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

Janey L. Wiggs[1], **Gareth R. Howell**[2], **Kevin Linkroum**[1], **Wael Abdrabou**[1], **Emily Hodges**[3], **Catherine E. Braine**[2], **Louis R. Pasquale**[1], **Gregory J. Hannon**[3], **Jonathan L. Haines**[4], and **Simon W. M. John**[2,5]

Gareth R. Howell: gareth.howell@jax.org; Kevin Linkroum: kevin_linkroum@meei.harvard.edu; Wael Abdrabou: wael_abdrabou@meei.harvard.edu; Emily Hodges: hodges@cshl.edu; Catherine E. Braine: catherine.braine@jax.org; Louis R. Pasquale: louis_pasquale@meei.harvard.edu; Gregory J. Hannon: hannon@cshl.edu; Jonathan L. Haines: jonathan@chgr.mc.vanderbilt.edu; Simon W. M. John: simon.john@jax.org

[1]Department of Ophthalmology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA, USA

[2]The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine, USA

[3]Cold Spring Harbor Laboratory, Watson School of Biological Sciences and Howard Hughes Medical Institute, Cold Spring Harbor, NY, USA

[4]Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN, USA

[5]Howard Hughes Medical Institute, 600 Main Street, Bar Harbor, Maine, USA

Abstract

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.

INTRODUCTION

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

Corresponding author: Janey L. Wiggs, MD, PhD, Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, 617 573 6440 (office), janey_wiggs@meei.harvard.edu.

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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].

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 primary open angle glaucoma 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, Figure 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, central corneal thickness, slit lamp evaluation and funduscopic evaluation. Glaucoma was defined as intraocular pressure >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). 35bp 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 was 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. 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.

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 4–6 months old C57BL/6J mice and fixed overnight in 4% paraformaldehyde (PFA) at 4°C. Eyes were stored in 1X Phosphate buffered solution (1X PBS) at 4°C. Assessment of *Col15a1* RNA was performed as described previously [17]. Briefly, eyes were placed in 30% sucrose overnight, rinsed in 1X 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 1 μg/ml digoxigenin (DIG)-labeled *Col15a1* riboprobe (clone 3482829 from Open Biosystems; antisense: digested with *Sal1*, reverse transcribed with T7; sense: digested with *Not1*, reverse transcribed with SP6;). Peroxidase (POD)-conjugated anti-DIG antibody (Roche, Indianapolis, IN) followed by TSA-Cy-3 (PerkinElmer) was used for fluorescence detection. Imaging and photography was performed on the SP5 confocal microscope (Leica). At least three sections from six eyes were assessed.

RESULTS

Targeted genomic capture followed by high throughput sequencing was performed using DNA from ten 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, OAG14). The selective capture targeted all exons and conserved sequences from D9S1841 to D9S271 corresponding to the GLC1J interval (see methods). For each of the ten 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 ten individuals. After filtering by: 1) selecting variants present in both affected individuals from a family; 2) removing variants present in the unaffected individuals (if included); and 3) 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

frequency less than 0.01) expected for a causative mutation for this rare mendelian disorder. However, a *COL15A1* variant, R163H, was found in 3 of the 4 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 (Figure 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 4 of the additional families (OAG25, OAG26, OAG31 and OAG60) (Figure 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 6 of the 7 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) (Figure 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 suggest 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 ESV (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 2A). In both the Caucasian and African cases there was a trend for higher intraocular pressure (IOP) in the carriers of the risk genotype, that was nominally significant in the African-American sample (Table 2B). Central corneal thickness,

another clinical feature that influences IOP and glaucoma risk, was not different among the R163H genotype groups (Table 2C).

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 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 (Figure 3). 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 of primary open angle glaucoma 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 4 OAG families (OAG16, OAG60, OAG125, OAG746) (Figures 1 and 2). The youngest affected members of family OAG60 are heterozygous for both the *COL15A1* R163H variant and the *COL18A1* A1381T variant (Figure 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 Polyphen2 and SIFT (Table 1). Affected individuals heterozygous for A13811T 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 [28,297]. 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 intraocular pressure [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 four 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, since 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 Figure 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 versus 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 it would be interesting to screen additional Q368X families for *COL18A1* variants [37].

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 [38]. Our findings suggest that variation in *COL15A1* is one factor that could influence the disease severity in African Americans. Of the *COL15A1* variants examined in this study, R168H is the most common variant 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 diease (GERP, −0.757; Polyphen2, Benign) [39], the Met55Val *SUMO4* allele reported to modify susceptibility to diabetes (GERP, −0.47; Polyphen2, Benign [40], and the Ala736Val *TMPRSS6* allele that is reported to modify the penetrance and clinical expression of hereditary hemochromatosis (GERP 4.72; Polyphen2, Benign) [41].

