

## Myocilin Mutations in a Zambian Population Attending Selected Referral Eye Health Facilities

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**Abstract: Purpose:** Research has shown that primary open-angle glaucoma (POAG) in Zambia has an earlier age of onset and is more clinically severe than in Europe and the United States of America. Mutations of Myocilin have been reported to be associated with POAG in multiple populations. We therefore, investigated the role of myocilin gene mutations in Primary Open Angle Glaucoma in a Zambian population.

**Methods:** The unrelated POAG patients and unaffected controls seen at the University Teaching Hospitals Eye Hospital, Kitwe Teaching Eye Hospital and Lusaka Eye Hospital were recruited for this study. Glaucoma specialists from the participating institutions ascertained all POAG and control patients. Age, sex and ethnicity matched unaffected controls were obtained in patients with an IOP < 22 mm Hg, clear ocular media and normal-appearing optic nerve heads. A complete eye examination, including visual field assessment, was performed in all cases. Genomic DNA was extracted from whole peripheral blood, then subjected to polymerase chain reaction to amplify exons, flanking introns and promoter regions of the myocilin gene. The amplified products were screened for base mutations by autosequence based on the Sanger method. The study used the chi square test and conditional logistic regression to compare the cases and controls. Identified mutations were compared to known myocilin mutations. **Results:** Unrelated 165 POAG patients and unaffected 173 controls enrolled for the study. The analysis revealed four variants of myocilin mutations in 49 participants which included one synonymous (silent) mutation (Thr474Thr; 45/338) and three missense mutations (Ala446Thr; 16/338), (Leu158Arg; 4/338) and (Arg342Lys; 1/338). The prevalence of myocilin (MYOC) gene mutations in this study was 14.5% (49/338). The study observed two previously reported mutations, Ala446Thr and Arg342Lys, as glaucoma causing mutations. The variant (Lys158Arg) observed in the study was a novel finding. These mutations were detected in age, sex and ethnically matched controls. The missense mutation, Ala446Thr, was found in eight cases and eight controls. Twenty (20) controls and 25 cases had the synonymous or silent (neutral) mutation, (Thr474Thr). **Conclusions:** The myocilin mutations represent a

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prevalence of 14.5 in a Zambian population. The Leu158Arg seems to represent novel glaucoma, causing missense mutation. Mutations in myocilin appear to play a big role in the pathogenesis of POAG in a Zambian population.

**Keywords:** Myocilin, Mutation, Primary Open Angle Glaucoma, Zambian population, Sanger method.

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## INTRODUCTION

Glaucoma is a group of non-communicable heterogeneous disorders that cause progressive apoptosis of retinal ganglion cells leading to optic nerve degeneration, excavation and corresponding visual field defects (VFD) [1, 2]. Glaucoma is asymptomatic and as such, most of the time, the patients are unaware of the disease [2]. It is usually discovered during the evaluation for other eye conditions or when the disease is advanced [3, 4]. The disease eventually leads to visual impairment and blindness.

Glaucoma may be classified based on the anterior chamber angle as Primary Open Angle Glaucoma (POAG) or Primary Congenital Glaucoma (PCG) or Primary Angle Closure Glaucoma (PACG) [5]. In a study conducted in Zambia at the University Teaching Hospital (UTH) eye department in 2013, the prevalence of POAG was remarkably higher (19.0%) than what has been reported in African-derived persons in East Baltimore, Barbados and other African indigenous surveys in West, East and Southern Africa [6]. The high prevalence in this study was attributed to the early onset of POAG which was noticed in participants aged 20 to 39 years and also due to the fact that this was a Hospital based study. These findings still gave an idea on how the glaucoma problem was a big problem in the Zambian setting.

Genetic mutations and central corneal thickness are other risk factors for POAG still being explored [7]. Juvenile-Onset Open-Angle Glaucoma (JOAG) is said to be a rare subset of POAG. In a study conducted at the UTH, JOAG prevalence was found to be 8.6% in the black Zambian population, suggesting that JOAG is a common condition in the black Zambian people [8]. Genetic risk factors are known to contribute to POAG, although it has a complex inheritance pattern that confounds many approaches used to study Mendelian traits [9].

Fifteen (15) POAG-associated loci and over 70 unique mutations [10] have been identified with many of these mutations being specific to a single population or ethnic group [9-13]. Within the loci several candidate genes have been identified including myocilin (MYOC) [14], optineurin [15], WD

repeat-containing protein 36 (WDR36) [16], and cytochrome p450 1B1 [17]. Of all these mutations, MYOC (MYOC; accession identifier NM\_000261) has been found to harbour more glaucoma-causing mutations than any other identified risk gene [10,18], with over 80 mutations identified in different populations [19,20].

