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EPHA2 polymorphisms in Estonian patients with age-related cataract

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ABSTRACT

Background: Ephrin receptors (Ephs) are tyrosine kinases that together with their ligands, ephrins, are considered important in cell–cell communication, especially during embryogenesis but also for epithelium homeostasis. Studies have demonstrated the involvement of mutations or common variants of the gene encoding Eph receptor A2 (*EPHA2*), in congenital cataract and in age-related cataract. This study investigated a number of disease-associated single nucleotide polymorphisms (SNPs) in *EPHA2* in patients with age-related cataract.

Materials and methods: The study included 491 Estonian patients who had surgery for age-related cataract, classified as nuclear, cortical, posterior subcapsular and mixed lens opacities, and 185 controls of the same ethnical origin. Seven SNPs in *EPHA2* (rs7543472, rs11260867, rs7548209, rs3768293, rs6603867, rs6678616, rs477558) were genotyped using TaqMan Allelic Discrimination. Statistical analyses for single factor associations used χ^2-test and logistic regression was performed including relevant covariates (age, sex and smoking).

Results: In single-SNP allele analysis, only the rs7543472 showed a borderline significant association with risk of cataract ($p = 0.048$). Regression analysis with known risk factors for cataract showed no significant associations of the studied SNPs with cataract. Stratification by cataract subtype did not alter the results. Adjusted odds ratios were between 0.82 and 1.16 (95% confidence interval 0.61–1.60).

Conclusions: The present study does not support a major role of EphA2 in cataractogenesis in an Estonian population.

INTRODUCTION

Cataract is an opacification of the eye lens leading to visual impairment and as such responsible for about 50% of all blindness globally.[1] In the western world, cataract surgery is the most common surgical procedure, leading to large costs for society. Whereas congenital cataract is largely inherited in a classical Mendelian manner, age-related cataract is a complex disease resulting from a combination of lifestyle-related factors and a large number of common genetic variations, *i.e.* single nucleotide polymorphisms (SNPs), each contributing with only a minor increase in risk of cataract. Twin studies have revealed that environmental effects are less important than what was previously believed and that genetic factors have the highest impact on risk of cataract.[2, 3] For cortical cataract, heredity accounted for 58%, environment for 26% and age for 16% of clinical variance.[2] For nuclear sclerosis, corresponding numbers were 48%, 14% and 38%.[3] A large genome-wide association study for age-related cortical cataract performed on subjects from the Beaver Dam Eye Study, showed linkage in 1p36,[4] a region associated with a type of congenital posterior polar cataract.[5, 6] Knockout of the Eph receptor A2 gene (*EPHA2*), which is located in chromosome 1p36, results in cataract in mice[7] and both congenital cataracts and age-related cataract in humans have shown association with mutations or SNPs in this gene.[7-10] The purpose of the present study was to investigate possible correlations between several SNPs in the *EPHA2* gene and cataract in an Estonian population.

MATERIALS AND METHODS

Patients

Patients with age-related cataract (n=491) and controls (n=185), all recruited from two ophthalmic clinics in Tartu and the South Estonian area, were recruited to the study after informed consent. The Ethical Commission at the University of Tartu in Estonia approved of the study and the tenets of the Declaration of Helsinki were followed. The type of cataract was determined prior to surgery using biomicroscopy and ophthalmoscopy and classified into the following subtypes; cortical cataract (n=151), posterior subcapsular cataract (n=119), nuclear cataract (n=75) and mixed cataract (n=146). Patients with secondary cataracts were excluded and control subjects were only included it they were free from cataract, uveitis, and glaucoma. Data on smoking habits was also obtained for all individuals.

