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Ophthalmic Epidemiol. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

Ophthalmic Epidemiol. 2018 June ; 25(3): 215–219. doi:10.1080/09286586.2017.1406122.

Exome Array Analysis of Nuclear Lens Opacity

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Abstract

Purpose—Nuclear cataract is the most common subtype of age-related cataract, the leading cause of blindness worldwide. It results from advanced nuclear sclerosis, or opacity in the center of the optic lens, and is affected by both genetic and environmental risk factors, including smoking. We sought to understand the genetic factors associated with nuclear sclerosis through interrogation of rare and low frequency coding variants using exome array data.

Methods—We analyzed Illumina Human Exome Array data for 1,488 participants of European ancestry in the Beaver Dam Eye Study who were without cataract surgery for association with nuclear sclerosis grade, controlling for age and sex. We performed single-variant regression analysis for 32,138 variants with minor allele frequency (MAF) ≥0.003. In addition, gene-based analysis of 11,844 genes containing at least two variants with MAF < 0.05 was performed using a gene-based unified burden and non-burden sequence kernel association test (SKAT-O). Additionally, both single-variant and gene-based analyses were analyzed stratified by smoking status.

Results—No single-variant test was statistically significant after Bonferroni correction ($p < 1.6 \times 10^{-6}$; top single nucleotide polymorphism (SNP): rs144458991, $p = 2.83 \times 10^{-5}$). Gene-based tests were suggestively associated with the gene RNF149 overall ($p = 8.29 \times 10^{-6}$) and among never smokers ($N = 790$, $p = 2.67 \times 10^{-6}$).

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Supplemental data for this article can be accessed on the publisher's website.

Declaration of interest

None of the authors have any proprietary interests or conflicts of interest related to this submission.

Submission

This work has not been published previously and is not under consideration for publication at other journals. The manuscript was submitted to JAMA Ophthalmology and was rejected. Reviewer comments and our responses are contained in our cover letter.

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Conclusions—This study did not find a significant genetic association with nuclear sclerosis, the possible association with the RNF149 gene highlights a potential candidate gene for future studies that aim to understand the genetic architecture of nuclear sclerosis.

Keywords

Nuclear sclerosis; nuclear cataract; nuclear lens opacity; exome array; genetics

Introduction

Age-related cataract is the leading cause of blindness worldwide,[1] and affects 17% of individuals in the United States over the age of 40 years.[2] Nuclear cataract is the most common subtype of age-related cataract,[3,4] and it results from advanced nuclear sclerosis, or opacity of the center of the optic lens. Older age, female sex, and family history increase the risk of nuclear cataract, as does smoking, which is a major modifiable risk factor.[5] Our previous analysis has demonstrated the importance of accounting for smoking when examining the genetics basis of nuclear sclerosis.[6]

Genetics also contribute to nuclear cataract, with heritability estimates of 35–48%.[5–9] A genome-wide association study (GWAS) for nuclear cataract in Asians identified significant SNPs on chromosome 3 in *KCNAB1* and on chromosome 21 near *CRYAA*,[10] a gene that codes for a protein expressed in the eye that affects lens opacity. In addition, several studies have examined if variation in *GJA3, GJA8, MIP, HSF4, LIM2*, and *CRYAA*,[11–13] which have established associations with congenital cataract, also play a role in age-related cataract. The results of these studies are inconsistent or lack replication.[14] One GWAS examined all age-related cataract subtypes combined in over 7,000 individuals and identified variants of suggestive, but not genome-wide significance.[15]

Despite these studies, the genetics of nuclear lens opacity is largely unknown. We sought to better understand nuclear sclerosis genetics through interrogation of rare and low frequency coding variants using exome array data.

