Myocilin predictive genetic testing for Primary Open Angle Glaucoma leads to early identification of at-risk individuals

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Abbreviations/acronyms:

ANZRAG	Australian and New Zealand Registry of Advanced Glaucoma
BCVA	Best corrected visual acuity
CCT	Central corneal thickness
CDR	Cup-to-disc ratio
IOP	Intraocular pressure
MD	Mean deviation
POAG	Primary Open Angle Glaucoma

ABSTRACT

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 four 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 versus 47.5 ± 16.7 years, $P = 0.018$). The mean highest intraocular pressure (17.6 ± 3.6 versus 32.2 ± 9.7 mmHg, $P < 0.001$), cup-to-disc ratio (0.48 ± 0.13 versus 0.65 ± 0.27, $P = 0.006$) and mean deviation on visual field testing (-1.2 ± 1.2 versus -10.0 ± 10.3, $P < 0.001$) were all significantly worse in Clinical cases compared with Genetic cases. Individuals with *MYOC* common p.Gln368Ter variant were further analysed 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. This is the first study to demonstrate the clinical utility of predictive genetic testing for *MYOC* glaucoma.

INTRODUCTION

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.[3] Symptoms are usually not apparent until substantial irreversible damage has occurred. Therefore we need to facilitate early diagnosis in order 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 2-4% of unselected POAG patients and in 8-36% 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 an earlier age of 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, with the p.Gln368Ter variant the most common.[12] Although clear genotype-phenotype correlations exist, inter- and intra-familial phenotypic variability is also 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 or 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 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 of 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. 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 of less than 0.5% on a reliable Humphrey 24-2 field, or a mean deviation (MD) of less than -22 dB, or in the absence of visual field testing, best-corrected visual acuity (BCVA) worse than 20/200 due 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. Non-advanced 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 as defined by IOP >21 mmHg or had pre-perimetric glaucoma with no glaucomatous field changes.

Advanced and non-advanced POAG cases recruited in the ANZRAG were screened for *MYOC* as previously described.[10] Glaucoma suspects who did not meet the advanced or non-advanced 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 counselling were offered to first-degree family members over the age of 18 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 patient at the time of their first presentation. All participants with *MYOC* variants were categorized into two 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). Participants' clinical parameters recorded at the time of their 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 four groups according to the severity of disease at the time of their first presentation: normal, glaucoma suspect, non-advanced glaucoma, and advanced glaucoma, as described above.

Data were analysed for all participants with *MYOC* disease-causing variants identified in the ANZRAG that satisfied inclusion criterion. BCVA was transformed in decimal fractions for analysis purposes. Due 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 analysed with PASW Statistics, Rel. 18.0.1.2009. Chicago: SPSS Inc. 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 (75%) participants included in the study. They consisted of 43 (59%) Clinical cases and 30 (41%) Genetic cases. 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 versus 47.5 ± 16.7 years, $P = 0.018$). At their first examination, 25 (83%) Genetic cases were unaffected and 5 (17%) were glaucoma suspect whereas among

Clinical cases 19 (44%) were glaucoma suspect, 12 (28%) had non-advanced glaucoma and 12 (28%) had advanced glaucoma (Figure 1). Among the Genetic cases, unaffected individuals were significantly younger compared to glaucoma suspects (42.5 ± 10.4 versus 55.8 ± 13.7 years, $P = 0.037$).

The mean highest IOP (17.6 ± 3.6 versus 32.2 ± 9.7 mmHg, $P < 0.001$), highest CDR (0.48 ± 0.13 versus 0.65 ± 0.27, $P = 0.006$), worst MD (-1.2 ± 1.2 versus -10.0 ± 10.3, $P < 0.001$), and worst BCVA (0.96 ± 0.30 versus 0.70 ± 0.38, $P = 0.004$) were all significantly less severe among Genetic cases compared with Clinical cases (Figure 2). The mean CCT was similar between the groups (561.3 ± 37.2 versus 538.7 ± 42.6, $P = 0.52$). Elevated IOP at presentation was recorded for 91% (39/43) of Clinical cases versus 10% (3/30) of Genetic cases. We conducted the same analyses including only one relative per family to account for the characteristics that individuals from the same family may share and obtained similar results (not shown).

