McCreary J. Regional distribution of calcium in alloxan diabetic rabbit lens. *Curr Eye Res*. 1989;8:517–521.

32. Hennessey JA, Boczek NJ, Jiang YH, et al. A CACNA1C variant associated with reduced voltage-dependent inactivation, increased CaV1.2 channel window current, and arrhythmogenicity. *PLoS One*. 2014;9:e106982.

33. Ferreira MA, O’Donovan MC, Meng YA, et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet*. 2008;40:1056–1058.

34. Ripke S, O’Dushlaine C, Chambert K, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet*. 2013;45:1150–1159.

35. Pollreisz A, Schmidt-Erfurth U. Diabetic cataract-pathogenesis, epidemiology and treatment. *J Ophtalmol*. 2010;2010:608751.

36. Kinoshita JH, Kador P, Catiles M. Aldose reductase in diabetic cataracts. *JAMA*. 1981;246:257–261.

37. Lorenzo M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Exp Diabetes Res*. 2007;2007:61058.

38. Sorbinil Retinopathy Trial Research Group. A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. *Arch Ophtalmol*. 1990;108:1234–1244.

39. Peissig PL, Rasmussen LV, Berg RL, et al. Importance of multi-modal approaches to effectively identify cataract cases from electronic health records. *J Am Med Inform Assoc*. 2012;19:225–234.

40. Lin HJ, Huang YC, Lin JM, et al. Single-nucleotide polymorphisms in chromosome 3p14.1-3p14.2 are associated with susceptibility of type 2 diabetes with cataract. *Mol Vis*. 2010;16:1206–1214.

41. Ritchie MD, Verma SS, Hall MA, et al. Electronic medical records and genomics (eMERGE) network exploration in cataract: several new potential susceptibility loci. *Mol Vis*. 2014;20:1281–1295.