MYOC is composed of 3 exons. The majority of myocilin mutations are missense variants located in the third exon in the olfactomedin-like domain [21, 22]. Despite extensive research, it remains unclear how myocilin mutations lead to glaucoma [23, 24].

He *et al.* (2009) however, reported that MYOC interacts with trabecular meshwork (TM) cells through the overexpression of Pro370Leu mutant MYOC which renders TM vulnerable to cellular injury and death [25]. So far MYOC mutations have an overall frequency of 2%-5% in all populations worldwide, but mostly in Caucasian populations [20, 24, 25]. A study conducted in the UK on Caucasians showed a prevalence of 2.2% [26]. Two studies conducted on African American population have reported frequency of 1.4% and 2.6% of probable MYOC disease-causing mutations [27, 28]. In African populations, probable disease-causing myocilin mutations were found in 1.75% of Moroccan POAG subjects and 4.4% of Ghanaian and South African POAG subjects [29-31]. Current data on sub-Saharan Africa is limited to two studies conducted on West and South Africans [30, 31].

As Africa has more genetic diversity than anywhere else on earth [32], Novel MYOC mutations were likely to be found in sub-Saharan Africa populations. The studies conducted in Ghana and South Africa by Challa *et al.* (2002) and Whighan *et al.* (2011) respectively, demonstrated the diversity by reporting novel glaucoma causing mutations not reported in any Caucasians cases [30,31]. The synonymous mutations have been reported in smaller frequencies (less than 1.0%) in the studies conducted so far.

No glaucoma genetic studies have been reported in the Zambian population. Our study attempted to establish the contribution of myocilin

mutations to POAG in the Zambian population. It also tried to identify novel mutations for future functional work and genetic screening. Therefore, we investigated the role of myocilin mutations in POAG in a Zambian population.

## METHODS

All participants gave written informed consent for participation in the study. Permission to carry out the study was obtained from the Zambia Ministry of Health and the facilities namely University Teaching Hospitals Eye Hospital, Kitwe Teaching Eye Hospital and Lusaka Eye Hospital. This study adhered to the tenets of the Declaration of Helsinki. The University of Zambia Biomedical and Research Ethics Committee (UNZABREC) reviewed and granted authority to conduct the research.

This was a case-control study. The cases and controls were matched for age, gender and ethnicity. The age difference needed to be  $\pm$  two years old, because this difference could not have anatomical differences between the matched participants. For the purposes of the study, ethnicity was categorised based on the officially recognised seven national groupings of Tonga, Nyanja, Lozi, Kaonde, Luvale, Lunda and Bemba. For each participant, this was determined by the language their mother spoke.

Subjects with POAG were unrelated and met the inclusion criteria of: 1) age of equal to or greater than 18 years; 2) glaucomatous optic neuropathy in at least one eye; and 3) Visual Field (VF) loss consistent with optic nerve damage in at least one eye. Glaucomatous optic neuropathy was defined as a cup-to-disc ratio greater than 0.7 or focal loss of the nerve fibre layer resulting in a notch, associated with a glaucomatous Visual Field Defect (VFD). Optic disc asymmetry  $\geq 0.2$ , or notching or focal thinning of the optic disc rim were among other features considered in the diagnosis of glaucoma. Visual Fields were performed using standard Humphrey's

Visual Field (HVF) perimetry. The anterior chamber angle in all the POAG cases was measured using gonioscopy. Intraocular Pressure (IOP) was measured by applanation tonometry. The exclusion criteria for POAG subjects included the diagnosis of a secondary form of glaucoma or a history of ocular trauma. The normal controls were recruited specifically for this study and the visual field was part of the eye exam. The control subjects were unrelated and met the criteria of: 1) no first degree relative with glaucoma; 2) applanation IOP less than 22 mmHg in both eyes without treatment; 3) no evidence of glaucomatous optic neuropathy in either eye and 4) normal VF in both eyes. All the participants presented as outpatients and underwent a thorough examination by an experienced ophthalmologist.

