Variations in COL15A1 and COL18A1 influence age of onset of primary open angle glaucoma

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Primary open angle glaucoma (POAG) is a genetically and phenotypically complex disease that is a leading cause of blindness worldwide. Previously we completed a genome-wide scan for early-onset POAG that identified a locus on 9q22 (GLC1J). To identify potential causative variants underlying GLC1J, we used targeted DNA capture followed by high throughput sequencing of individuals from four GLC1J pedigrees, followed by Sanger sequencing to screen candidate variants in additional pedigrees. A mutation likely to cause early-onset glaucoma was not identified, however COL15A1 variants were found in the youngest affected members of 7 of 15 pedigrees with variable disease onset. In addition, the most common COL15A1 variant, R163H, influenced the age of onset in adult POAG cases. RNA in situ hybridization of mouse eyes shows that Col15a1 is expressed in the multiple ocular structures including ciliary body, astrocytes of the optic nerve and cells in the ganglion cell layer. Sanger sequencing of COL18A1, a related multiplexin collagen, identified a rare variant, A1381T, in members of three additional pedigrees with early-onset disease. These results suggest genetic variation in COL15A1 and COL18A1 can modify the age of onset of both early and late onset POAG.

Conflict of interest

None of the authors have any conflict of interests with the content of this manuscript.

Primary open angle glaucoma (POAG) is a phenotypically and genetically complex disease that is a major cause of blindness worldwide (1). While irreversible optic nerve degeneration is a common disease feature, other phenotypic features are highly variable including age of onset. Most patients are affected as older adults, however a subset of glaucoma patients develop the disease before age 35 (juvenile-onset POAG or JOAG). At any age glaucoma is chronically progressive and current treatments can slow but not stop the disease-related optic nerve deterioration.

POAG can be inherited as an autosomal dominant trait or as a multifactorial complex trait. Generally, JOAG pedigrees exhibit autosomal dominant inheritance while inheritance of adult onset cases is more complex. Mutations in MYOC, coding for myocilin, are found in approximately 30% of JOAG families (2, 3). While most MYOC mutations cause severe early-onset glaucoma, several mutations, especially the GLN368X nonsense mutation, are associated with milder disease of later onset (4, 5). Interestingly, the age of disease onset in some families carrying adult-onset MYOC mutations is highly variable ranging from before age 20 to after age 50 (6). The factors responsible for this variability are not known, although the influence of modifier genes is an important possibility (7, 8).

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In an effort to identify novel JOAG genes we have previously completed a genome-wide linkage study using 25 two and three generation JOAG families that identified a large 11.4 Mb linkage interval on chromosome 9q22 (GLC1J) (9). In this study we use targeted DNA capture followed by high throughput sequencing of selected JOAG families with evidence of linkage to GLC1J, including those with adult-onset MYOC mutations and variable age of disease onset, to identify genes relevant to JOAG.

Materials and methods

Families and cases

The study was approved by the Massachusetts Eye and Ear Infirmary Institutional Review Board and adheres to the tenets set forth in the Declaration of Helsinki. Informed consent was obtained from all study participants. Fifteen early-onset POAG pedigrees consisting of a minimum of two affected individuals in two generations were included in this study. A family was defined as early-onset glaucoma if at least one affected family member developed glaucoma, according to the features described below, at or before age 40. Of the families included in this study 14 were Caucasian of European-ancestry and 1 was African-American (OAG26, Fig. 1). We also evaluated 368 European-derived Caucasians and 33 African-American, all with adult-onset POAG. All study participants underwent a complete ocular examination including measurements of visual acuity, intraocular pressure (IOP), central corneal thickness, slit lamp evaluation and funduscopic evaluation. Glaucoma was defined as IOP >22 mmHg in both eyes, glaucomatous optic nerve damage in both eyes, and visual-field loss in at least one eye. Early-onset of juvenile glaucoma was defined as onset of the glaucoma features described above at or before age 40 and adult-onset glaucoma is defined as onset of glaucoma case clinical features after age 40. Age of onset is the age when a health care provider gave the patient the diagnosis of glaucoma.

