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Distribution of COL8A2 and COL8A1 gene variants in Caucasian primary open angle glaucoma patients with thin central corneal thickness

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Purpose: One approach to identify genes that contribute to common complex ocular disorders such as primary open angle glaucoma (POAG) is to study the genetic determinates of endophenotypes that are defined by underlying pre-disposing heritable quantitative traits such as central corneal thickness (CCT). Collagen VIII is a major component of Descemet’s membrane and studies in mice have indicated that targeted inactivation of the genes encoding the collagen type 8 alpha 1 (Col8a1) and collagen type 8 alpha 2 (Col8a2) subunits (COL8A1 and COL8A2) results in thinning of the corneal stroma and of Descemet’s membrane. The purpose of this study is to evaluate COL8A1 and COL8A2 as candidate genes for thin CCT in human POAG patients.

Methods: 100 Caucasian POAG patients were enrolled in this study. The entire COL8A1 and COL8A2 coding sequence was determined in 8 patients with CCT<513 µm (one standard deviation (36 microns) below the mean (550 microns) and 8 patients with CCT>586 µm (one standard deviation above the mean). Selected COL8A2 exons containing variants of interest were sequenced in the full POAG cohort. Association and quantitative trait analyses were performed.

Results: Three patients with CCT less than 513 µm and advanced POAG were found to have missense changes in COL8A2; two patients had a previously identified mutation, R155Q and one had a novel change, P678L (p=0.0035, Fisher’s exact test). Missense changes were not found in any of the patients with CCT>513 µm and missense changes in the COL8A1 gene were not found in any patient. One common COL8A2 SNP, rs274754 was also statistically associated with CCT (p=0.018).

Conclusions: In this study we have identified COL8A2 missense changes in a group of Caucasian patients with very thin CCT and advanced POAG. These results suggest that DNA sequence variants in the COL8A2 gene may be associated with thin corneas in some glaucoma patients. Further study of COL8A2 variants in other patient populations, especially those with thinner CCT such as African-Americans would provide further support for a role of COL8A2 in corneal thickness and in glaucoma.

Primary open angle glaucoma (POAG) is phenotypically and genetically complex. One approach to identify genes that contribute to common complex traits is to study the genetic determinants of endophenotypes that are defined by underlying pre-disposing quantitative traits [1]. Mapping genes influencing the related quantitative trait, rather than the complete complex phenotype, has several important advantages including objective phenotype definitions and a possible reduction in the underlying molecular heterogeneity. Several quantitative traits with significant heritability are associated with POAG including intraocular pressure (IOP), optic nerve vertical cup-to-disc ratio, optic nerve area, and central corneal thickness (CCT) [2-9].

The ocular hypertension treatment trial (OHTS) initially identified thin CCT as a risk factor for progression from ocular hypertension to glaucoma [10]. Subsequently, other studies have suggested that thin CCT is associated with increased severity of visual field loss and more rapid progression of visual field loss [11-15]. Central corneal thickness is a normally distributed, highly heritable quantitative trait in human populations [16], with individuals of African race having lower CCT than Caucasian populations [17,18]. The increased incidence of POAG in African populations is consistent with the increased risk of disease associated with thin CCT.

Candidate gene studies and a recent genome-wide association study have identified several genes that may contribute to CCT variation, including the genes for type I collagen [19] and the genes coding for collagen 5 alpha 1 (COL5A1), autogenous vein graft remodeling associated protein 8 (AVGR8), and A-kinase anchor protein 13 (AKAP13) [20]. These results suggest that genes coding for
proteins that maintain corneal stromal integrity may be good candidates for genetic determinants influencing the trait. Collagen VIII (COL8) is a major component of Descemet’s membrane, and is composed of two subunits, collagen VIIIA1 (COL8A1) and collagen VIIIA2 (COL8A2) which form homotrimers [21]. Targeted inactivation of the COL8A1 and COL8A2 genes in mice results in anterior segment dysgenesis and thin corneal stroma [22], suggesting that COL8A1 and/or COL8A2 may contribute to the development of thin CCT. Further support for a role of COL8A2 in corneal thickness comes from a more recent study documenting thin corneas in mice with a COL8A2 missense change, G257D [23]. The purpose of this study is to evaluate COL8A1 and COL8A2 as candidate genes for thin CCT in human POAG patients.