After the clinical examination, 5 ml peripheral blood was obtained using peripheral venepuncture from all of the 338 participants and DNA was extracted using the QIAGEN MINI COLUMN Kit per the manufacturer's instructions at the University Teaching Hospitals Trogan laboratory. Extracted DNA was kept in at least 5 aliquots and stored at  $-80^{\circ}\text{C}$  for sequencing later. DNA concentrations were measured using a NanoDrop 1000 spectrophotometer and normalised to 10 ng/ml then dispatched on ice for storage at  $-80^{\circ}\text{C}$  at the University of Zambia, School of Veterinary Medicine, and Department of Disease Control Virology Molecular Laboratory within Lusaka for MYOC gene screening. The gel electrophoresis and spectrophotometry determined the quality and quantity of purified genomic DNA, respectively.

Primers flanking the entire coding sequence of MYOC were procured from the Commercial Laboratory in the United Kingdom (UK). Table 1 below provides a list of the primers that were used in the study to determine the role of POAG genes in the Zambian population.

**Table-1: List of PCR Primers for MYOC (Myocilin) Exon Sequencing in a Zambian population**

| Myocilin Exon | Forward primer sequence | Reverse primer sequence | PCR product size (bp) | Covered genomic region       |
|---------------|-------------------------|-------------------------|-----------------------|------------------------------|
| Exon 1a       | ATCTTGCTGGCAGCGTGAA     | TCTCTGGTTTGGGTTTCC      | 614                   | chr1:171,621,342–171,621,955 |
| Exon 1b       | GACAGCTCAGCTCAGGAAGG    | GAAGGTGATCGCTGTGCTTT    | 663                   | chr1:171,620,991–171,621,653 |
| Exon 2        | AGCAAAGACAGGGTTTCACC    | AGGGCTTTGTTAGGGAAAGG    | 554                   | chr1:171,607,517–171,608,071 |

The targeted region covered at least 80 base pairs into each intron to screen for potential mutations affecting exon splicing. Dream Taq DNA polymerase was used for all the polymerase-chain

reactions (PCR). The PCR amplifications were performed in Applied Biosystems Veriti 96 Well Thermal Cycler by Life Technologies Model number 9902 made in Singapore in 2015. Completed PCR

reactions were purified and sequenced using the Sanger method. Sequencing was performed in a Hitachi Applied Biosystems 3500 genetic analyser model 622-0010 of 2016. The raw sequence data obtained was edited using the Genetyx version 12 software package. After that, blasting and genetic analysis and were performed based on MYOC sequence (GenBank NM\_000261) from the NCBI. Sequence alignment of the MYOC wildtype and mutant sequences was done using Clustal Omega. The Expasy Bioinformatics Resource Portal was used to translate the mutant MYOC to the protein sequence.

Statistical analysis: Chi-square test was applied to test the association of MYOC alleles and other variables namely sex, age group, MYOC mutations, family history of glaucoma, ethnicity and area of residence. Odds ratio (OR) had corresponding 95% confidence intervals and p-values were applied to measure association with age, sex, CCT, MYOC mutations, positive family history (exposure) and POAG (outcome). The OR was used to compare the odds of genetic mutation expression and having POAG. The disease severity at presentation using a worse eye VFD and with

binocular field defects at presentation was analysed separately using chi square or Pearson's correlation. The case-control confounders were ruled out using the conditional bivariate. Conditional multiple logistic regressions was applied to test the association between the presence of POAG and the variables (genetic mutation, age, sex, history of glaucoma).

## RESULTS

A total of 165 cases and 173 controls participated in the study. The cases and controls were matched for age, sex and ethnicity. The male participants made up 51.2% and females 48.8%. The participants spoke the languages including Tonga, Nyanja, Lozi, Kaonde, Luvale, Lunda and Bemba. The distribution of languages was similar in cases and controls. The Bemba speaking people were the majority (48.2%), followed by the Nyanja speaking (21.3%), then Tonga speaking (13.6%) and the least was the Lunda speaking (2.9%). In terms of age group, the participants aged below 40 years of age made up 31.4%, and these above 65 years represented 26.6%. Table 2 below shows participants' demographics.