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. Intraocular pressure (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 don't 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 4 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.

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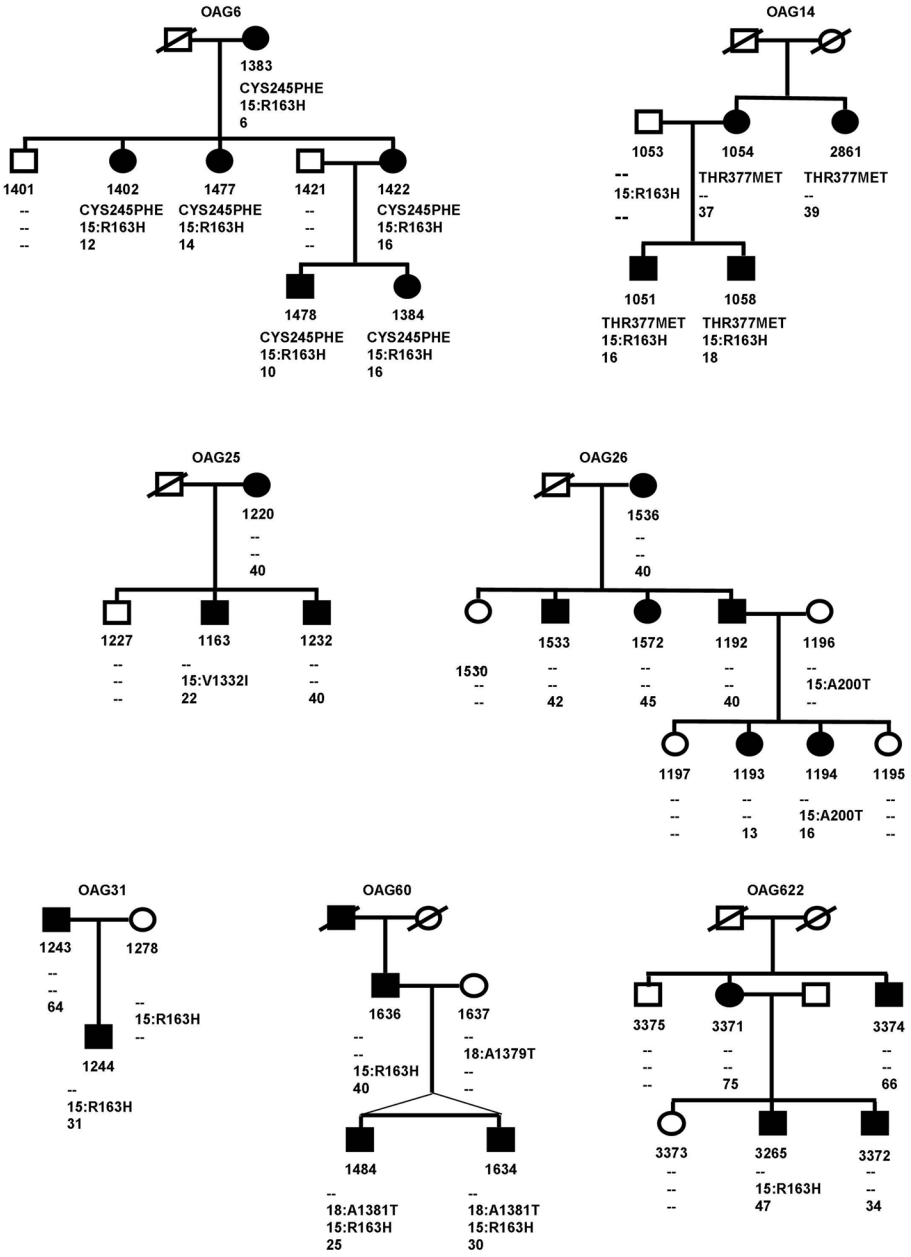

Figure 1. Glaucoma pedigrees with *COL15A1* variants

Affected pedigree members are shown as solid circles (females) or squares (males). The *MYOC* mutation (OAG6, 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 as determined 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.