SNPs and genotyping

In this study, we chose disease-associated SNPs of the *EPHA2* gene (gene ID: 1969).[7, 11, 12] All the SNPs were genotyped using genomic DNA extracted from whole blood samples. TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) were used according to the TaqMan® Allelic Discrimination technology,[13] on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the SDS 2.3 software supplied with the instrument. One of the SNPs (rs6678616) was custom made, using the Custom TaqMan® Assay Design Tool (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

Cataract patients and controls were analyzed with regard to differences in age, sex, smoking habits and allele frequencies, using Student's t-test and Pearson's chi-square test (or Fisher's exact test when appropriate). Single marker associations were analyzed using binary logistic regression including relevant risk factors for age-related cataract; age, sex and smoking as covariates in an additive model (homozygote for major allele=0, heterozygote=1 and homozygote for minor allele=2).[14-17] IBM® SPSS® Statistics 20.0 (IBM Corp., Armonk, NY,

USA) was used for statistical analyses and a p-value of ≤0.05 was considered statistically significant. All SNPs were analyzed for deviation from Hardy-Weinberg equilibrium with χ^2-test.[18]

RESULTS

Demographic data of cataract patients and controls are summarized in Table 1. Mean age was significantly lower in the control group but there was no difference in gender distribution between groups. As previously shown for this study population, the nuclear cataract subtype had a higher frequency of current smokers (data not shown).[19] All the SNPs in the *EPHA2* gene (Table 2) had a Hardy-Weinberg equilibrium p-value of >0.05 and the genotyping call rate was >99% for all the SNPs. Genotype distribution and frequencies of minor alleles are shown in Tables 3 and 4. Allele frequencies for the examined SNPs corresponded well to previously reported frequencies in European populations according to the NCBI SNP database, except for rs7548209 and rs477558 which differed slightly from the CEU population (Table 4). In univariate analyses only the rs7543472 showed a borderline/significant association with risk of cataract (p=0.048). When including relevant covariates (age, sex and smoking) in a multivariate analysis, no significant associations between the studied SNPs and cataract diagnosis were evident (Table 4). Stratification by cataract subtype did not change the results (data not shown).

DISCUSSION

The Eph receptor family constitutes 25% of all known human tyrosine kinase receptors.[20] The natural ligands of the Eph receptors are termed ephrins; ephrin A and ephrin B, where ephrin-A1-A5 are linked to the membrane via glycosylphosphatidylinositol and ephrin-B1-B3 have a transmembrane domain and a cytoplasmic tail.[21] The Eph receptors are also divided in two classes based on sequence homology of their extracellular domains; EphA receptors, which bind to ephrin-A ligands and EphB receptors which bind to ephrin-B ligands.[22] Exceptions are ephrin-A5 which binds to EphB2[23] and EphA4 which interacts with both ephrin-A and ephrin-B ligands.[24, 25] Interactions between the Ephs and the ephrins take place in the interface of adjacent cells, either via forward signaling by the Eph kinases, or via reverse signaling by ephrins on opposing cells.[26] This bidirectional signaling is believed to be important in cell contact-depending communication and for tissue assembly, especially during embryonic development.[20] Recent work has suggested that the Eph receptors also play essential roles in remodeling of epithelial tissue postnatally and that they may be important in epithelium homeostasis.[20]

Although expression of the Eph receptors are especially abundant in embryonic tissue, almost all Ephs have been found in adult cells, predominantly in different types of epithelial tissue like the lens.[27] Cheng et al have demonstrated that Epha2/Src signaling is essential for the differentiation process of the lens epithelial cells at the equator, and lenses from *EPHA2*$^{-/-}$ mice exhibit disorganized meridional rows, altered shape of the equatorial lens cells and disrupted alignment of lens.[28] Knockdown of *EPHA2* also resulted in micro- and sperophakia (small and more speric lenses) with disturbances in refractive power and decentration of the lens sutures.[29] Furthermore, loss of ephrin-A5, a ligand of EphA2, caused disruption of lens fiber organization and cataract development in 87% of ephrin-A5$^{-/-}$ mice.[30] It was also shown that ephrin-A5 interacts with EphA2 to regulate adherens junctions by recruitment of β-catenin to N-cadherin.[30]