**Table-2: Socio-demographic characteristics of the case-control study participants at the UTHs EH, KTEH, and LEH, n = 338**

| Variable         | Cases<br>% (N <sup>o</sup> ) | Controls<br>% (N <sup>o</sup> ) | Total Number of Participants (%) |
|------------------|------------------------------|---------------------------------|----------------------------------|
| <b>GENDER</b>    |                              |                                 |                                  |
| Male             | 50.9 (84)                    | 51.4 (89)                       | 173 (51.2)                       |
| Female           | 49.1 (81)                    | 48.6 (84)                       | 165 (48.8)                       |
| <b>AGE GROUP</b> |                              |                                 |                                  |
| <40              | 30.3 (50)                    | 32.4 (56)                       | 106 (31.4)                       |
| 40-44            | 8.5 (14)                     | 7.5 (13)                        | 27 (8.0)                         |
| 45-49            | 5.5 (9)                      | 5.2 (9)                         | 18 (5.3)                         |
| 50-54            | 9.7 (16)                     | 9.8 (17)                        | 33 (9.8)                         |
| 55-59            | 7.3 (12)                     | 6.9 (12)                        | 24 (7.1)                         |
| 60-64            | 12.1 (20)                    | 11.6 (20)                       | 40 (11.8)                        |
| ≥65              | 26.7 (44)                    | 26.6 (46)                       | 90 (26.6)                        |
| <b>ETHNICITY</b> |                              |                                 |                                  |
| Tonga            | 13.9 (23)                    | 13.3 (23)                       | 46 (13.6)                        |
| Nyanja           | 21.8 (36)                    | 20.8 (36)                       | 72 (21.3)                        |
| Lozi             | 4.2 (7)                      | 4.0 (7)                         | 14 (4.1)                         |
| Kaonde           | 3.8 (6)                      | 3.5 (6)                         | 12 (3.6)                         |
| Luvale           | 6.1 (10)                     | 6.4 (11)                        | 21 (6.2)                         |
| Lunda            | 3.0 (5)                      | 2.9 (5)                         | 10 (3.0)                         |
| Bemba            | 47.3 (78)                    | 49.1 (85)                       | 163 (48.2)                       |
| <b>TOTAL</b>     | <b>100 (165)</b>             | <b>100 (173)</b>                | <b>338 (100)</b>                 |

The POAG cases had a mean age of  $51.3 \pm 17.9$  years, while the mean age for the controls was  $55.3 \pm 15.8$  years. Among the study cases, 84 (50.9%) were males and 81 (49.1%) were females (Table 2). There was no statistical difference between the two sexes. Among the study controls, 84 (48.6%) were males, and 89 (51.4%) were females (Table 2).

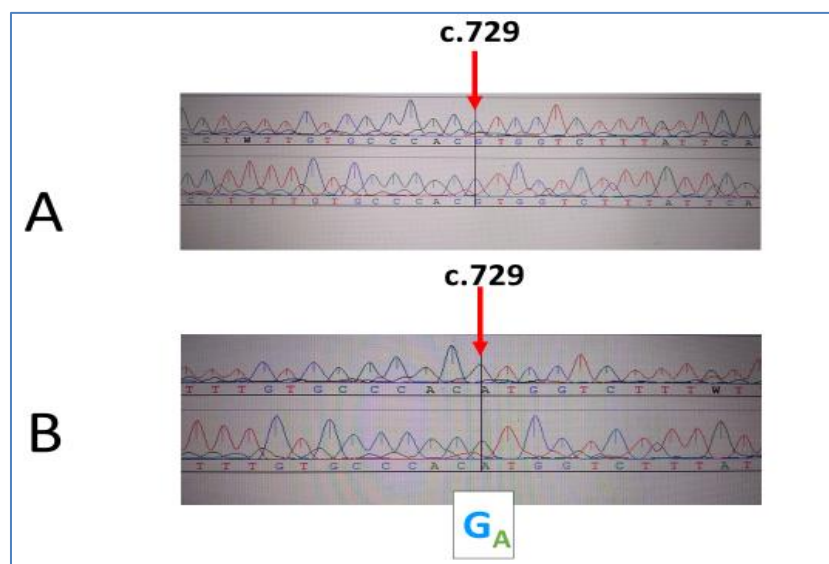
The analysis revealed four polymorphisms of myocilin mutations in 49 participants of the 338,

which gave a prevalence of MYOC gene mutations in this study of 14.5% (49/338). The mutations included one silent mutation (Thr474Thr; 45/338) and three missense mutations (Ala446Thr; 16/338), (Leu158Arg; 4/338) and (Arg342Lys; 1/338). All the 16 participants with the missense mutation Ala446Thr (16/338), were part of the 45 who had a silent mutation. The codon usage in the mutation changed to codons code for different amino acids, as shown in Table 3 below.

