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Myocilin Predictive Genetic Testing for Primary Open-Angle Glaucoma Leads to Early Identification of At-Risk Individuals

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Purpose: To assess the difference in severity of disease in primary open-angle glaucoma (POAG) patients with a *Myocilin* (*MYOC*) disease-causing variant who presented through normal clinical pathways (Clinical cases) versus those who were examined following genetic testing (Genetic cases).

Design: Retrospective clinical and molecular study.

Participants: Seventy-three *MYOC* mutation carriers identified through the Australian and New Zealand Registry of Advanced Glaucoma.

Methods: Individuals were classified based on how they first presented to an ophthalmologist: Clinical cases were referred by their general practitioner or optometrist, and Genetic cases were referred following positive results from genetic testing for the previously identified familial *MYOC* variant (cascade genetic testing). All cases were then sub-classified into 4 groups (unaffected, glaucoma suspect, glaucoma, advanced glaucoma) according to the severity of disease at the time of their first examination by an ophthalmologist.

Main Outcome Measures: Glaucoma clinical parameters and age at presentation.

Results: At their first examination, 83% of Genetic cases were unaffected and 17% were glaucoma suspect, whereas among Clinical cases 44% were glaucoma suspect, 28% had glaucoma, and 28% had advanced glaucoma. Genetic cases were significantly younger at presentation than Clinical cases (40.6±12.5 vs. 47.5±16.7 years; $P = 0.018$). The mean highest intraocular pressure (32.2±9.7 vs. 17.6±3.6 mmHg; $P < 0.001$), cup-to-disc ratio (0.65±0.27 vs. 0.48±0.13; $P = 0.006$), and mean deviation on visual field testing (−10.0±10.3 vs. −1.2±1.2; $P < 0.001$) were all significantly worse in Clinical cases compared with Genetic cases. Individuals with common *MYOC* p.Gln368Ter variant were further analyzed separately to account for the phenotypic variability of different disease-causing variants. All findings remained significant after adjusting for the common *MYOC* p.Gln368Ter variant.

Conclusions: Our findings demonstrated that *MYOC* cascade genetic testing for POAG allows identification of at-risk individuals at an early stage or even before signs of glaucoma are present. To our knowledge, this is the first study to demonstrate the clinical utility of predictive genetic testing for *MYOC* glaucoma. *Ophthalmology 2016;■:1−7 © 2016 by the American Academy of Ophthalmology*

Glaucoma is the leading cause of irreversible and preventable blindness worldwide.[1] It refers to a heterogeneous set of progressive eye disorders characterized by optic disc cupping and corresponding visual field defects.[2] Primary open-angle glaucoma (POAG) is the most common subset and affects 3% of the Australian population above the age of 50 years.[3] Symptoms are usually not apparent until substantial irreversible damage has occurred. Therefore it is important to facilitate early diagnosis to prevent vision loss. Approximately half of those affected remain undiagnosed,[3,4] suggesting that current screening strategies lack efficacy.

POAG has a strong genetic component.[5] Individuals with an affected first-degree relative are 9 times more likely to develop glaucoma compared with the general population.[6] The *Myocilin* (*MYOC*) gene was the first gene associated with POAG.[7,8] *MYOC* disease-causing variants have been identified in 2% to 4% of unselected POAG patients and in 8% to 36% of POAG patients diagnosed before 40 years of age.[9−11] The variants are inherited in an autosomal dominant fashion with high penetrance, and carriers usually demonstrate elevated intraocular pressure (IOP) with a younger age at onset than POAG patients without *MYOC* variants.[10] There is an enrichment of *MYOC* variants in patients with advanced POAG, indicating a progression to a more severe disease, particularly without treatment.[10] Since the discovery of the *MYOC* gene in 1997, over 80 disease-causing variants have been described; the p.Gln368Ter variant is the most common.[12] Although clear genotype−phenotype correlations exist, inter- and intrafamilial phenotypic variability is also

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well acknowledged. The p.Gln368Ter variant has a variable age-related penetrance, with 50% of carriers diagnosed with glaucoma by 50 years of age.[13] Other disease-causing variants such as p.Pro370Leu and p.Gly367Arg are more severe and are associated with complete penetrance by 50 years of age.[9,10,14,15] The exact mechanism of *MYOC* variants leading to disease has not yet been fully elucidated. There is evidence to suggest that the abnormal gene protein products accumulate in the trabecular meshwork, contributing to outflow obstruction and ultimately increasing IOP.[16,17]

POAG is treated by lowering IOP; it is an effective strategy to slow progression or to prevent disease development, provided that patients are identified early in the disease process.[18,19] Lowering IOP is achieved with medical therapy, with laser, or with incisional surgical interventions. In the era of personalized medicine, the ability to predict disease development can allow tailored, specific treatment plans for individuals. Considering the difficulties in diagnosing glaucoma early, the younger age at onset for *MYOC* carriers compared with the general population, and the availability of effective preventive measures for treating POAG, genetic testing of relatives for the previously identified familial *MYOC* variant (cascade genetic testing) offers the potential to improve patient care and to prevent glaucoma blindness.[20,21] No previous study has examined the possible clinical benefits of *MYOC* cascade genetic testing.

