AQPI and SLC4A10 as candidate genes for primary open-angle glaucoma

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Purpose: Recent evidence supports the role of reduced cerebrospinal fluid (CSF) pressure in the pathogenesis of primary open-angle glaucoma (POAG). We investigated the association of variants in two candidate genes that are important in CSF production, aquaporin 1 (AQPI) and solute carrier family 4, sodium bicarbonate transporter, member 10 (SLC4A10), with POAG in the Caucasian population.

Methods: POAG subjects (n=382) met the criteria of glaucomatous optic neuropathy with consistent visual field loss. Intraocular pressure was not used as an inclusion criterion. Control subjects (n=363) did not meet any of the inclusion criteria and had no family history of glaucoma. Eleven tagging single nucleotide polymorphisms (SNPs) for AQPI and SLC4A10 were genotyped in the POAG and control subjects, using allelic discrimination assays. Genotype frequencies were compared between the POAG and control subjects, using logistic regression adjusted for gender.

Results: There was no statistically significant difference in genotype frequencies between POAG and control subjects for any of the tested SNPs in AQPI and SLC4A10 (p>0.05).

Conclusions: There was no association between common sequence variants in the AQPI or SLC4A10 genes and POAG in the Caucasian population. This is the first study to investigate the association between these two candidate genes and increased risk for POAG.

Primary open-angle glaucoma (POAG; OMIM 137760) is the most common form of glaucoma, which is the leading cause of irreversible blindness worldwide [1,2]. POAG is characterized by a chronic optic neuropathy and progressive loss of retinal ganglion cells, leading to specific visual field defects in the absence of a known secondary cause [3,4]. Several risk factors have been associated with POAG, including elevated intraocular pressure (IOP), advanced age, black race, and family history [5]. To date, four causative genes for POAG from 11 candidate chromosomal loci have been identified, including MYOC (myocilin), OPTN (optineurin), WDR36 (WD repeat domain 36), and CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1), but these four genes together account for less than 10% of POAG cases [6-10].

Recent studies have provided strong support for the theory that reduced cerebrospinal fluid (CSF) pressure may play a role in the pathogenesis of POAG. In a retrospective review of patients with a history of lumbar puncture at the Mayo Clinic (Rochester, Minnesota), Berdahl et al. [11] reported that the mean CSF pressure was significantly lower in POAG patients when compared with nonglaucomatos control patients. A prospective study conducted by Ren et al. [12] confirmed that the mean CSF pressure is lower in POAG patients and in normal tension glaucoma (NTG) patients when compared with nonglaucomatos control patients. A decreased CSF pressure can lead to an increased translaminar pressure difference, which is defined as the pressure difference between IOP and CSF pressure [13]. When the translaminar pressure difference is abnormally increased, axoplasmic flow is disrupted, leading to retinal ganglion cell death and optic disc changes characteristic of glaucoma [14-18]. These findings lend support to the hypothesis that a decreased CSF pressure resulting in an increased translaminar pressure difference may contribute to the pathogenesis of glaucoma.

The candidate gene approach is used to study genes that are hypothesized to play a role in the etiology of a complex human disease with genetic contributions. This approach has been successful in identifying genes, such as complement factor H in age-related macular degeneration [19]. In this study we investigated aquaporin 1 (AQPI) and solute carrier family 4, sodium bicarbonate transporter, member 10 (SLC4A10) as candidate genes for POAG. AQPI maps to chromosomal location 7p14 and SLC4A10 maps to chromosomal location 2q23-q24, neither of which is at a known chromosomal locus for POAG listed by the Human Genome Organization [20-23]. Both of these genes are expressed in the choroid plexus and have been shown to be important in CSF production [20-29]. Knockout mouse models of these genes demonstrate a significant reduction in CSF production and intracranial pressure [22,28].
We hypothesized that \textit{AQP1} and \textit{SLC4A10} have a role in the pathogenesis of POAG because of their function in CSF production and their effect on the translaminar pressure difference. In this study, we investigated the association between sequence variants of these genes with increased risk for POAG in the Caucasian population.