Exon capture and sequencing

For high throughput sequencing, genomic DNA was prepared from 10 individuals in four pedigrees (OAG25: 1220, 1232, 1227; OAG6: 1383, 1401, 1402; OAG622: 3374, 3372; OAG14: 1051, 1054). DNA was enriched for functional elements in the 11.4 Mb GLC1J interval of human chromosome 9 (between D9S1841 and D9S271) using SureSelect DNA Capture Arrays (Agilent Technologies, Santa Clara, CA). Functional elements were defined as being exons and conserved sequences as annotated in the UCSC human genome browser (NCBI 36); 35 bp single end sequencing was performed on a Genome Analyzer IIX (Illumina Inc, San Diego, CA) (10). Sequencing quality control, read alignment and variant identification/annotation were performed using MAQ, BWA and Samtools (11, 12).

Sanger sequencing

Genomic DNA was prepared from either peripheral blood samples or buccal cell samples using established techniques. The entire coding regions of COL15A1 and COL18A1 were sequenced using DNA from the youngest affected individual in 15 early-onset glaucoma pedigrees. Genomic DNA was sequenced using primers designed to amplify the coding exons as well as the adjacent splice sites. Polymerase chain reaction (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) according to standard protocols.

Pathogenicity and statistical analysis

Pathogenicity was evaluated with three commonly used software programs, POLYPHEN2 (13), sift (14, 15) and pmut (16). The mean age of onset, standard deviation and variance for the cases with and without COL15A1 and COL18A1 variants were compared with a two-tailed, unequal variance t-test. A p-value of <0.05 was considered statistically significant.

Mouse strains and RNA in situ hybridization

All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic research. All mice were housed in a 14 h light to 10 h dark cycle. The Jackson Laboratory Animal Care and Use Committee approved all of the experiments in this study. Eyes, with the intra-orbital portion of the optic nerve still attached, were enucleated from 4 to 6 months old C57BL/6 J mice and fixed overnight in 4% paraformaldehyde (PFA) at 4°C. Eyes were stored in 1× phosphate-buffered solution (1× PBS) at 4°C. Assessment of Col15a1 RNA was performed as described previously (17). Briefly, eyes were placed in 30% sucrose overnight, rinsed in 1× PBS and frozen in OCT; 10 μm sections of eyes were obtained using a cryostat. Sections were incubated overnight at 65°C with hybridization solution containing 1μg/ml digoxigenin (DIG)-labeled Col15a1 riboprobe (clone 3482829 from Open Biosystems; antisense: digested with SalI, reverse transcribed with T7; sense: digested with NotI , reverse transcribed with SP6). Peroxidase (POD)-conjugated anti-DIG antibody (Roche, Indianapolis, IN) followed by TSA-Cy-3 (PerkinElmer, Waltham, MA) was used for fluorescence detection. Imaging and photography were performed on the SP5 confocal microscope (Leica, Buffalo Grove, IL). At least three sections from six eyes were assessed.