**Table-3: List of Polymorphisms, Original Base, Mutation Base, Original and Mutated Codon as well as Original and Mutated Amino Acids**

| Nucleotide sequence change | Amino Acid Change (Polymorphism) | Original Base | Mutation base | Original (Normal) Codon | Mutated Codon | Original Amino Acid | Amino Acid Causing Mutation |
|----------------------------|----------------------------------|---------------|---------------|-------------------------|---------------|---------------------|-----------------------------|
| c.723G>A                   | Ala446Thr                        | Adenine       | Guanine       | GCA                     | ACA           | Alanine (Ala)       | Threonine (Thr)             |
| c.39T>G                    | Leu158Arg                        | Thymine       | Guanine       | CTG                     | CGG           | Leucine (Lys)       | Arginine (Arg)              |
| c.721G>A                   | Arg342Lys                        | Guanine       | Adenine       | AGA                     | AAA           | Arginine (Arg)      | Lysine (Lys)                |
| c.729G>A                   | Thr474Thr                        | Guanine       | Adenine       | ACA                     | ACG           | Threonine (Thr)     | Threonine (Thr)             |

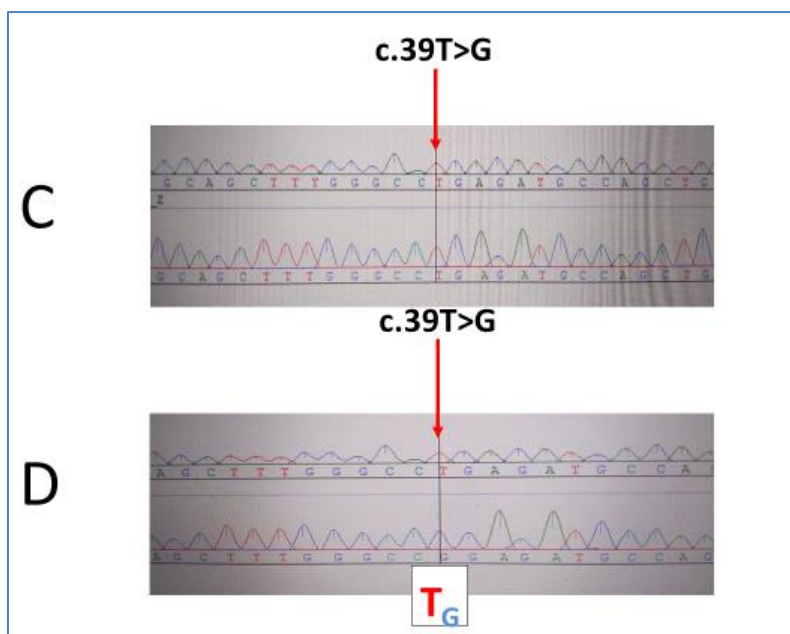
Figures 1 and 2 below shows the picture results of two of the four mutations identified.



**Fig-1: Mutation analysis of MYOC in 45 patients. MYO mutation c.729G>A. \*Genomic sequence of the MYOC gene from normal individuals A to mutated individual B at position 729, heterozygous c.729G>A, (Thr474Thr).**

All these changes, c.723G>A (Ala446Thr), c.39T>G (Leu158Arg), c.721G>A (Arg342Lys) and c.729G>A (Thr474Thr) appeared to be associated with glaucoma. Ala446Thr was observed in eight controls and eight cases, whereas Leu158Arg was observed four cases and not in the control participants. Both Arg342Lys and Ala446Thr were

observed in one control only. Leu158Arg, a novel missense mutation, was observed in four cases and no controls. Leu158Arg and Ala446Thr MYOC mutations appeared to cause a clinically distinguishable form of glaucoma when compared to cases that did not carry mutations in MYOC.



**Fig-2: Mutation analysis of MYOC in 4 patients. MYO mutation c.39T>G \*Genomic sequence of the MYOC gene from normal individuals C to mutated individual D at position 39, heterozygous c.39T>G, (Leu158Arg)**

The study identified, Thr474Thr, silent variant in 20 controls and 25 cases (Table 4). There was no statistical difference between the cases and controls when it came to the presence of Thr474Thr;  $p=0.342$ . Arg342Lys variant was found in no cases, but in one control. The variant c.729G>A was equally distributed among the controls, eight (50%) and the cases, eight (50%). Sixteen participants were found to carry multiple non-synonymous variants.

The 16 individuals carried Ala446Thr and Thr474Thr and one of the controls also Arg342Lys to make it three variants; Thr474Thr, Arg342Lys and Ala446Thr. In all, 7.7% (29/338) of the cases carried missense mutations (Leu158Arg, Arg342Lys and Ala446Thr). All these findings are illustrated in Table 4 below.

