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

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:4874822">http://nrs.harvard.edu/urn-3:HUL.InstRepos:4874822</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Distribution of COL8A2 and COL8A1 gene variants in Caucasian primary open angle glaucoma patients with thin central corneal thickness

T. Desronvil,1 D. Logan-Wyatt,1 W. Abdurabou,1 M. Triana,1 R. Jones,1 S. Taheri,1 E. Del Bono,1 L.R. Pasquale,1 M. Olivier,2 J.L. Haines,3 B.J. Fan,1 J.L. Wiggs1

1Department of Ophthalmology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston, MA; 2Rosalind Franklin University of Medicine and Science, Chicago, IL; 3Center for Human Genetics Research, Vanderbilt Medical School, Nashville TN

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 alpha1 (Col8a1) and collagen type 8 alpha2 (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 alpha1 (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 notch
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
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 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

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

Table 2. QTL association analysis of common SNPs and CCT in Caucasian POAG patients.
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
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 rs7338360 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 predisposing 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.

ACKNOWLEDGMENTS
Supported in part by NIH/NEI grants EY015872 and P30 EY014104, the Massachusetts Lions Eye Research Fund and Research to Prevent Blindness.

REFERENCES


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: 17628666]


Purecell 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]