**METHODS**

**Subjects:** This study was reviewed and approved by the Institutional Review Board of Duke University Medical Center (Durham, NC) and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all study participants. Study subjects were recruited from the Duke University Eye Center (Durham, NC) for a total of 382 subjects with POAG and 363 control subjects. Subjects with POAG were unrelated and met the following inclusion criteria: 1) age of onset greater than 30 years; 2) glaucomatous optic neuropathy in both eyes; and 3) visual field loss consistent with optic nerve damage in at least one eye [30]. Glaucomatous optic neuropathy was defined as a cup-to-disc ratio greater than 0.7 or focal loss of the nerve fiber layer resulting in a notch, associated with a glaucomatosus visual field defect. Visual fields were performed using standard automated perimetry or frequency doubling test [2]. IOP was not used as an inclusion criterion. The exclusion criteria for POAG subjects included the diagnosis of a secondary form of glaucoma or a history of ocular trauma. The control subjects were examined by the same glaucoma subspecialist (RRA) who examined the POAG subjects. The control subjects were unrelated and met the following criteria: 1) no first-degree relative with glaucoma; 2) IOP less than 21 mmHg in both eyes without treatment; 3) no evidence of glaucomatous optic neuropathy in either eye; and 4) normal visual field in both eyes.

**Genomic DNA genotyping:** Genomic DNA was extracted from peripheral blood samples via alcohol and salt precipitation using Gentra Systems PUREGENE DNA Purification Kit (Qiagen, Valencia, CA). Based on the genotype data from the HapMap Project, HaploView software version 4.1 (Broad Institute, Boston, MA) was used to select 11 tagging single nucleotide polymorphisms (SNPs) for \textit{AQP1} and \textit{SLC4A10} in the Caucasian population, using an \( r^2 \) threshold of 0.6 and a minor allele frequency threshold of 0.05 [31]. TaqMan allelic discrimination assays were used for genotyping with Assays-On-Demand and Assays-By-Design products, according to the standard protocols from the manufacturer (Applied Biosystems, Foster City, CA) [32]. For quality control purposes, two Centre d'Etude du Polymorphisme Humain standards (CEPH, Paris, France) and quality control samples were placed within and across 384-well plates (Applied Biosystems, Foster City, CA), and laboratory personnel were blinded to the location of these samples. Genotype submission to the database required matching genotypes for all quality control samples and at least 95% genotyping efficiency.

**Statistical analysis:** Analysis of Hardy–Weinberg equilibrium was performed separately for POAG and control subjects with Genetic Data Analysis (GDA) software [33]. Pairwise linkage disequilibrium (LD) between SNPs was calculated with the GOLD software [34]. Genotype frequencies in POAG and control subjects were compared by logistic regression with adjustment for gender using SAS software (SAS Institute Inc., Cary, NC). SNP genotypes were coded according to a log-additive risk model, which assumes that the risk from carrying a single copy of the variant (minor) allele is midway between that of zero copies (reference genotype) and of two copies on the logarithmic scale. Power calculations were performed using QUANTO software according to previously described methods, assuming a population prevalence of 10% and a log-additive risk model [35].

**RESULTS**

A total of 382 POAG subjects and 363 control subjects were included in the Caucasian data set. Of the POAG subjects, 15 subjects had NTG, defined as a maximum IOP <22 mmHg. The mean age of onset of POAG in the experimental subjects was 57.6±14.2 years, and the mean age of control subjects at the time of ophthalmologic exam was 64.8±9.3 years. The experimental group was 49.7% female, and the control group was 59.8% female.

A total of 11 tagging SNPs were selected to cover the LD blocks of \textit{AQP1} and \textit{SLC4A10}, using HaploView software (Figure 1). All of these were in Hardy–Weinberg equilibrium (\( p>0.01 \)) in the Caucasian controls. We did not observe statistically significant pair-wise LD (with an \( r^2 \) cut-off of 0.6) between the four tagging SNPs of \textit{AQP1} or the seven tagging SNPs of \textit{SLC4A10}. No significant genotype frequency differences between cases and controls were detected in either \textit{AQP1} or \textit{SLC4A10} (Table 1).

For the three SNPs with lower minor allele frequencies (ranging from 7% to 16% for rs765840, rs1399650, and rs1979112), our Caucasian data set had >94% power at a two-tailed significance level of 5% to detect an odds ratio of 2 or greater. For all other SNPs, our Caucasian data set had >90% power at a two-tailed significance level of 5% to detect an odds ratio of 1.5 or greater.