In humans, mutations in the *EPHA2* gene have been demonstrated in congenital posterior polar or cortical cataracts.[7, 9, 10] In addition, several SNPs in *EPHA2* have shown association with age-related cataract in population of European and Asian ancestry. [7, 8, 10-12] Based on the linkage seen between cortical cataract and one locus on chromosome 1p36 including *EPHA2* in genome-wide scan on a subset of participant from the Beaver Dam Eye Study, some of these association studies were only performed on subjects with cortical cataract.[7, 12] However, a few additional studies investigating possible associations with SNPs in *EPHA2* and other/all types of lens opacities have confirmed that less common variants of the *EPHA2* gene are mainly seen in cortical cataracts and to some extent also in posterior subcapsular cataract.[10, 11] The phenotype seen in lenses from *EPHA2*$^{-/-}$ mice, in families with congenital cataract due to *EPHA2* mutations and in a large portion of older individuals with cataract-associated SNPs in *EPHA2* is thus consistent.

In the present study, no significant associations were found between cataract and the investigated SNPs in *EPHA2*, neither when considering different types of lens opacities nor when comparing the whole group of cataract patients with controls. This may be an effect of too small groups, even though significant associations between the same variants of *EPHA2* and cataract have been demonstrated in other studies.[10, 12] The adjusted odds ratios obtained in this study, between 0.82 and 1.16 (95% confidence interval 0.61-1.60), do indicate that any possible effects on risk of cataract and the *EPHA2* SNPs investigated here are rather small in the studied population.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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TABLES

TABLE 1. Demographics of patients with cataract and controls.

Parameter	Cataract					Controls	p-values*
	All cases	Cortical	Mixed	PSC	Nuclear		
No of subjects	491	151	146	119	75	185	
Age (years)	72 ± 8.7	72 ± 8.4	72 ± 8.7	71 ± 8.2	74 ± 9.5	66 ± 6.9	<0.001
Sex							
Female	342 (69.7)	114 (75.5)	98 (67.1)	83 (69.7)	47 (62.7)	134 (72.4)	0.51
Male	149 (30.3)	37 (24.5)	48 (32.9)	36 (30.3)	28 (37.3)	51 (27.6)	
Smoking							
Current smoker	71 (14.5)	17 (11.3)	22 (15.1)	14 (11.8)	18 (24.0)	18 (9.7)	0.13
Ever smoker	123 (25.1)	31 (20.5)	42 (28.8)	26 (21.8)	24 (32.0)	42 (22.7)	0.55

Data presented as absolute numbers (%) or mean ± SD. PSC: posterior subcapsular cataract.
* p-values were calculated with χ^2-test for categorical parameters and Student's t-test for age (all cases versus controls).

TABLE 2. Overview of SNPs studied in *EPHA2*.

rs-ID	Genome position* Chr: 1	Allele major > minor[a]	Gene location	SNP type	TaqMan assay
rs7543472	16440392	T > C	Down stream	-	C__30571916_10
rs11260867	16441728	C > G	Down stream	-	C____385507_10
rs7548209	16448622	G > C	Down stream	-	C____486603_10
rs3768293	16467924	T > G	Intron 3-4	-	C__11556891_10
rs6603867	16474701	G > A	Intron 3-4	-	C__11556899_10
rs6678616	16475123	G > A	Exon 3	Synonymous	custom[b]
rs477558	18219827	G > A	Upstream	-	C___3082756_20

* Positions are based on NCBI Build 37 and are given according to sense sequences (on the chromosomes) relative to the human reference sequences.
[a] Major and minor alleles in our studied population
[b] Primers and probe were designed according to antisense strand of the gene; forward primer: GCACTTCTTGTAGTAGACACGGA, reverse primer: CCAGGATATCGGTGCCTGTG , probe: TGGCGCT[G/A]CTCTC.