Results

Targeted genomic capture followed by high throughput sequencing was performed using DNA from ten
Fig. 1. Glaucoma pedigrees with COL15A1 variants. Affected pedigree members are shown as solid circles (females) or squares (males). The MYOC mutation (OAG6 and OAG14), the collagen variant and the age of disease onset are listed directly below the pedigree figure. The symbol (---) indicates that the individual does not have a MYOC variant (first line below the figure), a collagen variant (second line below the figure) or that the age of onset is not known or the individual is unaffected (third line below the figure). The youngest affected members of OAG60 carry both a COL18A1 variant (A1381T) and a COL15A1 variant (R163H). The age of onset data for OAG60 was included in the comparison of the mean age of onset with and without COL18A1 A1381T but not for COL15A1 R163H as all the affected members of the family carried the COL15A1 R163H variant. Similarly, for family OAG6 the age of onset data was not included in the comparison of the mean age of onset with and without COL15A1 R163H as all the affected members of the family were heterozygous for the COL15A1 R163H variant. Individual 1193 in OAG26 was diagnosed with glaucoma in the same year as her sister (1194) when she was 13 and her sister was 16. However, 1193 was minimally affected at that point while her sister (the carrier of the R163H variant) was severely affected by advanced optic nerve damage suggesting that she had an earlier onset of disease. Individual 3265, heterozygous for COL15A1 R163H (family OAG622) and 3372 were diagnosed at the same time when 3265 was 47 and 3372 was 34, however 3265 had advanced disease at the time of diagnosis and 3372 was minimally affected suggesting that 3265 had onset disease at an earlier age.
individuals (eight affected and two unaffected) who were members of four independent JOAG families selected based on linkage to the GLC1J locus. Interestingly, two of the selected families also carried adult-onset MYOC mutations (OAG6 and OAG14). The selective capture targeted all exons and conserved sequences from D9S1841 to D9S271 corresponding to the GLC1J interval (see Materials and methods). For each of the 10 individuals, sequence reads were aligned to the human reference genome (NCBI build 36) and greater than 95% of target bases were covered by at least 6 reads. Between 530 and 771 sequence variants with allele ratios of greater than 0.3 were identified using SAMtools for the 10 individuals. After filtering by: (i) selecting variants present in both affected individuals from a family; (ii) removing variants present in the unaffected individuals (if included); and (iii) removing synonymous changes, a total of 21 missense variants were identified in 18 genes. There were no nonsense, splice site or insertion/deletion variants that met the filtering criteria. Of the 21 missense changes, none had the population frequency (minor allele variants were identified in 18 genes. There were no synonymous changes, a total of 21 missense variants were identified in 18 genes. There were no nonsense, splice site or insertion/deletion variants that met the filtering criteria. Of the 21 missense changes, none had the population frequency (minor allele frequency less than 0.01) expected for a causative mutation for this rare mendelian disorder. However, a COL15A1 variant, R163H, was found in three of the four Caucasian families (OAG6, OAG14 and OAG622) despite a minor allele frequency of 0.125 in Caucasians with European ancestry (NHLBI ESP variant server (EVS), http://evs.gs.washington.edu/EVS/). We investigated the segregation of the COL15A1 R163H variant in all available family members using Sanger sequencing. While R163H did not uniquely segregate with disease status, the variant was consistently found in the affected individuals with the earliest age of disease onset in the family (Fig. 1), suggesting that COL15A1 R163H may impact age of disease onset.

To further investigate the effect of COL15A1 variants on age of disease onset, we sequenced the entire COL15A1 coding sequence in 11 additional families with variable onset of disease, as well as the affected member with earliest age of onset from OAG25, using Sanger sequencing. Two of the additional families also had MYOC mutations (families OAG16 and OAG125). We identified a COL15A1 variant in four of the additional families (OAG25, OAG26, OAG31 and OAG60) (Fig. 1). All of these families are Caucasian except for family OAG26 which is African-American. In total we identified a COL15A1 variant in 7 of the 15 families evaluated and found that affected individuals who carried a COL15A1 variant had onset of disease on average at age 25.0 (±12.2, standard deviation) compared with an average onset of disease at age 44.0 (±15.9, standard deviation) for those affected family members without COL15A1 variants (p = 0.013, two-tailed, unequal variance t-test). The COL15A1 variant in six of the seven families originates in the unaffected parent, consistent with the hypothesis that the COL15A1 variant is not causative but a modifier variant that can modify the age at disease onset.

In total, our analysis identified three different COL15A1 variants, R168H (5 families), A200T (one family) and V1332I (one family) (Fig. 1, Table 1). The R168H and A200T missense changes both affect evolutionarily conserved amino acids [GERP (Genomic Evolutionary Rate Profiling) score 1.22 and 3.12, respectively] and are located in the N-terminal thrombospondin domain of the COL15A1 protein. The V1332I variant is also evolutionarily conserved (GERP score 2.94) and is located in the endostatin-like domain. Pathogenicity testing using POLYPHEN2, SIFT and PMUT suggests that both the R168H and A200T variants can affect protein function (Table 1). V1332I was not identified as pathological by any of the three pathogenicity programs; however, the evolutionary conservation indicated by the positive GERP score supports a potential impact of the variant on the protein function. All three variants are present in the NHLBI ESP (http://evs.gs.washington.edu/EVS/) and all three are more common in African-Americans than European Americans (Table 1).