**DISCUSSION**

Recent studies have shown that CSF pressure is reduced in POAG subjects as compared to control subjects, suggesting that CSF pressure may play a role in the pathogenesis of POAG [11,12]. Decreased CSF pressure leads to an increase in the translaminar pressure difference. Elevations of IOP also increase translaminar pressure differences, which have been shown to disrupt axoplasmic flow and cause retinal ganglion...
cell death in a glaucomatous pattern [14-18]. Two genes that are expressed in the choroid plexus and have been shown to be important in CSF production are \(AQP1\) and \(SLC4A10\) \[20-29\]. These two genes were selected out of many other genes implicated in CSF production because of their selective expression in the choroid plexus, their functional significance in water and ion transport, and their demonstrated role in CSF production in knockout mouse models \[20-29\]. However, we did not detect an association between common tagging SNPs of \(AQP1\) or \(SLC4A10\) and POAG in this study.

In this study we focused on the potential role of genes associated with CSF production in glaucoma. It is important to note that \(AQP1\) is also expressed in the trabecular meshwork and Schlemm’s canal cells located within the conventional aqueous outflow tract of the eye \[36\], while \(SLC4A10\) is not \[37\]. \(AQP1\) is believed to improve cell viability in the setting of mechanical strain, but the degree to

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**Table 1. Minor allele frequencies of tagging SNPs in the \(AQP1\) and \(SLC4A10\) genes in Caucasian POAG and control subjects**

| Candidate gene | SNP     | Allele | Controls (n=363) | POAG (n=382) | p-value* |
|---------------|---------|--------|-----------------|--------------|----------|
| \(AQP1\)     | rs1004317 | G      | 0.404           | 0.399        | 0.786    |
|               | rs17159702 | G      | 0.292           | 0.265        | 0.297    |
|               | rs765840 | A      | 0.067           | 0.077        | 0.468    |
|               | rs1049305 | C      | 0.412           | 0.367        | 0.078    |
| \(SLC4A10\)  | rs1399650 | C      | 0.157           | 0.152        | 0.739    |
|               | rs2892769 | C      | 0.451           | 0.439        | 0.639    |
|               | rs1551051 | T      | 0.267           | 0.237        | 0.146    |
|               | rs1979112 | G      | 0.121           | 0.141        | 0.221    |
|               | rs1227929 | T      | 0.409           | 0.411        | 0.901    |
|               | rs1913807 | T      | 0.207           | 0.213        | 0.574    |
|               | rs4500960 | A      | 0.490           | 0.483        | 0.726    |

The asterisk indicates that the p-value is from logistic regression with adjustment for sex, using log-additive coding of SNP genotypes; SNPs: single nucleotide polymorphisms; POAG: primary open-angle glaucoma; \(AQP1\): aquaporin-1; \(SLC4A10\): solute carrier family 4, sodium bicarbonate transporter, member 10.
which AQP1 contributes to bulk outflow in the conventional outflow tract of the eye is uncertain [38-40].

It is known that AQP1 and SLC4A10 are major contributors to CSF production. Due to the functional importance of AQP1 and SLC4A10 in CSF production, sequence variants within these two genes might be expected to be more prevalent in NTG patients since both a retrospective and a prospective study showed a trend of NTG patients having lower CSF pressure than POAG subjects [12,41]. Our Caucasian data set is composed primarily of POAG subjects with high tension glaucoma, and only 15 subjects had NTG. Therefore, this study did not have sufficient power to detect an association between AQP1 or SLC4A10 and an increased risk of NTG.

Our large sample size provided adequate statistical power to detect a moderate or strong association between common sequence variants of these two genes and an increased risk of POAG in the Caucasian population. However, we cannot rule out the presence of such an association in populations of different ancestry. It is also possible that only rare sequence variants in these genes, which our study was not designed to detect, have an appreciable effect on CSF production and pressure. Additionally, mutations identified in different regions of AQP1 have been shown to reduce water and ion transport function [42-43]. Targeted mutations, such as an exon deletion in SLC4A10 knockout mice, resulted in an 88% reduction in brain ventricle size from decreased CSF production as compared to wild-type mice [28]. The effect of knockout models of these genes on the optic nerve has not been described to date. Further studies would be necessary to identify naturally occurring mutations in AQP1 and SLC4A10 that lead to decreased CSF pressure.

In conclusion, we did not find an association between common sequence variants in AQP1 and SLC4A10 and high tension POAG in our Caucasian data set. Further studies are needed to determine if variants within these two genes are associated with NTG only or if rare sequence variants with a greater effect on CSF pressure may play a role in POAG in populations of Caucasian or non-Caucasian origin.

