Screening for Glaucomatous Disc Changes Prior to Diagnosis of Glaucoma in Myocilin Pedigrees

Sonya L. Bennett, FRANZCO; Alex W. Hewitt, MBBS; Johan L. Poulsen, MBChB; Lisa S. Kearns, BOrthOphSci(Hons); James E. Morgan, DPhil, FRCOphth; Jamie E. Craig, DPhil, FRANZCO; David A. Mackey, MD, FRANZCO

Objective: To investigate whether structural differences of the optic nerve head are evident in young people who do not have manifest glaucoma but are known to carry myocilin mutations.

Methods: A case-control design was adopted. Subjects from Australian pedigrees known to have either the Gln368STOP myocilin mutation (cutoff age, <40 years) or the Thr377Met myocilin mutation (cutoff age, <30 years) were examined for signs of glaucoma. Stereoscopic disc photographs were digitalized. Analysis of the optic disc area, optic cup area, and neuroretinal rim area was performed using digital stereoscopy with a Z-screen. Mutation analysis was conducted using direct sequencing. The t test, corrected for multiple comparison testing, was used in analysis.

Results: A total of 29 myocilin mutation-carrying (case) and 33 mutation-free (control) individuals were reviewed. The mean ± SD ages were 19.9 ± 9.0 and 22.1 ± 9.5 years in the mutation and mutation-free groups, respectively (P = .35). There was no significant difference in intraocular pressure between mutation carriers and non-carriers (P = .44). There were no statistically significant differences in the mean disc, neuroretinal rim, and cup areas between the groups. The mean ± SD neuroretinal rim area was 1.24 ± 0.24 mm² in the noncarrier group and 1.25 ± 0.23 mm² in the mutation group (P = .46). No notch, nerve fiber layer defect, or neuroretinal rim hemorrhage was noted in any eye examined.

Conclusions: Although confounded by penetrance and expressivity, no quantified structural difference in the optic nerve head was observed in individuals who had a myocilin mutation prior to the diagnosis of glaucoma.

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Worldwide, primary open-angle glaucoma (POAG) is one of the leading causes of blindness. Primary open-angle glaucoma is characterized by progressive excavation of the optic nerve head with corresponding visual field loss. Approximately half of the cases in the community are undiagnosed. The spectrum of POAG compounds the difficulty of population-based screening. A continuum exists between clinically undetectable POAG, asymptomatic disease, and functional impairment. Within this “glaucoma continuum,” retinal ganglion cell loss and retinal nerve fiber layer damage precede visual field deficit by at least 5 years. It is known that the prevalence of POAG increases with age; however, there is no sound evidence base delineating the age for the commencement of screening.

Genetic screening offers an innovative means for identifying people predisposed to disease development. Mutations in the myocilin (MYOC) gene account for approximately 3% to 4% of unselected POAG cases. The primary pathogenetic mechanisms by which a mutation in the MYOC gene results in glaucoma are still not fully understood. There is evidence that MYOC mutations cause a gain in function, whereby abnormal gene products may accumulate in trabecular meshwork cells, contributing to outflow obstruction and resulting in intraocular pressure (IOP) elevation. The Gln368STOP and Thr377Met MYOC mutations are the most common MYOC mutations identified in Australia. Our previous investigations of pedigrees containing these mutations revealed that the mean ± SD age for the diagnosis of glaucoma or ocular hypertension was 52.4 ± 12.9 and 40.4 ± 11.0 years for the Gln368STOP and Thr377Met MYOC mutation carriers, respectively. The Thr377Met MYOC mutation penetrance rate for ocular hypertension or glaucoma in Australia is approximately 88% at age 30 years, while the age-related penetrance for ocular hypertension or glaucoma is 72% at age 40 years for the Gln368STOP MYOC mutation.
mum IOP readings are typically higher in patients carrying the Thr377Met mutation, who typically have a mean ± SD IOP of 31.7 ± 9.9 mm Hg, rather than the Gln368STOP MYOC mutation, which usually manifests with a mean ± SD IOP of 28.4 ± 4.7 mm Hg.13,14

We performed cascade genetic screening in Australian pedigrees known to carry either the Gln368STOP or Thr377Met MYOC mutation. The aim of this study was to investigate whether structural differences of the optic nerve head are evident in young people who do not have manifest glaucoma but are known to carry common MYOC mutations. The main interest of this work was to investigate the early changes of the optic nerve in subjects at an established high risk of developing glaucoma compared with subjects who likely have a baseline risk.