To further support a role of COL15A1 in age of onset of POAG, we evaluated the most common COL15A1 variant, R163H, in an adult-onset POAG cohort consisting of 368 Caucasians with European ancestry and a separate set of 33 African-American adult-onset glaucoma cases. (The other COL15A1 variants are too rare to be analyzed in this adult-onset glaucoma cohort). For the Caucasian group, we found that the mean age of disease onset was 54.2 (±7.1, standard deviation) in cases homozygous for the R163H AA genotype (homozygous Histidine) compared with 60.9 (±11.6, standard deviation) for those cases homozygous for the R163H GG genotype (homozygous Arginine) (p = 0.007, two-tailed, unequal variance t-test). In the African-American sample the mean age of disease onset was 50.4 (±5.6, standard deviation) for the R163H AA genotype compared with 61.8 (±7.3, standard deviation) mean age of onset for the R163H GG genotype (p = 0.034, two-tailed, unequal variance t-test) (Table 2). In both the Caucasian and African cases there was a trend for higher IOP in the carriers of the risk genotype, that was nominally significant in the African-American sample (Table 2). Central corneal thickness, another clinical feature that influences IOP and glaucoma risk, was not different among the R163H genotype groups (Table 2).

It is not clear how variations in COL15A1 may impact age of onset of POAG. Previous data has shown expression of COL15A1 in the trabecular meshwork and Schlemm’s canal (18). To gain a more complete understanding of the ocular structures potentially impacted by COL15A1 variations we performed RNA in situ hybridization of mouse eyes (see Materials and methods). Our data shows that Col15a1 is expressed in the multiple ocular structures including the ciliary body, astrocytes of the optic nerve and cells in the ganglion cell layer (Fig. 2). Therefore, variations in COL15A1 may impact structures involved in the maintenance of aqueous humor and neural structures.

COL15A1 is highly homologous to a similar multiplexin collagen, COL18A1 (19). To determine if variants in COL18A1 also impact the age of onset
Table 1. Features of COL15A1 and COL18A1 variants

| Variants | COL15A1 | COL18A1 |
|----------|---------|---------|
|          | R163H   | A200T   | V1332I  | A1381T  |
| dbSNP id | rs2075662 | rs61999303 | rs10519 | rs144147445 |
| Protein domain | Thrombospondin | Thrombospondin | Endostatin-like | Endostatin |
| GERP     | 1.22    | 3.12    | 2.94    | 4.14    |
| MAF EA   | 0.15    | 0.00    | 0.06    | 0.01    |
| MAF AA   | 0.32    | 0.03    | 0.23    | 0.003   |
| Pathogenicity |
| POLYPHEN2 | Benign (0.011) | Possibly damaging (0.322) | Benign (0.002) | Probably damaging (1.00) |
| SIFT     | Tolerated (0.06) | Damaging (0.00) | Tolerated (0.23) | Damaging (0.00) |
| PMUT     | Pathological (0.507) | Neutral (0.09) | Neutral (0.03) | Neutral (0.239) |

GERP, Genomic Evolutionary Rate Profiling; MAF, minor allele frequency; EA, European ancestry; AA, African-American.

The POLYPHEN2 measures of pathogenicity are: BENIGN, possibly damaging and probably damaging based on the false discovery rate. SIFT measures of pathogenicity are tolerated (>0.05) and damaging (<0.05) and a scale of 0–1.0. PMUT pathogenicity is either pathological (>0.5) or neutral (<0.5) on a scale of 0–1.0.

Table 2. COL15A1 R163H and adult-onset primary open angle glaucoma disease features

A. Age of onset

| Genotype | Mean age of onset (SD) | p-Valuea |
|----------|------------------------|---------|
| Caucasian (368) |
| AA (13)  | 54.2 ± 7.7             | 0.0076  |
| AG (86)  | 61.7 ± 11.9            |         |
| GG (269) | 60.9 ± 12.1            |         |
| African-American (33) |
| AA (5)   | 50.4 ± 5.6             | 0.034   |
| AG (16)  | 58.1 ± 9.1             |         |
| GG (12)  | 61.8 ± 11.5            |         |

B. IOP

| Genotype | Mean IOP (SD) | p-Valueb |
|----------|---------------|---------|
| Caucasian (368) |
| AA (13)  | 25.2 ± 4.1    | 0.10    |
| AG (86)  | 22.9 ± 3.2    |         |
| GG (269) | 22.6 ± 3.0    |         |
| African-American (33) |
| AA (5)   | 26.4 ± 3.2    | 0.03    |
| AG (16)  | 28.1 ± 3.5    |         |
| GG (12)  | 23.0 ± 3.3    |         |