**METHODS**

**Patients:** This study was approved by the institutional review board of the Massachusetts Eye and Ear Infirmary, Boston, MA. After informed consent, 100 Caucasian POAG patients from the Massachusetts Eye and Ear Infirmary glaucoma service were enrolled in this study. Central corneal thickness (CCT) was measured using an ultrasonic pachymeter (DGH Technology, Inc., Exton, PA). The recorded value was an average of three measurements for each eye. The number used for CCT for each patient was the average of each eye. The following criteria were used to establish POAG affected status: 1) IOP≥22 mmHg in both eyes on 2 occasions, or IOP≥19 mmHg in both eyes on treatment with 2 or more glaucoma medications; 2) Visual field loss in at least one eye on a reliable visual field (reliable visual field is defined by fixation loss ≤33%, false positive rate ≤20% and false negative rate ≤20%) that is in a distribution consistent with nerve fiber layer loss and corresponds to changes in the optic nerve; and 3) Optic nerve damage in at least one eye characterized by two of the following: vertical cup/disc ratio >0.7, superior or inferior neuroretinal rim <0.1, focal notching of the superior or inferior neuroretinal rim, nerve fiber bundle defect with a width of 2 or more retinal vein diameters located 1 disc diameter from the optic nerve, asymmetry of the cup/disc ratio >0.2 without asymmetric refraction, and disc hemorrhage. Patients with known corneal disease and patients who had undergone corneal surgery, including refractive surgery, were excluded from this study.

**DNA sequencing:** Genomic DNA was prepared from buccal cell samples using established techniques (Gentra, Minneapolis, MN). Initially, the entire coding region of COL8A1 and COL8A2 was sequenced in 8 patients with CCT<513 μm (one standard deviation from the mean of 550±36 μm) and 8 patients with CCT>586 μm (one standard deviation from the mean). Genomic DNA was sequenced using primers (Table 1) designed to amplify the coding exons as well as the adjacent splice sites for both the COL8A1 and COL8A2 genes. PCR was performed in a thermal cycler (model 2720; Applied Biosystems Inc., Foster City, CA) set at the following parameters: 50 °C for 2 min, 95 °C for 10 min,
92 °C for 15 s, and 58 °C for 1 min for a total of 60 cycles. PCR products were directly sequenced on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) with BigDye Terminators (Applied Biosystems) according to standard protocols. Selected COL8A2 exons containing variants of interest were sequenced in the entire POAG cohort using the same techniques.

**Statistical methods:** The association of missense changes in COL8A2 and CCT<513 µm was assessed using Fisher’s exact test. Quantitative trait analysis for rs274754 and rs3738360 was performed using PLINK (version 1.07) [24], and p-values were adjusted for gender and age of enrollment using logistic regression.

### RESULTS

**Identification of sequence variants in COL8A1 and COL8A2:**

100 Caucasian POAG patients with a mean CCT of 550±36 µm (range 488–676 µm) were evaluated for this study. The mean CCT in our POAG sample is similar to that observed in the normal Caucasian population [25]. The mean age at enrollment was 68 years and the population was 51% female. Initially we sequenced the entire coding sequence for both the COL8A1 and COL8A2 genes in 8 patients with CCT<513 µm (one standard deviation from the mean) and in 8 patients with CCT>586 µm (one standard deviation from the mean). Three patients with CCT less than 513 µm were found to have missense changes in COL8A2; two patients had a previously identified mutation, R155Q and one had a novel change, P678L. Missense changes were not found in any of the patients with CCT>586 µm and missense changes were not found in any patients in the COL8A1 gene. Two common COL8A2 SNPs, rs274754 and rs3738360 were also found in both groups of patients. The entire cohort was further genotyped for R155Q, P678L, rs274754, and rs3738360 by genomic sequencing. Missense changes were not identified in any of the remaining POAG patients. The location of each variant in the COL8A2 gene is shown in Figure 1.

**Quantitative trait analysis:** The two common COL8A2 SNPs (rs274754 and rs3738360) were genotyped in the entire cohort and analyzed for association with CCT. The genotype frequencies, mean CCT values for each genotype and associated p-values for these two SNPs are shown in Table 2. SNP rs274754, located in the first intron is statistically associated with CCT (p=0.018), while SNP rs3738360, located in the 3’ UTR does not demonstrate an association in this group of individuals.