**Table-4: List of Coding Variants and Amino Acid Change Identified from MYOC Exon Sequencing in the Zambian Population, n = 49**

| Nucleotide sequence change | AA change | Reported pathogenicity | Observed in POAG (n=165) | Observed in controls (n=173) | Total |
|----------------------------|-----------|------------------------|--------------------------|------------------------------|-------|
| c.723G>A                   | Ala446Thr | Glaucoma causing       | 8                        | 8                            | *16   |
| c.39T>G                    | Leu158Arg | Novel                  | 4                        | 0                            | 4     |
| c.721G>A                   | Arg342Lys | Glaucoma causing       | 0                        | 1                            | *1    |
| c.729G>A                   | Thr474Thr | Novel                  | 25                       | 20                           | 45    |
| TOTAL                      |           |                        | 29                       | 20                           | 49    |

Note: \*The 16 and 1 individual were part of 45 participants who also had silent MYOC mutation. The 45 was the number Considered for counting so that there was no duplication in terms of numbers.

Of the 49 MYOC mutations, the cases had 29 (59.2%), and the controls had 20 (40.8%) (Table 5). The participants who were Lozi seemed to have a higher prevalence of 21.4% compared to the Luvale people who were the lowest at 4.8% (Table 5). There was a statistical difference ( $p=0.034$ ), of

prevalence within the ethnic groupings. The silent MYOC mutation (Thr474Thr) occurred in 45 participants (25 cases and 20 controls) and it manifested more in Bemba, Nyanja and Tonga ethnic communities (Table 5).

**Table-5: Myocilin Mutation in Cases and Controls Vs Ethnicity, n= 49**

| Variable         | Cases    |           |               | Controls |           |               | Total     | Prevalence within Ethnic Groups (%) |
|------------------|----------|-----------|---------------|----------|-----------|---------------|-----------|-------------------------------------|
|                  | MYOC 1a  | MYOC 2    | MYOC 2 silent | MYOC 1a  | MYOC 2    | MYOC 2 silent |           |                                     |
| <b>ETHNICITY</b> |          |           |               |          |           |               |           |                                     |
| Tonga            | 1        | 3         | 5             |          | 2         | 3             | 9         | 19.6 (9/46)                         |
| Nyanja           | 2        | 2         | 9             |          | 3         | 3             | 14        | 19.4 (14/72)                        |
| Lozi             |          | 1         | 2             |          | 0         | 1             | 3         | 21.4 (3/14)                         |
| Kaonde           |          |           | 2             |          | 0         |               | 2         | 16.7 (2/12)                         |
| Luvale           |          | 1         | 1             |          | 0         |               | 1         | 4.8 (1/21)                          |
| Lunda            |          |           | 1             |          | 0         | 1             | 2         | 20.0 (2/10)                         |
| Bemba            | 1        | 1         | 5             |          | 3         | 12            | 18        | 11.0 (18/163)                       |
| <b>TOTAL</b>     | <b>4</b> | <b>*8</b> | <b>25</b>     |          | <b>*8</b> | <b>20</b>     | <b>49</b> | <b>14.5 (49/338)</b>                |

Note: \*The eight individuals with MYOC 2 in cases and controls adding up to 16, were part of 45 participants who also had silent MYOC mutation. The 45 was the number considered for counting so that there was no duplication in terms of numbers.

The mutation Thr474Thr variant was observed in five ethnic groups and was not significantly over or underrepresented in any one group ( $p=0.406$ ).

In terms of sex, 28 (57.1%) of the mutations were in male cases, and 42.9% occurred in female

controls, Table 6. There was no statistical difference between the two male and female groups,  $p=0.800$ . The MYOC mutations were most prevalent in the participants below the age of 40 years, 16 (32.7%), followed by those above 65 years, 12 (24.5%). The lowest was the 45 – 49 years age group with two (4.1%) participants.