Once a mutation-carrying patient with POAG has been identified, each of his or her first-degree relatives can be tested for the same mutation. If these relatives are identified as carrying the at-risk mutation, they are followed up closely for early clinical signs of glaucoma, and their first-degree relatives are also tested. Thus, cascade genetic screening moves out in a stepwise direction from the index case until all the relatives who carry the mutation are identified.

Cascade screening was performed for pedigrees with either the Gln368STOP or the Thr377Met MYOC mutations identified through the Glaucoma Inheritance Study in Tasmania. All recruited subjects underwent a comprehensive clinical examination, which included anterior segment examination; gonioscopy; IOP measurement by Goldmann applanation tonometry; perimetry; pachymetry; refraction; and a mydriatic optic disc assessment.

Stereoscopic optic disc photographs were taken (Nidek fundus camera 3-Dx/F; Nidek, Gamagori, Japan) and then digitized at a high resolution (2102 × 1435 pixels, 2900 pixels per inch, 16-bit color) using a Nikon CoolScan IV ED slide scanner (Nikon Corp, Tokyo, Japan). Analysis of the optic disc area, optic cup area, and neuroretinal rim area was performed stereoscopically using custom software (StereoDx) with a Z-screen (StereoGraphics Corporation, Beverly Hills, Calif).15,16 The technical details of this system have been described elsewhere.15,16 In brief, the Z-screen comprises a glass plate incorporating a liquid crystal membrane, which is laid over the computer monitor screen. The phase of the liquid crystal film is altered at 60 Hz so that the observer, when wearing passive Polaroid glasses, can restrict the view of the component image of the stereopair to the correct eye. The images of the stereopair are each displayed at 60 Hz, with the monitor refreshing at 120 Hz to provide a flicker-free stereoscopic image. The stereoscopic depth of the mouse cursor can be adjusted to coincide with the plane of the E schnig rim. The inner border of the neuroretinal rim is defined as the point at which a plane, lying at the level of the E schnig rim, intersects with the surface of the retinal nerve fiber layer. Image magnification was corrected on the basis of keratometry readings; refraction; and camera specifications using established methods to provide scaled estimates of disc parameters.17,18 Measurements were performed by a single grader who was masked to the mutation status of each subject.

Glaucoma was defined as the presence of an abnormal visual field test result with a corresponding optic disc change that was characteristic of glaucoma. Able subjects underwent automated visual field assessment using the Humphrey computerized perimeter (Humphrey Field Analyzer II; Zeiss-Humphrey, Dublin, Calif). Visual field test results were deemed appropriate if the rate of false-positive or false-negative responses was less than 20%. G laucomatous visual field defects were defined as having a total mean defect of less than 2 dB with, on repeat testing, at least 2 adjacent points in a location typical for glaucoma, having a pattern deviation of less than 1%.

Age cutoff criteria were set at approximately 1 SD lower than the mean age of glaucoma diagnosis for each mutation. Hence, for the purposes of this study, the age limit for recruitment was set at 40 years for the Gln368STOP pedigrees and at 30 years for the Thr377Met pedigrees. Subjects were excluded if they had glaucoma previously diagnosed or a refractive error more than ±6 diopters.

This study was approved by the relevant ethics committees of the Royal Victorian Eye & Ear Hospital and the Royal Hobart Hospital. Written informed consent was obtained from each subject, and this study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

DATA ANALYSIS

A case-control design was adopted. Case subjects were the offspring of index cases identified to also carry their respective pedigree’s MYOC mutation. Subjects from these same pedigrees found not to have inherited these mutations were defined as control subjects. The optic disc features of MYOC mutation carriers and noncarriers were compared. Additionally, disc features were compared on a mutation-by-mutation basis. Vertical and horizontal cup and disc measurements as well as each 60° neuroretinal rim segment were analyzed separately. The t test with a Bonferroni correction for multiple-comparison testing was performed using Intercooled Stata 7.0 for Windows (StataCorp, College Station, Tex). Power calculations were performed using the PS program version 1.0.17 for Windows.19