Materials and methods

Study population

The Beaver Dam Eye Study is a population-based cohort of predominantly European Ancestry individuals, aged 43–84, residing in Beaver Dam, Wisconsin between 1987 and 1988. Participants attended a baseline examination (N = 4,926) and up to four subsequent examinations five years apart where ocular and demographic data were collected; full details have been published previously.[16] We selected unrelated individuals with extreme baseline ocular phenotypes (intraocular pressure and refractive error) for genotyping. The present study was limited to individuals who had not undergone cataract surgery and had genotype, nuclear sclerosis, age, sex, and smoking data (N = 1,488, Supplemental Table S1). This study followed the Declaration of Helsinki recommendations and has been approved by the Institutional Review Board at the University of Wisconsin. All participants provided informed consent.

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Outcome assessment

Nuclear sclerosis grade was ascertained from slit-lamp photographs of the lens, taken with a Topcon SL5 photo slit-lamp camera (Topcon America Corp., Paramus, New Jersey). Two separate graders compared the photographs to four standard photographs, which they then graded 1–5, 5 being the densest opacity. Full grading protocol details have been published elsewhere.[17,18] The most severe nuclear sclerosis grade in either eye at the visit prior to lens replacement surgery or censoring was used.

Genotyping and quality control

We genotyped 1,908 participants using the Illumina HumanExome BeadChip (San Diego, CA, USA) at the Genetic Resources Core Facility at Johns Hopkins Institute of Genetic Medicine. Genotypes were called using the Illumina GenTrain clustering algorithm in GenomeStudio.

All samples had a call rate >98%. Individuals with sex inconsistencies (n = 15) and Mendelian errors (n = 2) or missing values for nuclear sclerosis, age, sex, or smoking at the visit prior to cataract surgery or censoring (n = 312) were excluded. Cryptic relatedness and unexpected duplicates were determined by identifying pairs of individuals with identical by descent sharing >20%, representing first and second-degree relatives. The individual from each relative pair with the lower quality score (n = 85) was excluded.

We genotyped 242,901 variants. We used the GenGen package v 1.01 (https://github.com/WGLab/GenGen/releases) to convert allele coding and SeattleSeq 138 version 9.09 using GRCh37/hg19 (http://snp.gs. washington.edu/SeattleSeqAnnotation138) to annotate variants with gene names. Non-autosomal variants (n = 5,465), SNPs with a call rate<98% (n = 6,121), and monomorphic variants (n = 138,061) were removed. Principal components analysis using SMARTPCA in EIGENSTRAT was performed to assess population stratification.[19] Six individuals did not cluster with HapMap European ancestry controls (CEU), and were excluded. There was high concordance among HapMap samples (99.82%) and blind sample duplicates (99.99%). After quality control analysis using PLINK, 1,488 individuals and 93,254 SNPs remained (Table S1). We did not exclude SNPs from analysis based upon departure from Hardy-Weinberg Equilibrium (HWE). However, HWE was computed for all analyzed SNPs, and none of our top SNPs in single-variant or gene-based analyses provided evidence of departure from HWE ($p < 0.001$).

Single-variant analysis

Single-variant analysis was performed to detect if individual SNPs (n = 44,578) with a minor allele frequency (MAF) ≥0.003 were associated with nuclear sclerosis. This cutoff was used to ensure adequate representation (at least 10 copies) of each minor allele. We had >80% power to detect a beta of 0.6 or greater for MAF = 0.005, and 25% power to detect similar betas for a MAF = 0.001 (Quanto power calculator assuming continuous trait, additive inheritance, mean = 3.2, SD = 0.8). A linear regression of nuclear sclerosis grade on age and sex was performed (Stata v13.1, StataCorp, College Station, Texas), and the residuals were used as the quantitative outcome in the analytical models. For single-variant analysis, a modified Bonferroni significance threshold was used ($p = 1.6 \times 10^{-6}$) based on 32,138

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independent SNPs (pairwise $r^2 > 0.2$). As smoking is a major risk factor for nuclear cataract, we stratified analyses by smoking status: never smokers versus past or current smokers.