Probands and siblings

We then analyzed separately the probands and their siblings, including 38 Clinical and 9 Genetic cases. The mean age at presentation was similar in both groups (48.29 ± 17.0 years Clinical versus 45.3 ± 15.2 years Genetic, $P = 0.401$). At presentation 16 were glaucoma suspect, 11 had non-advanced glaucoma and 11 had advanced glaucoma among Clinical cases, whereas 5 were unaffected and 4 were glaucoma suspect among Genetic cases.

The mean highest IOP (20.2 ± 3.2 versus 32.2 ± 10.0 mmHg, $P < 0.001$), highest CDR (0.46 ± 0.18 versus 0.66 ± 0.27, $P = 0.026$) and worst MD (-1.3 ± 1.1 versus -10.9 ± 10.4, $P = 0.017$) were all significantly less severe among Genetic cases compared with Clinical cases. Although not significant, the worst BCVA was also less severe in Genetic cases compared with Clinical cases (0.91 ± 0.27 versus 0.70 ± 0.39, $P = 0.128$). The mean CCT was significantly different between both groups (569.7 ± 29.6 Genetic versus 536.0 ± 42.8

Clinical). Elevated IOP was reported for 92% (35/38) of Clinical cases versus 22% of Genetic cases.

Probands and offsprings

Next, we analyzed probands and their offsprings, comprising 35 Clinical and 21 Genetic cases. The mean age at presentation was significantly lower among Genetic (41.7 ± 9.4 years) compared with Clinical cases (62.1 ± 17.1, $P = 0.002$). Among the Clinical cases, 14 were glaucoma suspect, 11 had glaucoma and 11 had advanced glaucoma at presentation whereas 20 Genetic cases were unaffected and 1 was a glaucoma suspect.

The mean highest IOP (16.5 ± 3.1 versus 32.2 ± 9.8 mmHg, $P < 0.001$), highest CDR (0.49 ± 0.11 versus 0.67 ± 0.26, $P = 0.004$), worst MD (-1.0 ± 1.0 versus -10.3 ± 10.0, $P < 0.001$) and worst BCVA (1.01 ± 0.31 versus 0.71 ± 0.38, $P = 0.003$) were all significantly less severe in Genetic cases compared with Clinical cases. The mean CCT was similar between both groups (553.5 ± 42.0 versus 533.5 ± 44.4, $P = 0.204$). Elevated IOP was recorded in 89% (31/35) of the Clinical cases compared with 5% (1/21) of the Genetic cases.

Carriers of *MYOC* p.Gln368Ter

Individuals with the p.Gln368Ter variant totaled 52 cases, 71% of the total study population. Of the 52 p.Gln368Ter cases, 28 (54%) were Clinical cases and 24 (46%) were Genetic cases. The mean current age was 68.4 ± 8.8 years (range 53-87 years) for Clinical cases and 44.7 ± 12.7 years (range 24-77 years) for Genetic cases. The mean age at presentation was significantly younger among Genetic cases compared with Clinical cases (40.5 ± 13.3 versus 55.0 ± 9.8 years, $P < 0.001$). Among Genetic cases, 19 were unaffected and 5 were glaucoma suspect at presentation whereas 12 Clinical cases were glaucoma suspect, 8 had non-advanced glaucoma and 8 had advanced glaucoma.

The mean highest IOP (18.0 ± 3.7 versus 29.9 ± 9.3 mmHg, $P < 0.001$), highest CDR (0.49 ± 0.14 versus 0.66 ± 0.27, $P = 0.016$), worst MD (-1.3 ± 1.2 versus -9.2 ± 10.0, $P = 0.010$), and worst BCVA (0.95 ± 0.29 versus 0.67 ± 0.41, $P = 0.009$) were all significantly less severe

among Genetic cases compared with Clinical cases with p.Gln368Ter (Figure 3). The mean CCT was significantly higher among Genetic cases compared with Clinical cases (569.4 ± 32.5 versus 530.1 ± 40.8, $P = 0.004$). Increased IOP at presentation was recorded for 86% (24/28) of Clinical cases versus 13% (3/24) of Genetic cases. Figure 4 shows higher IOP and lower MD with a later age at presentation for Clinical cases compared with Genetic cases.

Response to treatment

The IOP before and after treatment was available for 83% (35/42) of the glaucoma suspects and affected individuals included in the study who were on treatment. All individuals attained IOP within the normal range with IOP-lowering therapy. The mean highest IOP before treatment was 31.8 ± 1.4 mmHg (range 21-52 mmHg) versus 16.8 ± 0.4 mmHg (range 12-21 mmHg) after initiation of treatment ($P < 0.001$).