**COL8A2 missense carrier phenotypes:** Phenotypic information for the three COL8A2 missense carriers is shown in Table 2.

![Figure 1. Structure of the COL8A2 gene and protein domains. The COL8A2 gene consists of two exons separated by single intron. The protein contains NC2, NC1, C1q, and triple helical domains as indicated. The locations of the variants studied are indicated with arrows.](image-url)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>N</th>
<th>CCT µm (mean±SD)</th>
<th>p-trend</th>
<th>p-adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3738360</td>
<td>TT</td>
<td>78</td>
<td>550.6±38.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>14</td>
<td>545.5±30.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>92</td>
<td>p-trend=0.64; p-adj=0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs274754</td>
<td>TT</td>
<td>45</td>
<td>561.3±32.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>23</td>
<td>539.9±30.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>18</td>
<td>545.1±48.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>86</td>
<td>p-trend=0.047; p-adj=0.018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CCT: the average measures of central corneal thickness between left and right eyes; p-adj: p value adjusting for sex and age of enrollment.
in Table 3. All three patients have very thin CCT, and advanced POAG including elevated intraocular pressure, optic nerve degeneration, and significant visual field defects. None of these patients had undergone refractive surgery or any other type of corneal surgery before CCT measurement.

**Functional significance of COL8A2 missense variants:**
R155Q was found in two patients and P678L was found in one patient with CCT<513 µm. Both of these missense changes are evolutionarily conserved and are likely to be pathogenic. R155Q is located in the evolutionarily conserved triple helical domain. Although tests of functional significance suggest that the glutamine could be tolerated at position 155, the R155Q change has been previously associated with Fuch’s endothelial dystrophy (FED) [26] suggesting that the variant has a role in corneal disease. P678L

**DISCUSSION**
In this study we have identified COL8A2 missense changes in a group of Caucasian patients with very thin CCT and advanced POAG. R155Q was found in two patients and P678L was found in one patient with CCT<513 µm. Both of these missense changes are evolutionarily conserved and are likely to be pathogenic. R155Q is located in the evolutionarily conserved triple helical domain. Although tests of functional significance suggest that the glutamine could be tolerated at position 155, the R155Q change has been previously associated with Fuch’s endothelial dystrophy (FED) [26] suggesting that the variant has a role in corneal disease. P678L

![Figure 2. Evolutionary conservation of R155Q and P678L. Arrows indicate the position of Arginine 155 and Proline 678. Conserved amino acids are highlighted in yellow.](http://www.molvis.org/molvis/v16/a234)
is a novel COL8A2 missense change that is located in the 
highly conserved C1q domain. This variant is predicted to be 
pathologic by PolyPhen-2 and PMUT. Other COL8A2 
missense changes have also been found in FED (R34H, 
R304Q, Q455K, Q455V, and L450W) [26,30-33]. COL8A2 
mutations have not been found in patients with keratoconus 
or keratoglobus [34]. Our patients did not have clinical 
evidence of any of these corneal disorders.

As the previous reports relating COL8A2 missense 
changes to corneal disease have not included CCT 
measurement as part of the clinical assessment, it is not 
possible to determine if the patients carrying these gene 
variants also had thin CCT. However, given our results and 
the complex genetics of FED [35], it is likely that COL8A2 
missense changes are responsible for thin CCT and that 
COL8A2 variants may also be one factor that can contribute 
to FED. Indeed several reports support this hypothesis, 
including a report describing familial aggregation, but not 
perfect segregation, of a COL8A2 missense change and FED 
[36] and the identification of the R155Q change in one FED 
affected patient and two controls in a population from South 
India [37]. The R155Q variant is also relatively common in 
the Japanese, a population known to have thinner CCT [38, 
39].

We also evaluated two common SNPs, rs274754 located 
within intron 1 near the 5’ end of the gene and rs3738360 
located in the 3’UTR. Only SNP rs274754 was statistically 
associated with CCT, with the G allele associated with thinner 
CCT. Interestingly the G allele of rs274754 is more common 
in Africans (dbSNP), and individuals of African origin have 
thinner corneas and a higher incidence of POAG than 
Caucasians [40,41]. It would be of interest to evaluate the 
association between CCT and rs274754 and other common 
COL8A2 SNPs in a larger POAG sample and also in African 
populations.