C. CCT

| Genotype | Mean CCT (SD) | p-Valuec |
|----------|---------------|---------|
| Caucasian (368) |
| AA (13)  | 550 ± 10.1    | 0.94    |
| AG (86)  | 550 ± 11.2    |         |
| GG (269) | 552 ± 7.5     |         |
| African-American (33) |
| AA (5)   | 534 ± 9.8     | 0.87    |
| AG (16)  | 527 ± 10.1    |         |
| GG (12)  | 524 ± 12.1    |         |

CCT, central corneal thickness; IOP, intraocular pressure; SD, standard deviation.

a Mean age of onset of AA carriers compared with mean age of disease onset of GG carriers using a two-tailed, unequal variance, t-test.
b Mean IOP of individuals with the AA genotype compared with the GG genotype using a two-tailed, unequal variance t-test.
c Mean CCT of individuals with the AA genotype compared with the GG genotype using a two-tailed, unequal variance t-test.

of POAG we sequenced the entire COL18A1 coding region using Sanger sequencing in the same set of 15 families that were originally used to identify COL15A1 variants. We found a rare COL18A1 variant, A1381T, in four OAG families (OAG16, OAG60, OAG125, and OAG746) (Figs 1 and 3). The youngest affected members of family OAG60 are heterozygous for both the COL15A1 R163H variant and the COL18A1 A1381T variant (Fig. 1). The Alanine at position 1381 is a highly evolutionarily conserved amino acid located in the endostatin domain (GERP score = 4.14). The A1381T variant is predicted to be pathogenic by both
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Fig. 2. Col15a1 is expressed in multiple ocular structures. Assessment of expression by RNA *in situ* hybridization shows that Col15a1 (red, arrows) is expressed in the ciliary body (top panels), astrocytic columns in the optic nerve (middle panels) and retinal ganglion cells in ganglion cell layer (lower panels).

Polyphen2 and sift (Table 1). Affected individuals heterozygous for A1381T had a mean age of onset of 32.2 (±7.9, standard deviation) compared with 48.8 (±6.3, standard deviation) for the affected family members without the A1381T variant (p = 0.006, two-tailed, unequal variance *t*-test). Interestingly, the affected members of two of these families have the same MYOC variant, Q368X, known to be associated with adult-onset disease (4–6). This variant was too rare to be assessed in the adult-onset POAG groups.

Discussion

In this study we have identified genetic variants in the multiplexin collagens 15A1 and 18A1 in individuals with early-onset glaucoma. While the segregation of these variants did not support disease causality, all the variants were found in affected individuals with earlier disease onset compared with other affected family members without the collagen variants. These results suggest that these variants act as disease-modifying factors. We also showed that the most common variant, COL15A1 R163H influenced the age of onset of adult-onset POAG.

Collagen 15A1 and 18A1 are features of basement membranes (20, 21) and extracellular matrix (22, 23) and both have been shown to have ocular expression (24–26). Some mutations in COL18A1 are a cause of autosomal recessive Knobloch’s disease that is characterized by myopia and retinal degeneration (27). The C-terminal endostatin domain that includes the location of the A1381T variant is known to have anti-angiogenic properties and also promotes apoptosis (28, 29). Collagen 15A1 is expressed in the endothelial cells of Schlemm’s canal that is a component of the trabecular outflow pathways involved in removal of aqueous humor and regulation of IOP (18). A Col15a1 and Col18a1 double-knockout mouse has abnormalities of the ocular anterior segment that could influence glaucoma development including iris stromal atrophy (30). Collectively, these results suggest that variants in COL15A1 and COL18A1 could influence age of disease onset by compromising the basement membranes of structures involved in fluid outflow. In this study we show that Col15a1 is also expressed in neural structures, which could suggest that COL15A1 variants could also influence susceptibility of the optic nerve to degeneration. Further studies defining the molecular interactions of the multiplexin collagens would be necessary before a definitive role for these proteins in the development of glaucoma could be determined.