Established in 2007, the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) has gathered the largest cohort of patients with advanced glaucoma with the aim to identify genetic risk factors for glaucoma blindness.[22] The ANZRAG offers all participants with *MYOC* disease-causing variants the opportunity to have cascade genetic testing performed on all first-degree family members over the age of 18 years. Using the ANZRAG, this study aimed to assess the clinical utility of performing cascade genetic testing by comparing the disease severity of POAG patients with a *MYOC* disease-causing variant who presented through usual clinical care pathways with those who were examined following genetic testing.

Methods

Ethics committee approval was obtained through the Southern Adelaide and Flinders University Clinical Research Ethics Committee. The study adhered to the tenets of the Declaration of Helsinki and followed the National Health and Medical Research Council statement of ethical conduct in research involving humans. Informed consent was obtained from all participants.

Participant recruitment into the ANZRAG has been described previously.[22] Patients with all levels of glaucoma could be referred to the ANZRAG by clinicians. Advanced glaucoma was defined as central visual field loss related to glaucoma with at least 2 of the 4 central fixation squares having a pattern standard deviation probability less than 0.5% on a reliable Humphrey 24-2 field, or a mean deviation (MD) greater than −22 dB or, in the absence of visual field testing, best-corrected visual acuity (BCVA) worse than 20/200 owing to glaucoma. Participants also needed evidence of glaucoma in the less severely affected eye, characterized by glaucomatous visual field defects with corresponding optic disc rim thinning. Nonadvanced glaucoma was defined by glaucomatous visual field defects, with corresponding optic disc rim thinning, including an enlarged cup-to-disc ratio (CDR) (≥0.7) or CDR asymmetry (≥0.2) between both eyes. Glaucoma suspects had ocular hypertension, defined by IOP >21 mmHg, or had preperimetric glaucoma with no glaucomatous field changes.

Advanced and nonadvanced POAG cases recruited in the ANZRAG were screened for *MYOC* as previously described.[10] Glaucoma suspects who did not meet the advanced or nonadvanced criteria but had a combination of ocular hypertension, young age, and positive family history of glaucoma were also screened. Through the proband, cascade genetic testing and counseling were offered to first-degree family members older than 18 years who were either affected or unaffected.

This study retrospectively identified the manner in which patients with an underlying *MYOC* disease-causing variant first presented to an ophthalmologist and aimed to capture a clinical picture of the patients at the time of their first presentation. All participants with *MYOC* variants were categorized into 2 main groups: participants who were referred to an ophthalmologist for the first time by their general practitioner or optometrist (Clinical group) and those who were referred to an ophthalmologist for the first time following genetic testing results (Genetic group). Clinical parameters recorded at the time of participants' first presentation to an ophthalmologist were collected. The data collected included demographic information, IOP, CDR, central corneal thickness (CCT), BCVA, and reliable visual field testing parameters including MD. Once cases were classified according to their mode of presentation, they were further sub-classified into 4 groups according to the severity of disease at the time of their first presentation: normal, glaucoma suspect, nonadvanced glaucoma, and advanced glaucoma, as described previously.

Data were analyzed for all participants with *MYOC* disease-causing variants identified in the ANZRAG that satisfied inclusion criteria. BCVA was transformed into decimal fractions for analysis purposes. Owing to the phenotypic variations of underlying *MYOC* variants, additional analysis was also performed on participants carrying p.Gln368Ter only, as it is the most common disease-causing variant. Clinical data were analyzed with PASW Statistics, Rel. 18.0.1.2009 (SPSS Inc., Chicago, IL). Data are presented as mean ± standard deviation. The Mann-Whitney *U* test was used for the assessment of differences in nonparametric data and chi-square tests for categorical data.

Results

Ninety-seven participants with a *MYOC* disease-causing variant were identified in the ANZRAG. Of these, clinical details at presentation could be obtained for 73 participants (75%) included in the study. They consisted of 43 Clinical cases (59%) and 30 Genetic cases (41%). There were 39 (53%) female and 34 (47%) male patients. The mean current age was 60.9±17.7 years (range, 16−87 years) for Clinical cases and 44.7±11.9 years (range, 24−77 years) for Genetic cases. Genetic cases were significantly younger at presentation than Clinical cases (40.6±12.5 vs. 47.5±16.7 years; $P = 0.018$). At their first examination, 25 Genetic cases (83%) were unaffected and 5 (17%) were glaucoma suspect, whereas among Clinical cases 19 (44%) were glaucoma suspect, 12 (28%) had nonadvanced glaucoma, and 12 (28%) had advanced glaucoma (Fig 1). Among the Genetic cases, unaffected individuals were significantly younger compared with glaucoma suspects (42.5±10.4 vs. 55.8±13.7 years; $P = 0.037$).

The mean highest IOP (17.6±3.6 vs. 32.2±9.7 mmHg; $P < 0.001$), highest CDR (0.48±0.13 vs. 0.65±0.27; $P = 0.006$), worst MD (−1.2±1.2 vs. −10.0±10.3; $P < 0.001$), and worst BCVA (0.96±0.30 vs. 0.70±0.38; $P = 0.004$) were all significantly less severe among Genetic cases compared with Clinical