Gene-based analysis

We performed gene-based tests to improve power over single-variant analyses where multiple variants in a gene are associated with nuclear sclerosis. A unified burden and sequence kernel association test in the R package, SKAT-O was used.[20] We analyzed 11,844 genes, each with multiple variants, resulting in 63,598 SNPs with MAF<0.05 (Bonferroni threshold: 4.22×10^{-6}). We performed sensitivity analyses, using both standard weights $\sqrt{w_j}=\beta\,(MAF_j; \mathrm{a}_1=1,\ \mathrm{a}_2=25)$, which provide rare variants high weights, as well as Madsen–Browning weights, which modestly upweight the rare variants. We stratified by smoking status, and also examined candidate genes associated with congenital cataract and age-related cataract for association with nuclear sclerosis.

Results

Of the genotyped individuals, 1,488 passed quality control and had complete data for all variables. Approximately half (46.9%) of these individuals were current or former smokers (Table 1). Never smokers were slightly older and more likely to be female than current or former smokers. Nuclear sclerosis severity was similar between the smoking groups.

Single-variant analysis

None of the single-variant tests were statistically significant ($p < 1.6 \times 10^{-6}$; Table 2). The top SNP, rs144458991 (MAF = 0.0038), was on chromosome 11 in *SUV420H1* ($p = 2.83 \times 10^{-5}$). After stratification by smoking status, no variants were significant.

Gene-based analysis

Gene-based tests using Madsen–Browning weights showed no significant associations overall (Table S2). There was suggestive evidence of an association for the gene *RNF149* ($p = 8.29 \times 10^{-6}$; Table 3). Interestingly, this finding was exome-wide significant ($p < 4.2 \times 10^{-6}$) for never smokers ($p = 2.67 \times 10^{-6}$), but was not significant in past or current smokers ($p = 0.07$). No other genes were significant, including candidate genes from congenital cataract studies ($p > 0.05$, Table S3).

Replication of published SNPs

Neither of the significant variants from the GWAS in Asians (rs7615568, intronic to *KCNAB1*, and rs11911275, upstream of *CRYAA*) nor SNPs in linkage disequilibrium with them were present on this exome array, thus they could not be evaluated for replication.[10] No other SNPs in *KCNAB1* ($p > 0.81$) or *CRYAA* ($p > 0.10$) approached significance, nor did was either gene significant in gene-based tests (*CRYAA* had only one SNP and was not included in gene-based tests, *KCNAB1* $p = 0.30$).

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Discussion

We aimed to better understand the genetic underpinnings of nuclear sclerosis through exome array analysis. Neither single-variant nor gene-based analyses uncovered significant associations with nuclear sclerosis. After stratification by smoking status, we identified a suggestive association among never smokers with *RNF149*.

RNF149 codes the ring finger 149 protein, involved in ligase and protein ubiquitination, a process that tags molecules for proteasome-dependent degradation and has some evidence for expression in the retina.[21,22] We hypothesize that if variants in this gene inhibit ubiquitination and hence degradation of crystalline proteins, these proteins could accumulate in the lens, causing increased opacity. This effect may be greater in nonsmokers if smoking has a very strong effect on nuclear sclerosis risk, thus overpowering the effect of the gene.

Our study has several limitations. We did not identify a comparable replicate population (similar ancestry and age) with available genetic data for our regions of interest, which restricted our ability to confirm our suggestive association. The exome array primarily targets the coding region of the genome; thus, we were not able to evaluate noncoding variants where some variants affecting nuclear cataract may be present. Finally, the Beaver Dam Eye Study participants are primarily of European ancestry. Consequently, our findings may not be generalizable to other ancestral populations with different allele frequencies.

Nevertheless, our results implicate a potentially interesting gene for nuclear sclerosis that warrants attention, and should be evaluated for interaction with smoking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by U10EY006594 (B Klein, R Klein), R01EY021531, and the Research to Prevent Blindness unrestricted grant. SJL was supported by an institutional training grant from the NIH/NHLBI (T32 HL007024). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Table 1

Demographic and ocular features of Beaver Dam Eye Study participants.