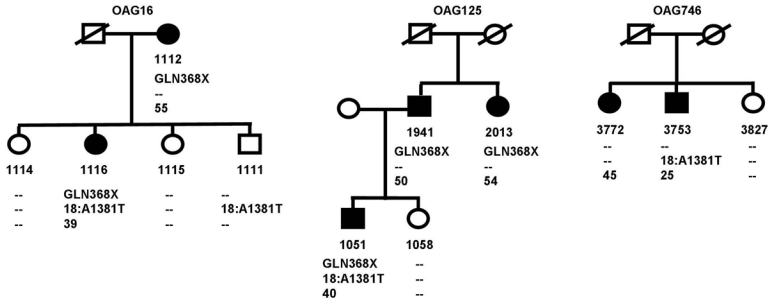

Figure 2. Glaucoma pedigrees with variants in *COL18A1*

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).

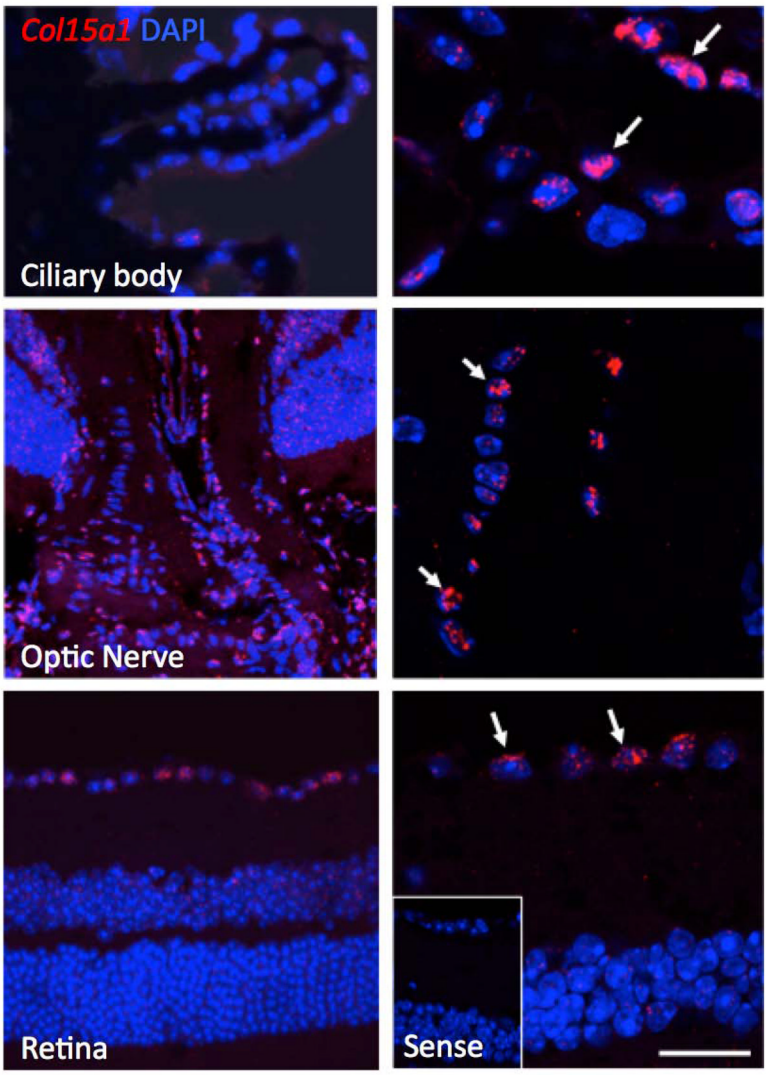

Figure 3. *Col15a1* is expressed in multiple ocular structures

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).

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 (.011)	Possibly Damaging (.322)	Benign (.002)	Probably Damaging (1.00)
SIFT	Tolerated (.06)	Damaging (0.00)	Tolerated (0.23)	Damaging (0.00)
PMUT	Pathological (.507)	Neutral (0.09)	Neutral (0.03)	Neutral (0.239)

Abbreviations: 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 to 1.0. PMUT pathogenicity is either Pathological (>0.5) or Neutral (<0.5) on a scale of 0 to 1.0.

Table 2

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

A. Age of onset			
	Genotype	**Mean Age of Onset (St. Dev)**	**p-value***
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. Intraocular pressure (IOP)			
	Genotype	**Mean IOP (St. Dev)**	**p-value***
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. Central corneal thickness (CCT)			
	Genotype	**Mean CCT (St. Dev)**	**p-value**
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	

* 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. St. Dev= Standard Deviation.

* Mean intraocular pressure (IOP) of individuals with the AA genotype compared with the GG genotype using a two-tailed, unequal variance t-test. St. Dev= Standard Deviation

Mean central corneal thickness (CCT) of individuals with the AA genotype compared with the GG genotype using a two-tailed, unequal variance t-test. St. Dev= Standard Deviation