LABORATORY TECHNIQUES

Mutation analysis was performed through direct sequencing. Genomic DNA was isolated from peripheral blood samples, and the MYOC exon 3 amplicon containing the MYOC 368 and 377 codons was amplified using previously published intronic primers.20 The polymerase chain reaction products were purified and sequenced. The sequencing reactions were carried out using the Applied Biosystems Big Dye Terminator kit (Applied Biosystems, Scoresby, Australia), with 30 cycles of 10 seconds at 95°C and 5 seconds at 50°C, followed by 4 minutes at 60°C, as specified by the manufacturer. Sequencing analysis was performed using an Applied Biosystems Prism 310 Genetic Analyzer and results were reviewed using Sequencher (Gene Codes Corporation, Ann Arbor, Mich).

A total of 30 MYOC mutation-carrying (case) and 33 mutation-free (control) subjects were recruited. Of the 31 subjects recruited from the 9 Gln368STOP MYOC pedigrees (G Tas2, G Tas209, G Tas287, G Tas309, G Tas88, G Qld11, G Vic122, G Vic124, and G Vic139), 14 (45.2%) were found to carry the MYOC mutation. No subject from the Gln368STOP MYOC pedigrees was required to be excluded because of new visual field findings suspicious for glaucoma. The Thr377Met MYOC mutation was identified in 16 (50.0%) of the 32 subjects who were recruited.
from 2 separate pedigrees (GVic1 and GVic20). One 26-year-old subject (who was later found to have the Thr377Met mutation) was excluded because of a previous diagnosis of glaucoma (GVic1, subject IV:1).14 The fundus image of 1 eye of another subject (with a Thr377Met MYOC mutation) was of poor quality and was excluded from analysis. Hence, optic discs from a total of 29 MYOC mutation carriers were available for analysis. At the .05 significance level, this cohort had 80% power to detect an 0.18-mm² difference in neuroretinal rim areas.

Demographic details of the study cohort are displayed in the Table. There was no significant difference in IOP between mutation carriers and noncarriers (P = .44). Three Thr377Met mutation carriers, aged between 22 and 30 years, were found to have a maximum recorded IOP higher than 21 mm Hg, though lower than 25 mm Hg. The neuroretinal rim area in these subjects’ eye with the smallest neuroretinal rim area ranged between 1.214 and 1.297 mm².

Vertical cup-disc ratios correlated highly with global disc areas (mutation carriers, y = 0.168x + 0.125; r² = 0.24; P = .006; nonmutation carriers, y = 0.132x + 0.247; r² = 0.21; P = .008). Across the whole cohort, the measured optic disc variables approximated a Gaussian distribution. Adjusting for age at the time of examination did not reveal any significance difference in optic disc feature between mutation carriers and noncarriers.

No notch, nerve fiber layer defect, or neuroretinal rim hemorrhage was noted in any eye examined (Figure 1 and Figure 2). Results from each 60° sector of the optic disc area from the worst eye, as judged by the eye with the smallest neuroretinal rim area, are presented in the Table. There was no statistically significant difference in cup-disc area ratios (Figure 3) or total rim area between Gln368STOP and Thr377Met mutation carriers and noncarriers (right eye, P = .08 and P = .29; left eye, P = .51 and P = .25, respectively). Combining both mutation

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Abbreviation: IOP, intraocular pressure.
*Calculated using Fisher exact test.
†Not significant following Bonferroni correction for multiple-comparison testing (P = .13).
‡Not significant following Bonferroni correction for multiple-comparison testing (P = .46).

Figure 1. Right and left optic disc examples of subjects from Gln368STOP MYOC pedigrees. A, Mutation carrier GQld11-14 aged 22 years. B, Mutation carrier GTas287-11 aged 7 years. C, Mutation carrier GVic139-5 aged 32 years. D, Mutation carrier GVic124-11 aged 37 years. E, Noncarrier GQld11-12 aged 7 years. F, Noncarrier GTas287-10 aged 29 years. G, Noncarrier GVic139-4 aged 34 years. H, Noncarrier GVic124-7 aged 31 years.
groups, there was no statistically significant difference in cup-disc area ratios between case and control subjects (right eye, \( P = .16 \); left eye, \( P = .34 \)).