DISCUSSION

Glaucoma can lead to irreversible blindness if left untreated and often remains undiagnosed until substantial damage has occurred. It is crucial to identify at-risk individuals at the earliest opportunity because there are medical and surgical treatment options that are effective for slowing down the progression of or even preventing glaucoma from developing.[18,19] *MYOC* disease-causing variants exhibit a strong age-dependent penetrance and affected individuals present with more advanced disease if not identified and treated early.[10] Despite evidence supporting clinical validity and patient's acceptance for *MYOC* genetic testing,[20,21] there is a lack of outcome measures and evidence-based clinical utility for genetic testing for the monogenic forms of glaucoma. This study is the first to investigate the clinical utility of cascade genetic testing for *MYOC* by examining the clinical parameters at time of presentation of *MYOC* carriers.

We showed that patients identified via cascade genetic testing presented 7 years younger than those identified following ophthalmic referral. The majority (83%) of carriers identified

through genetic testing were asymptomatic at the time of presentation whereas half of the patients who had an ophthalmic referral had early signs of glaucoma and the other half already had glaucoma, including 28% with advanced disease. All clinical parameters related to glaucoma (IOP, CDR and MD on visual field test) were significantly worse at presentation among Clinical cases compared with Genetic ones.

We conducted separate analyses on probands/siblings and probands/offsprings to evaluate whether the age difference affected our findings. As expected, the age at presentation was significantly younger in Genetic cases compared with Clinical cases within the probands/children group whereas the age at presentation was similar between Clinical and Genetic cases within the probands/siblings group. There were fewer siblings than offsprings in the Genetic group, which can be explained by a proportion of siblings already affected by glaucoma and not identified through genetic testing. In both analyses, the clinical parameters associated with glaucoma were significantly less severe in Genetic cases compared with Clinical cases. Forty-four percent of the siblings were identified as glaucoma suspect following genetic testing results. However the siblings in the Genetic group had better glaucoma parameters than the probands, despite the fact that they presented at a similar age than the probands and that almost half of them had early signs of glaucoma. These findings highlight the usefulness of cascade genetic testing irrespective of the age of the family members.

Genotype-phenotype correlations have been well described for *MYOC* variants.[10-12] In order to reduce the variability accounted for by disease-causing variants of different severity, we analysed individuals carrying only the most common *MYOC* variant (p.Gln368Ter) separately. p.Gln368Ter is usually associated with a moderate severity and displays an age-related penetrance with half of the carriers being diagnosed with glaucoma by 50 years of age and almost all carriers diagnosed by 75 years of age.[13] When considering p.Gln368Ter carriers only, individuals diagnosed early because of more severe *MYOC* variants are

excluded as shown by the older age at presentation among p.Gln368Ter carriers. Our results showed that p.Gln368Ter carriers identified through genetic testing presented 15 years younger than those who presented clinically. They also show better clinical parameters at presentation as illustrated by lower IOPs, CDR and MD on visual field test than their clinically diagnosed counterparts. Glaucoma suspects identified by ophthalmic presentation were on average in their early 50s, which is in accordance with the age-related penetrance for this variant. Unaffected individuals identified through genetic testing were on average in their late 30s (37.1 ± 2.5 years), an age group where a minority of p.Gln368Ter carriers are affected. This shows the ability of cascade genetic testing to identify gene carriers before they exhibit symptoms of the disease.

Among the individuals carrying variants other than p.Gln368Ter, some had a more severe disease with an early age of glaucoma onset. In these families, we would expect cascade genetic testing to have similar positive outcomes if conducted at an early age, and we have previously discussed the benefits of a genetic testing approach for minors in these families.[23] Our numbers were too small to analyze this group separately in this study but future studies should examine the clinical utility of genetic testing in individuals carrying *MYOC* variants associated with early glaucoma onset. Similarly, our findings could be extrapolated to other monogenic rarer forms of the disease such as *Optineurin* and *TBK1* glaucoma associated variants. However the utility of a genetic testing approach is currently less clear in the complex and more common forms of glaucoma that are the result of multiple genetic factors with small effect size.