MYOC mutations were present in the affected members of 4 of the 15 pedigrees evaluated in this study. Three families have MYOC mutations known to be associated with adult-onset disease: T377M in OAG14, and Q368X in OAG16 and OAG125 (6). The MYOC mutation in affected members of OAG6 (C245F) has not been previously described, although another missense change at the same location (C245Y) has been found in a Chinese family with a mean age of diagnosis of 18 ± 6.4 (31). However, as every affected member of OAG6 also carries COL15A1 R163H it is not possible to determine the age of disease onset associated with MYOC C245F or the impact of COL15A1 R163H on disease onset in this family. Accordingly, while we presented the OAG6 pedigree in Fig. 1 we did not include the age of disease onset data from this family in the comparison of mean age of onset in carriers of R163H vs affected individuals without R163H. Interestingly, the rare COL18A1 A1381T variant was found in early-onset glaucoma patients in both families with the Q368X mutation. Q368X is the most common and least severe MYOC mutation (32). MYOC mutations cause protein misfolding and subsequent retention of aggregated mutant and normal myocilin in the endoplasmic reticulum (ER) (33). In vitro solubility assays suggest that the mutations causing more severe disease are least soluble and are most likely to remain in the ER (34, 35). While the Q368X mutation is insoluble in the *in vitro* assay it may be at least partially secreted into the extracellular matrix (36) where it could potentially interact with collagen 18. Further experimentation would be necessary to confirm this hypothesis. Variable age of onset in families with MYOC Q368X has been previously reported and
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it would be interesting to screen additional Q368X families for COL18A1 variants (8).

All of the COL15A1 variants identified in this study are more common in African-Americans than in Caucasians with European ancestry. African-American race is an independent risk factor for open angle glaucoma and African-Americans are frequently affected at earlier ages with more severe disease than Caucasians (37). Our findings suggest that variation in COL15A1 is one factor that could influence the disease severity in African-Americans. While the in silico pathogenicity of this variant could be considered to be less compelling than the other variants described in this study, the GERP score and POLYPHEN2 values are well within the range of other relatively common disease-modifying alleles including the Val158Met COMT allele reported to modify age of onset of Parkinson’s disease (GERP, −0.757; POLYPHEN2, BENIGN) (38), the Met55Val SUMO4 allele reported to modify susceptibility to diabetes (GERP, −0.47; POLYPHEN2, BENIGN) (39), and the Ala736Val TMPRSS6 allele that is reported to modify the penetrance and clinical expression of hereditary hemochromatosis (GERP, 4.72; POLYPHEN2, BENIGN) (40).

There are several limitations to our study. First, age of onset is difficult to measure and is not readily available in most glaucoma cohorts. In our study we used the age that an individual was told by a health care provider that they had glaucoma. Because many patients are enrolled in studies retrospectively this number is subject to recall bias. Nevertheless we were able to demonstrate an interesting relationship between COL15A1 and COL18A1 variants and age of glaucoma onset. IOP is also difficult to measure because it varies according to time of day, previous medication and surgery, central corneal thickness and the methods used for pressure measurement. Another limitation is that for most of the families in this study we do not have information about the ‘primary’ or ‘causative’ gene, which could make it more difficult to assess the contribution of the potential-modifying effects of the COL15A1 and COL18A1 variants. In these early-onset families the assumption is that the primary mutation has penetrance similar to that of the MYOC mutations present in four of the investigated families and that one additional variant, either from COL15A1 or COL18A1 can impact the age of disease onset. In the adult-onset glaucoma cases, the assumption is that the disease-associated alleles have lower penetrance consistent with adult-onset disorders of complex inheritance, and in this instance the effects of the COL15A1 R163H variant are most apparent when the individual is homozygous for the risk alleles. Finally, while the COL15A1 variants influencing age of onset could have contributed to the observed linkage signal at 9q22, the possibility remains that another gene in this region also contributes to early-onset glaucoma. The methods used in this study would not identify variants in the 5′ and 3′ regulatory regions, deep intronic variants or large copy number variations.