One individual (mutation carrier GTas287-11) with the Gln368STOP mutation was found to have outlying cup-disc area ratios of 0.6 (Figure 1B). These measurements were not corrected for disc size, which in this individual was larger than normal. Because the individual was 7 years of age at the time of examination, reliable visual field test results were not available; however, repeat IOP readings have all been consistently lower than 19 mm Hg bilaterally.

**COMMENT**

We found no quantifiable difference in the structure of the optic nerve head, either in cup-disc area ratio or neuroretinal rim area, between young individuals who were carriers and those who were noncarriers of common MYOC mutations. Although confounded by penetrance and heterogeneity, the results from our study suggest that young patients with either the Gln368STOP or Thr377Met mutation cannot be clinically differentiated from family members who do not have these mutations. It is clear that these results are applicable only to MYOC glaucoma. It is certainly possible that other POAG-related genes, particularly those influencing normal-tension glaucoma, may not follow this natural history.

This study underscores the importance of baseline optic disc evaluation in predisposed cases. Observing and analyzing serial optic disc photographs and IOP measurements of these young individuals will enhance our understanding of the disease mechanisms underlying MYOC glaucoma. Airaksinen et al\(^2\) and Zeyen and Caprioli\(^3\) have demonstrated that in eyes with initially normal visual field test results from patients with glaucoma diagnosed by contralateral ocular findings, the rate of neuroretinal rim area loss is approximately 0.47% to 1.7% per year. It is possible that the trajectory of neuroretinal rim loss in MYOC glaucoma differs significantly from that of POAG caused by other mechanisms.

The finding of Gln368STOP mutation carriers having smaller vertical cup-disc ratios than their non–mutation carrying counterparts was unexpected and may either represent a statistical anomaly, given that the finding was no longer significant following correction for multiple testing, or it may be due to ascertainment bias. Considerable lengths were taken to ascertain the most optimal internal control set, that is a nonmutation carrier from the same family, thereby reducing any confounding bias introduced by coassociated traits. Ideally, to avoid confounding traits, only 1 randomly selected member per pedigree affected would be included; however, the resultant loss of power in this study would negate any such benefit.

Along with being one of the most sensitive features in differentiating patients with POAG from patients without POAG,\(^4,5\) a small neuroretinal rim area is a principal morphological predictive factor for disease development and progression.\(^6\) Further to this, the superior and inferior poles of the optic disc lose nerve fibers at a selectively greater rate.\(^7\) Mutation carriers were not found to have a smaller total
neuroretinal rim area than noncarriers and, importantly, no preferential loss of neuroretinal rim tissue was noted in the superior or inferior regions.

These results lend support to the hypothesis that MYOC glaucomatous optic nerve changes occur following a sustained elevation of IOP.\(^{20}\) In contrast, optineurin mutations are thought to induce retinal ganglion cell apoptosis.\(^{27}\) Large diurnal variations in IOP do occur in individuals with POAG and may dramatically influence the degree of optic disc damage.\(^{28}\) It is also possible that either intermittent spiking or a stepwise increase in IOP may occur prior to a sustained elevation in IOP. The optic disc features of the 7-year-old subject carrying the Gln368STOP MYOC mutation are suspicious of glaucomatous damage. It is interesting that he has never been found to have an elevated IOP and that other causes for optic neuropathy have been excluded. Along with MYOC glaucoma being principally associated with a high IOP, the prevalence of glaucomatous disc damage has also been shown to dramatically increase at an IOP higher than 21 mm Hg.\(^{3}\) Investigation of the diurnal IOP variation in MYOC cases is warranted.

In this study, we actively conducted cascade genetic screening for MYOC glaucoma. Genetic screening and counseling have been shown to be appreciated by families known to carry MYOC mutations.\(^{29}\) While population-based molecular screening for MYOC changes alone is not currently cost-effective, with the advance of genetic studies more genes implicated in POAG will be identified.\(^{30}\) Increasing our understanding of the genetic mechanisms of POAG will have a significant public health impact. Genetic screening will principally highlight those people who do not require vigilant predisease clinical screening.

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Correspondence: David A. Mackey, MD, FRANZCO, Clinical Genetic Unit, Eye Research Australia, Royal Victorian Eye & Ear Hospital, East Melbourne, Victoria 3002, Australia (dmackey@postoffice.utas.edu.au).

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REFERENCES