**Table-6: Myocilin Mutation in Cases and Controls versus Sex and Age Group (n = 49)**

| Variable                  | Cases       |            |                   | Controls    |            |                   | Total (%) |
|---------------------------|-------------|------------|-------------------|-------------|------------|-------------------|-----------|
|                           | Myocilin 1a | Myocilin 2 | Myocilin 2 silent | Myocilin 1a | Myocilin 2 | Myocilin 2 silent |           |
| <b>SEX</b>                |             |            |                   |             |            |                   |           |
| Male                      | 1           | 7          | 17                |             | 2          | 10                | 28 (57.1) |
| Female                    | 3           | 1          | 8                 |             | 6          | 10                | 21 (42.9) |
| <b>SUB-TOTAL</b>          | <b>4</b>    | <b>*8</b>  | <b>25</b>         |             | <b>*8</b>  | <b>20</b>         | <b>49</b> |
| <b>AGE GROUP IN YEARS</b> |             |            |                   |             |            |                   |           |
| <40                       | 3           | 1          | 6                 |             | 3          | 7                 | 16 (32.7) |
| 40-44                     |             | 0          | 1                 |             | 1          | 3                 | 4 (8.2)   |
| 45-49                     |             | 0          | 1                 |             | 1          | 1                 | 2 (4.1)   |
| 50-54                     | 1           | 0          | 2                 |             | 0          | 2                 | 5 (10.2)  |
| 55-59                     |             | 1          | 4                 |             | 0          | 0                 | 4 (8.2)   |
| 60-64                     |             | 0          | 2                 |             | 2          | 4                 | 6 (12.2)  |
| ≥65                       |             | 6          | 9                 |             | 1          | 3                 | 12 (24.5) |
| <b>SUB-TOTAL</b>          | <b>4</b>    | <b>*8</b>  | <b>25</b>         |             | <b>*8</b>  | <b>20</b>         | <b>49</b> |

Note: \*The eight individuals with Myocilin 2 in cases and controls adding up to 16, were part of 45 participants who also had silent MYOC mutation. The 45 was the number considered for counting so that there was no duplication in terms of numbers.

When conditional logistic regression (conditioned on age, sex, ethnicity and area of residence), was conducted CCT, IOP and family history were associated with POAG. The glaucoma cases with a positive history of glaucoma in the family were 108 times more likely to develop glaucoma, and this was statistically significant ( $p =$

0.008). The cases also had 1.697 chances of having raised intraocular pressure, and this was equally statistically significant (0.002). There was no statistical association between MYOC mutations and POAG ( $p=0.921$ ,  $p=0.700$  and  $p=0.992$ ). All these findings are illustrated in Table 7 below.

**Table-7: Conditional Logistic Regression to Determine Factors Associated with POAG in Cases and Controls (n = 338)**

| Variable             | Description  | OR      | 95%CI |          | p-value | AOR       | 95%CI |         | p-value |
|----------------------|--------------|---------|-------|----------|---------|-----------|-------|---------|---------|
|                      |              |         | Lower | Upper    |         |           | Lower | Upper   |         |
| Age in Years         |              | 0.978   | 0.812 | 1.177    | 0.813   | 0.446     | 0.207 | 0.962   | 0.040   |
| Ethnicity            | Tonga        | 65.289  | 0     | 6280     | 0.61    | 1         | -     | -       | -       |
|                      | Lozi         | 426.627 | 0     | 3201     | 0.471   | 1         | -     | -       | -       |
|                      | Luvale       | 65.289  | 0.001 | 5658     | 0.471   | 1         | -     | -       | -       |
|                      | Bemba (Ref)  |         |       |          |         |           |       |         |         |
| CCT                  |              | 0.997   | 0.992 | 1.003    | 0.3     | 0.962     | 0.932 | 0.992   | 0.014   |
| IOP                  |              | 1.493   | 1.293 | 1.724    | 0       | 1.697     | 1.221 | 2.358   | 0.002   |
| History of Glaucoma  | Yes          | 43      | 5.921 | 312.256  | 0       | 108.208   | 3.49  | 3354.97 | 0.008   |
|                      | No (Ref)     |         |       |          |         |           |       |         |         |
| MYOC 1a              | Present      | 65.289  | 0.021 | 2025.58  | 0.308   | 15.723    | 0     | 9.031   | 0.921   |
|                      | Absent (Ref) |         |       |          |         |           |       |         |         |
| MYOC 2               | Present      | 1.143   | 0.414 | 3.152    | 0.796   | 2.038     | 0.055 | 75.895  | 0.700   |
|                      | Absent (Ref) |         |       |          |         |           |       |         |         |
| MYOC 2 Silent        | Present      | 1.437   | 0.759 | 2.721    | 0.265   | 0.989     | 0.11  | 8.864   | 0.992   |
|                      | Absent (Ref) |         |       |          |         |           |       |         |         |
| Past Medical History | Hypertension | 65.289  | 2.788 | 1528.808 | 0.009   | 15650.166 | 0.001 | 4894    | 0.273   |
|                      | Diabetes     | 65.289  | 5.137 | 829.805  | 0.001   | 1695.154  | 0.002 | 1618    | 0.291   |
|                      | Tuberculosis | 65.289  | 0.001 | 565.749  | 0.471   | 8.152     | 0     | 7271    | 0.954   |
|                      | None (Ref)   |         |       |          |         |           |       |         |         |