POAG is inherited as a complex non-Mendelian trait that 
is likely to result from multiple genetic and environmental 
Factors. Identifying genes responsible for POAG pre-
disposing endophenotypes, such as thin CCT, is one path 
toward defining the underlying complex genetic architecture 
of the disease. This study suggests that COL8A2 gene variants 
can contribute to thin CCT. Further studies identifying genes 
that contribute to POAG endophenotypes will help define the 
molecular events underlying the complex phenotype as well 
as lead to gene-based tests for screening and diagnosis.

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REFERENCES

1. Charlesworth J, Kramer PL, Dyer T, Diego V, Samples JR, 
Craig JE, Mackey DA, Hewitt AW, Blangero J, Wirtz MK.
The path to open-angle glaucoma gene discovery: 
endophenotypic status of intraocular pressure, cup-to-disc 
ratio, and central corneal thickness. Invest Ophthalmol Vis 

2. Klein BE, Klein R, Lee KE. Heritability of risk factors for 
primary open angle glaucoma: the Beaver Dam Eye Study. 
14691154]

3. van Koolwijk LM, Despriet DD, van Duijn CM, Pardo Cortes 
LM, Vingerling JR, Aulchenko YS, Oostra BA, Klaver CC, 
Lemié JG. Genetic contributions to glaucoma: heritability of 
intraocular pressure, retinal nerve fiber layer thickness, and 
48:3669-76. [PMID: 17652737]

4. Chang TC, Congdon NG, Wojciechowski R, Muñoz B, Gilbert 
D, Chen P, Friedman DS, West SK. Determinants and 
heritability of intraocular pressure and cup-to-disc ratio in a 
defined older population. Ophthalmology 2005; 
112:1186-91. [PMID: 15939473]

5. Schwartz JT, Reuling FH, Feinleib M. Size of the physiologic 
cup of the optic nerve head. hereditary and environmental 

6. Randas WD, van Koolwijk LM, Ikram MK, Jansonius NM, de 
Jong PT, Bergen AA, Isaacs A, Amin N, Aulchenko YS, 
Wolfs RC, Hofman A, Rivadeneira F, Oostra BA, 
Utterlinden AG, Hysi P, Hammond CJ, Lemié JG, 
Vingerling JR, Klaver CC, van Duijn CM. A genome-wide 
association study of optic disc parameters. PLoS Genet 2010; 
6:e1000978. [PMID: 20548946]

7. Macgregor S, Hewitt AW, Hysi PG, Ruddle JB, Medland SE, 
Henders AK, Gordon SD, Andrew T, McEvoy B, Sanfilippo 
PG, Carbonaro F, Tah V, Li YJ, Bennett SL, Craig JE, 
Montgomery GW, Tran-Viet KN, Brown NL, Spector TD, 
Martin NG, Young TL, Hammond CJ, Mackey DA. Genome-wide 
association identifies ATOH7 as a major gene 
determining human optic disc size. Hum Mol Genet 2010; 
19:2716-24. [PMID: 20395239]

8. Toh T, Liew SH, MacKinnon JR, Hewitt AW, Poulsen JL, 
Spector TD, Gilbert CE, Craig JE, Hammond CJ, Mackey 
DA. Central corneal thickness is highly heritable: the twin eye 
[PMID: 16186354]

Heritability of central corneal thickness in Chinese: the 
Guangzhou Twin Eye Study. Invest Ophthalmol Vis Sci 

10. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham 
EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, 
Wilson MR, Kass MA. The Ocular Hypertension Treatment 
Study: baseline factors that predict the onset of primary open-
[PMID: 12049575]

11. Herndon LW, Weizer JS, Stinnett SS. Central corneal thickness 
as a risk factor for advanced glaucoma damage. Arch 

12. Hewitt AW, Cooper RL. Relationship between corneal 
thickness and optic disc damage in glaucoma. Clin 
Experiment Ophthalmol 2005; 33:158-63. [PMID: 
15807824]


15. Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z. Predictors of long-term progression in the early manifest glaucoma trial. Ophthalmology 2007; 114:1965-72. [PMID: 17628686]


24. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559-75. [PMID: 17701901]