Open angle glaucoma is genetically and phenotypically complex and is likely to result from combinations of multiple genes and environmental factors. The results from this study suggest that both high-penetrant disease-causing alleles and low-penetrant disease-associated alleles may be influenced by genetic-modifying factors. The discovery of alleles that can modify the severity of disease-causing mutations can provide valuable information about the pathogenesis of disease and possibly suggest novel therapeutic approaches. Further evaluation of the effects of these collagen variants in glaucoma in animal models could identify important therapeutic targets. Additionally further study of the phenotypic features associated with these variants could lead to the development of clinically useful diagnostic tests.

Fig. 3. Glaucoma pedigrees with variants in COL18A1. Affected pedigree members are shown as solid circles (females) or squares (males). The MYOC mutation (OAG16, OAG125), the collagen variant and the age of disease onset are listed directly below the pedigree figure. The symbol (−−) indicates that the individual does not have a MYOC variant (first line below the figure), a collagen variant (second line below the figure) or that the age of onset is not known or the individual is unaffected (third line below the figure).
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References

1. Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. J Clin Invest 2010: 120 (9): 3064–3072.
2. Wiggs JL, Allingham RR, Vollrath D et al. Prevalence of mutations in TIGR/Myocilin in patients with adult and juvenile primary open-angle glaucoma. Am J Hum Genet 1998: 63 (5): 1549–1552.
3. Fingert JH, Héon E, Liebmann JM et al. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet 1999: 8 (5): 899–905.
4. Angius A, Spinelli P, Ghiolotti G et al. Myocilin Gln368stop mutation and advanced age as risk factors for late-onset primary open-angle glaucoma. Arch Ophthalmol 2000: 118 (5): 674–679.
5. Allingham RR, Wiggs JL, De La Paz MA et al. Gln368STOP myocilin mutation in families with late-onset primary open-angle glaucoma. Invest Ophthalmol Vis Sci 1998: 39 (12): 2288–2295.
6. Fingert JH, Stone EM, Sheffield VC. Alward WL. Surv Ophthalmol 2002: 47 (6): 547–561.
7. Vincent AL, Billingsley G, Buys Y et al. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet 2002: 70 (2): 448–460.
8. Craig JE, Baird PN, Healey DL et al. Evidence for genetic heterogeneity within eight glaucoma families, with the GLC1A Gln368STOP mutation being an important phenotypic modifier. Ophthalmology 2001: 108 (9): 1607–1620.
9. Wiggs JL, Lynch S, Yuagi G et al. A genomewide scan identifies novel early-onset primary open-angle glaucoma loci on 9q22 and 20p12. Am J Hum Genet 2004: 74 (6): 1314–1320.
10. Hodges E, Smith AD, Kendall J et al. High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. Genome Res 2009: 19 (9): 1593–1605.
11. Li H, Handsaker B, Wysoker A et al. 1000 genome project data processing subgroup. The sequence alignment/map format and SAMtools. Bioinformatics 2009: 25 (16): 2078–2079.
12. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009: 25 (14): 1754–1760.
13. Adzhubei IA, Schmidt S, Peshkin L et al. A method and server for predicting damaging missense mutations. Nat Methods 2010: 7 (4): 248–249.
14. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet 2006: 7: 61–80.
15. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 2012: 40 (Web Server issue): W242–W245.
16. Ferrer-Costa C, Gelpí JL, Zamakola L, Parraga I, de la Cruz X, Orozco M. PMUT: a web-based tool for the annotation of pathological mutations on proteins. Bioinformatics 2005: 21 (14): 3176–3178.
17. Howell GR, Macalaino DG, Sousa GL et al. Molecular clustering identities complement and endothelin induction as early events in a mouse model of glaucoma. J Clin Invest 2011: 121 (4): 1429–1444.