TABLE 3. Genotype frequencies of SNPs in *EPHA2* in cataract and controls.

rs-ID	Genotype	Cataract					Controls	p-values*
		All cases	Cortical	Mixed	PSC	Nuclear		
		n = 491	n = 151	n = 146	n = 119	n = 75	n = 185	
	CC	30 (6.1)	6 (4.0)	7 (4.8)	9 (7.6)	8 (10.7)	12 (6.5)	
rs7543472	CT	163 (33.2)	42 (27.8)	54 (37.0)	40 (33.6)	27 (36.0)	58 (31.4)	0.897
	TT	298 (60.7)	103 (68.2)	85 (58.2)	70 (58.8)	40 (53.3)	115 (62.2)	
	GG	16 (3.3)	4 (2.6)	5 (3.4)	4 (3.4)	3 (4.0)	6 (3.2)	
rs11260867	CG	158 (32.2)	36 (23.8)	50 (34.2)	42 (35.3)	30 (40.0)	58 (31.4)	0.978
	CC	317 (64.6)	111 (73.5)	91 (62.3)	73 (61.3)	42 (56.0)	121 (65.4)	
	CC	29 (5.9)	12 (7.9)	9 (6.2)	4 (3.4)	4 (5.3)	6 (3.2)	
rs7548209	CG	173 (35.2)	59 (39.1)	51 (34.9)	39 (32.8)	24 (32.0)	74 (40.0)	0.249
	GG	289 (58.9)	80 (53.0)	86 (58.9)	76 (63.9)	47 (62.7)	105 (56.8)	
	GG	59 (12.0)	23 (15.2)	17 (11.6)	10 (8.4)	9 (12.0)	15 (8.1)	
rs3768293	GT	214 (43.6)	64 (42.4)	60 (41.1)	54 (45.4)	36 (48.0)	84 (45.4)	0.349
	TT	218 (44.4)	64 (42.4)	69 (47.3)	55 (46.2)	30 (40.0)	86 (46.5)	
	AA	56 (11.4)	22 (14.6)	16 (11.0)	9 (7.6)	9 (12.0)	14 (7.6)	
rs6603867	AG	210 (42.8)	65 (43.0)	59 (40.4)	51 (42.9)	35 (46.7)	88 (47.6)	0.267
	GG	225 (45.8)	64 (42.4)	71 (48.6)	59 (49.6)	31 (41.3)	83 (44.9)	
	AA	43 (8.8)	16 (10.6)	11 (7.5)	8 (6.7)	8 (10.7)	11 (5.9)	
rs6678616	AG	199 (40.5)	63 (41.7)	59 (40.4)	47 (39.5)	30 (40.0)	83 (44.9)	0.368
	GG	249 (50.7)	72 (47.7)	76 (52.1)	64 (53.8)	37 (49.3)	91 (49.2)	
	AA	107 (21.8)	31 (20.5)	33 (22.6)	24 (20.2)	19 (25.3)	47 (25.4)	
rs477558	AG	237 (48.3)	72 (47.7)	76 (52.1)	54 (45.4)	35 (46.7)	86 (46.5)	0.603
	GG	199 (29.4)	48 (31.8)	37 (25.3)	41 (34.5)	21 (28.0)	52 (28.1)	

Data presented as absolute numbers (%). PSC: posterior subcapsular cataract.
* p-values were calculated with χ^2-test for categorical parameters (all cases versus controls).

TABLE 4. Minor allele frequencies of SNPs in *EPHA2*.

rs-ID	Minor allele*	Cataract n = 2x491	Controls n = 2x185	OR (95% CI)[a]	p-value[b]
rs7543472	C	21.2 %	26.2 %	0.82 (0.62-1.10)	0.19
rs11260867	G	18.1 %	22.2 %	0.84 (0.61-1.16)	0.30
rs7548209	C	24.5 %	20.5 %	1.16 (0.85-1.60)	0.34
rs3768293	G	32.8 %	33.5 %	0.96 (0.73-1.27)	0.79
rs6603867	A	29.0 %	31.9 %	0.98 (0.75-1.30)	0.91
rs6678616	A	29.0 %	28.4 %	0.98 (0.73-1.30)	0.87
rs477558	A	47.3 %	45.1 %	1.05 (0.82-1.35)	0.71

* Minor allele in our studied population.
[a] Odds ratio (OR) adjusted for age, sex and smoking. 95% CI; confidence interval.
[b] p-values were calculated using logistic regression with age, sex and smoking as covariates in an additive model.