ACKNOWLEDGMENTS

We would like to thank the study participants and study staff at the Duke Center for Human Genetics and the Department of Ophthalmology at Duke University Eye Center for their assistance. This research was supported by NIH grants R01EY015543 (R.R.A.), R01EY013315 (M.A.H.), R01EY019126 (M.A.H.), and by Duke University’s CTSA grant TL1RR024126 from NCRR/NIH.

REFERENCES

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP. Global data on visual impairment in the year 2002. Bull World Health Organ 2004; 82:844-51. [PMID: 15640920]

2. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90:262-7. [PMID: 16488940]

3. Allingham RR, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: A review. Exp Eye Res 2009; 88:837-44. [PMID: 19061866]

4. Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open-angle glaucoma. N Engl J Med 2009; 360:1113-24. [PMID: 19279343]

5. Leske MC. Open-angle glaucoma—an epidemiologic overview. Ophthalmol Epidemiol 2007; 14:166-72. [PMID: 17896292]

6. Monemi S, Spaeth G, DaSilva A, Popinchalk S, Ilitch E, Liebmann J, Ritch R, Héon E, Crick RP, Child A, Sarfarazi M. Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. Hum Mol Genet 2005; 14:725-33. [PMID: 15677485]

7. Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Héon E, Krupin T, Ritch R, Kreuzer D, Crick RP, Sarfarazi M. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science 2002; 295:1077-9. [PMID: 11834836]

8. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. Identification of a gene that causes primary open angle glaucoma. Science 1997; 275:668-70. [PMID: 9005853]

9. Fan BJ, Wang DY, Lam DS, Pang CP. Gene mapping for primary open angle glaucoma. Clin Biochem 2006; 39:249-58. [PMID: 16332362]

10. Vasiiliou V, Gonzalez FF. Role of CYP1B1 in glaucoma. Annu Rev Pharmacol Toxicol 2008; 48:333-58. [PMID: 17914928]

11. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. Ophthalmology 2008; 115:763-8. [PMID: 18452762]

12. Ren RJJ, Tian G, Zhen Y, Ma K, Li S, Wang H, Li B, Zhang X, Wang N. Cerebrospinal fluid pressure in glaucoma. Ophthalmology. 2009In press

13. Morgan WH, Yu DY, Cooper RL, Alder VA, Cringle SJ, Constable IJ. The influence of cerebrospinal fluid pressure on the lamina cribrosa tissue pressure gradient. Invest Ophthalmol Vis Sci 1995; 36:1163-72. [PMID: 7730025]

14. Jonas JB, Berenshtein E, Holbach L. Anatomic relationship between lamina cribrosa, intraocular space, and cerebrospinal fluid space. Invest Ophthalmol Vis Sci 2003; 44:5189-95. [PMID: 14638716]

15. Anderson DR, Hendrickson A. Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. Invest Ophthalmol Vis Sci 1974; 13:771-83. [PMID: 4137635]

16. Levy NS. The effects of elevated intraocular pressure on slow axonal protein flow. Invest Ophthalmol Vis Sci 1974; 13:691-5. [PMID: 4137262]

17. Minckler DS, Tso MO, Zimmerman LE. A light microscopic, autoradiographic study of axoplasmic transport in the optic nerve head during ocular hypotony, increased intraocular pressure, and papilledema. Am J Ophthalmol 1976; 82:741-57. [PMID: 63246]

18. Quigley H, Anderson DR. The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. Invest Ophthalmol Vis Sci 1976; 15:606-16. [PMID: 60300]
19. Hageman GS, Anderson DH, Johnson LV, Hancock LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT, Merriam JF, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005; 102:7227-32. [PMID: 15870199]

20. Boassa D, Yool AJ. Physiological roles of aquaporins in the choroid plexus. Curr Top Dev Biol 2005; 67:181-206. [PMID: 15949534]

21. Nielsen S, Smith BL, Christensen EI, Agre P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 1993; 90:7275-9. [PMID: 8346245]

22. Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. FASEB J 2005; 19:76-8. [PMID: 15533949]

23. Speake T, Freeman LJ, Brown PD. Expression of aquaporin 1 and aquaporin 4 water channels in rat choroid plexus. Biochim Biophys Acta 2003; 1609:80-6. [PMID: 12507761]

24. Boassa D, Stamer WD, Yool AJ. Ion channel function of aquaporin-1 natively expressed in choroid plexus. J Neurosci 2005; 26:7811-9. [PMID: 16870726]