Through our cascade testing program, we make genetic testing available to all first-degree relatives but we do not contact relatives directly to promote autonomous and noncoercive decisions. This approach yields a response rate of 50% which is similar to other adult-onset conditions with treatment options and high penetrance genes such as inherited cancers and cardiomyopathies.[24,25] Individuals with a family history are more likely to access screening

for glaucoma.[26] However, in our cohort 79% (33/42) of individuals who presented clinically had a family history, including 67% (16/24) who presented with glaucoma. This suggests that family history may not be enough of a risk factor to diagnose at-risk individuals early. Additionally, we previously showed that the majority of newly identified *MYOC* carriers had never seen an eye specialist,[10] supporting genetic testing as an effective way to identify at-risk individuals in *MYOC* families in a more timely manner.

In this study, 25 individuals had no signs of glaucoma on examination following genetic results. These individuals were significantly younger than those identified as glaucoma suspects following genetic results. *MYOC* variants are highly penetrant: Age-related penetrance is complete at 50 years old for *MYOC* variants associated with an early age of onset[9,10,14,15] and almost complete at 75 years old for the common p.Gln368Ter variant.[13] Therefore, these unaffected individuals are expected to develop glaucoma at some stage. Interestingly, we are aware of two individual who subsequently converted to glaucoma suspect in the Genetic group on follow-up. Long-term studies that follow at-risk asymptomatic individuals are still needed to assess clinical outcomes, the progression of the disease and the best treatment strategies for *MYOC* carriers.

Cascade genetic screening for glaucoma is a promising avenue to prevent glaucoma blindness. A previous study demonstrated the acceptability of predictive genetic testing for *MYOC* glaucoma.[20] Data from the ANZRAG have recently shown that families perceived strong benefits to cascade testing as it leads to the possibility of preventive measures.[21] We have previously shown that *MYOC* disease-causing variants are more prevalent in the advanced stages of glaucoma.[10] As a result early diagnosis is important as carriers may require earlier interventions and more aggressive management of their IOP. Our findings also confirm that *MYOC* carriers respond to IOP-lowering therapy. Personalized medicine using genetic information to predict disease development and to tailor preventative interventions for each patient is an evolving field.[27] Although current glaucoma therapies are effective in

lowering IOP in patients with *MYOC* disease-causing variants, targeted therapies for *MYOC* glaucoma are emerging; studies have shown a reduction in the glaucomatous phenotype of *MYOC*-transgenic mice treated with topical ocular sodium 4-phenylbutyrate[28] and *MYOC*-transgenic mice with CRISPR-Cas9 mediated genome editing (Jain A, Zode G, Buge K, et al. CRISPR-Cas9 mediated genome editing of *Myocilin* in hereditary glaucoma. Presented at ASHG Annual Meeting, October 7, 2015; Baltimore). The identification of *MYOC* carriers will become even more important with the development of therapies targeted for *MYOC* glaucoma.

This study has some potential limitations. First, there might be a recruitment bias as patients who are more likely to have undiagnosed glaucoma are also the ones who will not seek genetic testing and are less likely to be screened.[21] The ANZRAG recruits individuals with both advanced and non-advanced POAG but has a recruitment bias toward more advanced disease, which could have resulted in an overestimation of the severity in the Clinical group. Second, this is a retrospective study and clinical details at the time of initial diagnosis were missing for 25% of participants with a *MYOC* variant. Many of them had been diagnosed decades ago, and as such, records of the initial presenting details no longer existed or were irretrievable. However, a randomized clinical trial to study the efficacy of genetic testing for glaucoma leading to better visual outcome would be impossible to conduct. So although a retrospective study collecting clinical evidence has limitations, this is the first study to report such findings.

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Figure legends:

Figure 1: Diagram of the study showing the number of participants in the Clinical and Genetic groups and their glaucoma status at first presentation. Clinical cases were referred by their general practitioner or optometrist, and Genetic cases were referred following genetic test results.

Figure 2: Comparison of the clinical characteristics between Clinical and Genetic cases with a *MYOC* variant. IOP: Intraocular pressure, CDR: cup-to-disc ratio, MD: mean deviation from a reliable visual field test. ** $P \leq 0.01$, *** $P \leq 0.001$

Figure 3: Comparison of the clinical characteristics between Clinical and Genetic cases with the p.Gln368Ter *MYOC* variant. IOP: Intraocular pressure, CDR: cup-to-disc ratio, MD: mean deviation from a reliable visual field test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure 4: Clinical details in relation to the age at presentation between Clinical and Genetic cases with the p.Gln368Ter *MYOC* variant. IOP: Intraocular pressure, MD: mean deviation from a reliable visual field test.