Variable	Overall	Never smokers	Current or former smokers
N (%)	1488	698 (46.9%)	790 (53.1%)
Age (years), mean (SD)	70.3 (9.3)	71.6 (9.2)	69.1 (9.2)
Female, N (%)	871 (58.5%)	502 (71.9%)	369 (46.7%)
Nuclear sclerosis score, mean (SD)	3.20 (0.79)	3.26 (0.81)	3.15 (0.78)

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Table 2

Top single-variant analysis results, overall and stratified by smoking status.[a]

SNP	Chr	Position	Gene	MAF	Minor Allele	Beta	P value
Overall (N = 1488)							
rs144458991	11	67925546	*SUV420H1*	0.0038[b]	G	0.88	2.83E-05
rs2308327	10	131565170	*MGMT*	0.1188	G	−0.15	4.86E-05
rs7642805	3	175592927	intergenic	0.3406	G	0.10	4.94E-05
rs12805648	11	7509566	*OLFML1*	0.1709	A	0.13	5.59E-05
rs2308321	10	131565064	*MGMT*	0.1188	G	−0.15	5.95E-05
Never smokers (N = 790)							
rs306481	19	56487603	*NLRP8*	0.4451	A	−0.14	3.59E-05
rs148139055	16	83991313	*OSGIN1*	0.0031[c]	C	0.96	3.84E-05
rs10445526	18	66605389	*CCDC102B*	0.2998	G	−0.15	5.82E-05
rs143346525	1	27877117	*AHDC1*	0.0047[d]	A	−1.00	5.99E-05
rs1470859	7	47913579	*PKD1L1*	0.168	A	−0.17	1.19E-04
Past or current smokers (N = 698)							
rs112380460	17	11166786	*SHISA6*	0.0037[e]	A	1.28	3.08E-06
rs2852464	12	52710721	*KRT83*	0.3705	G	0.16	3.09E-05
rs2066509	6	53365063	*GCLC*	0.0075[f]	A	0.83	4.83E-05
rs1140085	4	3015553	*GRK4*	0.1463	A	−0.19	1.08E-04
rs755639	2	127860149	*BIN1*	0.4165	C	0.14	1.45E-04

[a] Bonferroni corrected significance threshold: $p < (0.05/32{,}138 \text{ variants}) = 1.6 \times 10^{-6}$

[b] ExAc MAF: 0.0028, 1000 genomes CEU MAF = 0.0

[c] ExAc MAF: 0.0036, 1000 genomes CEU MAF = 0.0

[d] ExAc MAF: 0.0018, 1000 genomes CEU MAF = 0.0

[e] ExAc MAF: 0.0028, 1000 genomes CEU MAF = 0.0051

[f] ExAc MAF: 0.0054, 1000 genomes CEU MAF = 0.0051

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Table 3

Top gene-based analysis results overall, and corresponding significance in never smokers and past or current smokers.[a,b]

	Overall (N = 1488)		Never smokers (N = 790)		Past and Current smokers (N = 698)	
Gene	**P value**	**N SNPs**	**P value**	**N SNPs**	**P value**	**N SNPs**
RNF149	8.29E-06	2	**2.67E-06**	2	0.069	2
ATP9B	3.11E-04	4	5.28E-04	4	0.20	2
GPSM1	3.67E-04	5	0.061	4	4.37E-04	5
ACOT11	5.47E-04	14	6.85E-03	13	0.02	11
CCDC51	6.31E-04	2	0.064	2	2.36E-03	2

[a] Bonferroni corrected significance threshold: $p < (0.05/11{,}844 \text{ genes}) = 4.2 \times 10^{-6}$. Significant genes are in bold.

[b] The variants that comprise the set for *RNF149* are rs184618462 (MAF = 0.0013), and rs143827530 (MAF = 0.0021).