Note: CCT = Central Corneal Thickness; IOP = Intraocular pressure; OR = Odds Ratio; AOR = Adjusted Odds Ratio  
CI = Confidence Interval

## DISCUSSION

The current data on sub-Saharan Africa POAG genetics is limited to two studies conducted on Ghana and South African [30, 31]. This first study in the Zambian population brings an addition to this pool of studies.

Myocilin occurs on locus GLC1A on chromosomal region 1q21-q31 consisting of three exons and encoding a 504 amino acid complex (peptide) [18]. The identified myocilin mutations have an overall frequency of 2% – 4% in all populations worldwide [32-34]. In this study, the prevalence was found to be 14.5%. The myocilin found in Zambia corresponded with that in NCBI. MYOC is composed of three exons.

In some African populations, probable disease-causing myocilin mutations exist in 1.75% of Moroccan POAG participants and 4.4% of Ghanaian and South African POAG participants [29-31].

This study observed probable glaucoma causing mutations suggesting the presence of this phenomenon in the general population. The finding

of this study was higher (14.5%) than that in African American studies at 2.6% and 1.4% by Fingert *et al.*, (1999) and Liu *et al.*, (2012) respectively [27,28]. However, extensive myocilin mutations studied in Caucasian populations have equally shown low frequency of myocilin mutations in adult-onset POAG participants of 2 to 5% [26, 35-37].

The genetic diversity in Africa is more than anywhere else on earth, making it the most likely environment for establishing novel MYOC mutations, especially in sub-Saharan Africa populations [31]. The studies conducted in Ghana and South Africa by Challa *et al.* (2002) and Whighan *et al.* (2011) respectively, reported novel glaucoma causing mutations different from those found in the Caucasians cases [30, 31]. In this study, a novel missense mutation, Leu158Arg, was observed in four cases and was absent in all the 173 controls. The study also identified Ala446Thr variant in 2.4% cases and 2.1% controls. Previously Ala446Thr was identified as occurring in controls only [27,28]. This study identified the mutation Ala446Thr for the first time in the cases. Eight cases and eight controls had Ala446Thr polymorphism.

The study identified one novel myocilin mutations located in exon 2. The silent (neutral) mutation, Thr474Thr, was found in exon 2. The Thr474Thr mutation catered for 91.83% of the prevalence of MYOC mutations in this study. Contrary to the reports by other researchers, another mutation, Ala446Thr, was found in exon 2. It is unclear why these two variants seemed overrepresented in controls, but it is conceivable that they appeared to play a significant role in POAG. Their presence in the controls could imply that the controls could be at risk of developing glaucoma at some point in their lives. The two (Thr474Thr and Ala446Thr) occurred in the same participants and had a very significant relationship. Wherever there was Thr474Thr, there was Ala446Thr. Chances of having Ala446Thr increased eight times in the presence of Thr474Thr. However, further studies are needed to either ascertain or dismiss this postulation. Although the frequency of several sequence variants differed between cases and controls, these differences were not statistically significant.

It is also not clear why the variant, c.729G>A (Thr474Thr), was overrepresented in the controls. The participants who were Lozi speaking seemed to have a higher prevalence of 21.4% compared to the Luvale speaking who were the lowest at 4.8%. There was a statistical difference of prevalence within the ethnic groupings. The novel mutation, Lys158Arg, was found in three ethnic groups. The results of this study, although on the higher side, agree with previous work that suggests MYOC is not related to population differences in POAG [38].

## CONCLUSION

A significant number of black

these observations further while paying particular attention in examining the possible occurrence of other new glaucoma genes and mutations as well as investigating environmental factors and gene-gene interactions that may contribute to the disease the Zambian population in general. In general, the presence of high POAG genetic burden in this population suggests the need to set up a functional surveillance system that combines both community and facility-based approaches to profile the epidemiology of glaucoma continuously as a barometer to inform and shape existing primary preventive ophthalmological strategies.

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