18. Liton PB, Liu X, Stamler WD, Challa P, Epstein DL, Gonzalez P. Specific targeting of gene expression to a subset of human trabecular meshwork cells using the chitinase 3-like 1 promoter. Invest Ophthalmol Vis Sci 2005: 46 (1): 183–190.
19. Hägg PM, Muona A, Liétard J, Kivirikko S, Pihlajaniemi T. Complete exon-intron organization of the human gene for the alpha1 chain of type XV collagen (COL15A1) and comparison with the homologous COL18A1 gene. J Biol Chem 1998: 273 (28): 17824–17831.
20. Li D, Clark CC, Myers JC. Basement membrane zone type XV collagen is a disulfide-bonded chondroitin sulfate proteoglycan in human tissues and cultured cells. J Biol Chem 2000: 275 (29): 22339–22347.
21. Iozzo RV. Basement membrane proteoglycans: from cell to ceiling. Nat Rev Mol Cell Biol 2005: 6 (8): 646–656.
22. Rasi K, Piuhola J, Czabanka M et al. Collagen XV is necessary for modeling of the extracellular matrix and its deficiency predisposes to cardiomyopathy. Circ Res 2010: 107 (10): 1241–1252.
23. Nicolae C, Olsen BR. Unexpected matrix diseases and novel therapeutic strategies. Cell Tissue Res 2010: 339 (1): 155–165.
24. Määttä M, Heljasvaa R, Sormunen R, Pihlajaniemi T, Autio-Harjanne H, Tervo T. Differential expression of collagen types XVII/endostatin and XV in normal, keratoconus, and scarred human corneas. Cornea 2006: 25 (3): 341–349.
25. Hurskainen M, Eklund L, Hägg PO et al. Abnormal maturation of the retinal vasculature in type XVII collagen/endostatin deficient mice and changes in retinal glial cells due to lack of collagen types XV and XVIII. FASEB J 2005: 19 (11): 1564–1566.
26. Seppinen L, Pihlajaniemi T. The multiple functions of collagen XVIII in development and disease. Matrix Biol 2011: 30 (2): 83–92.
27. Menzel O, Bekkeheien RC, Reymond A et al. Knobloch syndrome: novel mutations in COL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. Hum Mutat 2004: 23 (1): 77–84.
28. Marneros AG, She H, Zambarakji H et al. Endogenous endostatin inhibits choroidal neovascularization. FASEB J 2007: 21 (14): 3809–3818.
29. Dityar AV, Pozdnayakova NV, Feldman NB, Lusenko SV, Severin SE. Endostatin: current concepts about its biological role and mechanisms of action. Biochemistry (Moscow) 2007: 72 (3): 235–246.
30. Ylikarppi R, Eklund L, Sormunen R et al. Double knockout mice reveal a lack of major functional compensation between collagens XV and XVIII. Matrix Biol 2003: 22 (5): 443–448.
31. Fan BJ, Leung DY, Wang DY et al. Novel myocilin mutation in a Chinese family with juvenile-onset open-angle glaucoma. Arch Ophthalmol 2006: 124 (1): 102–106.
32. Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open-angle glaucoma. N Engl J Med 2009: 360 (11): 1113–1124.
33. Zode GS, Kuehn MH, Nishimura DY et al. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. J Clin Invest 2011: 121 (9): 3542–3553.
34. Liu Y, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. Hum Mol Genet 2004: 13 (11): 1193–1204.
35. Zhou Z, Vollrath D. A cellular assay distinguishes normal and mutant TIGR/myocilin protein. Hum Mol Genet 1999: 8 (12): 2221–2228.
36. Aroca-Aguilar JD, Sánchez-Sánchez F, Martínez-Redondo F, Coca-Prados M, Escribano J. Heterozygous expression of myocilin glaucoma mutants increases secretion of the mutant forms and reduces extracellular processed myocilin. Mol Vis 2008: 14: 2097–2108.
37. Sommer A, Tielsch JM, Katz J et al. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. Arch Ophthalmol 1991: 109 (8): 1090–1095.
38. Klebe S, Golmard JL, Nalls MA et al. The Val158Met COMT polymorphism is a modifier of the age at onset in Parkinson’s disease with a sexual dimorphism. J Neurol Neurosurg Psychiatry 2013: 84 (6): 666–673.
39. Tang ST, Peng WJ, Wang CJ, Tang HQ, Zhang Q. Polymorphism M55V in gene encoding small ubiquitin-like modifier 4 (SUMO4) protein associates with susceptibility to type 1 (and type 2) diabetes. Diabetes Metab Res Rev 2012: 28 (8): 679–687.
40. Valenti L, Fracanzani AL, Rametta R et al. Effect of the A736V TMPRSS6 polymorphism on the penetrance and clinical expression of hereditary hemochromatosis. J Hepatol 2012: 57 (6): 1319–1325.