25. Praetorius J, Nejsum LN, Nielsen SA. SCL4A10 gene product maps selectively to the basolateral plasma membrane of choroid plexus epithelial cells. Am J Physiol Cell Physiol 2004; 286:C601-10. [PMID: 14592810]

26. Chen LM, Kelly ML, Rojas JD, Parker MD, Gill HS, Davis BA, Boron WF. Use of a new polyclonal antibody to study the distribution and glycosylation of the sodium-coupled bicarbonate transporter NCBE in rodent brain. Neuroscience 2008; 151:374-85. [PMID: 18061361]

27. Damkier HH, Nielsen S, Praetorius J. Molecular expression of SLC4-derived Na+-dependent anion transporters in selected human tissues. Am J Physiol Regul Integr Comp Physiol 2007; 293:R2136-46. [PMID: 17715183]

28. Jacobs S, Ruusuvuori E, Sipila ST, Haapanen A, Damkier HH, Kurth I, Hentschke M, Schweizer M, Rudhard Y, Laatikainen LM, Tyynelä J, Praetorius J, Voipio J, Hübner CA. Mice with targeted Slc4a10 gene disruption have small brain ventricles. Curr Top Dev Biol 2005; 67:181-206. [PMID: 16084106]

29. Brown PD, Davies SL, Speake T, Millar ID. Molecular mechanisms of cerebrospinal fluid production. Neuroscience 2004; 129:957-70. [PMID: 15561411]

30. Hauser MA, Allingham RR, Linkroum K, Wang J, LaRocque-Abramson K, Figueiredo D, Santiago-Turla C, del Bono EA, Haines JL, Pericak-Vance MA, Wiggs JL. Distribution of WDR36 DNA sequence variants in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2006; 47:2542-6. [PMID: 16723468]

31. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263-5. [PMID: 15297300]

32. Livak KJ. Allelic discrimination using fluorescent probes and the 5' nuclease assay. Genet Anal 1999; 14:143-9. [PMID: 10084106]

33. Weir BS. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sunderland, MA: Sinauer Associates, Inc.; 1996.

34. Abecasis GR, Cookson WO. GOLD--graphical overview of linkage disequilibrium. Bioinformatics 2000; 16:182-3. [PMID: 10842743]

35. Gauderman WJ. Sample size requirements for association studies of gene-gene interaction. Am J Epidemiol 2002; 155:478-84. [PMID: 11867360]

36. Stamer WD, Snyder RW, Smith BL, Agre P, Regan JW. Localization of aquaporin CHIP in the human eye: implications in the pathogenesis of glaucoma and other disorders of ocular fluid balance. Invest Ophthalmol Vis Sci 1994; 35:3867-72. [PMID: 7523327]

37. Hauser MA, Layfield D, Yang J, Wang T, Walter J, Hoffman E, Stamer D, Allingham RR. SAGE Expression Analysis of Trabecular Meshwork to Identify POAG Susceptibility Genes. ARVO Annual Meeting; 2005 May 1–5; Fort Lauderdale (FL).

38. Baetz NW, Hoffman EA, Yool AJ, Stamer WD. Role of aquaporin-1 in trabecular meshwork cell homeostasis during mechanical strain. Exp Eye Res 2009; 89:95-100. [PMID: 19268465]

39. Stamer WD, Peppel K, O'Donnell ME, Roberts BC, Wu F, Epstein DL. Expression of aquaporin-1 in human trabecular meshwork cells: role in resting cell volume. Invest Ophthalmol Vis Sci 2001; 42:1803-11. [PMID: 11431445]

40. Stamer WD, Chan DW, Conley SM, Coons S, Etherie CR. Aquaporin-1 expression and conventional aqueous outflow in human eyes. Exp Eye Res 2008; 87:349-55. [PMID: 18657536]

41. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. Invest Ophthalmol Vis Sci 2008; 49:5412-8. [PMID: 18719086]

42. Beitz E, Wu B, Holm LM, Schultz JE, Zeuthen T. Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci USA 2006; 103:269-74. [PMID: 16407156]

43. Chretien S, Catron JP. A single mutation inside the NPA motif of aquaporin-1 found in a Colton-null phenotype. Blood 1999; 93:4021-3. [PMID: 10383192]

44. Preston GM, Smith BL, Zeidel ML, Moulds JJ, Agre P. Mutations in aquaporin-1 in phenotypically normal humans without functional CHIP water channels. Science 1994; 265:1585-7. [PMID: 7521540]