Global Academic Journal of Medical Sciences

Available online at www.gajrc.com
DOI: 10.36348/gajms.2022.v04i02.003

ISSN: 2706-9036 (P)
ISSN: 2707-2533 (0)

Original Research Article

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

Muma I. M. Kangwa[1-3*], Bailey Robin[4], Nyalazi I. M. Jessie[2], Zulu George[2], Malisawa-Mumba S. Tyness[2], Musonda - Chinama M. L. Lillian[5], Muma-Sibande Kachikonyo[2], Kaonga Patrick[6] and Simulundu Edgar[7,8]

[1]Department of Surgery, School of Medicine, University of Zambia, Lusaka, Zambia
[2]University Teaching Hospitals Eye Hospital, Lusaka, Zambia
[3]Department of Ophthalmology, School of Medicine and Clinical Sciences, Levy Mwanawasa Medical University, Lusaka, Zambia
[4]London School of Hygiene and Tropical Medicine, London, UK
[5]Eye Department, Levy Mwanawasa University Teaching Hospital, Lusaka, Zambia
[6]Tropical Gastroenterology and Nutrition Group (TROPGAN), Internal Medicine Department, University Teaching Hospitals – Adult Hospital, Lusaka, Zambia
[7]Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[8]Macha Research Trust, Macha, Choma, Zambia

***Corresponding Author**
Kangwa I. M. Muma
Department of Surgery, School of Medicine, University of Zambia, Lusaka, Zambia

Article History
Received: 22.01.2022
Accepted: 01.03.2022
Published: 04.03.2022

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

Citation: Muma I. M. Kangwa, Bailey Robin, Nyalazi I. M. Jessie, Zulu George, Malisawa-Mumba S. Tyness, Musonda - Chinama M. L. Lillian, Muma-Sibande Kachikonyo, Kaonga Patrick & Simulundu Edgar (2022). Myocilin Mutations in a Zambian Population Attending Selected Referral Eye Health Facilities. *Glob Acad J Med Sci*; Vol-4, Iss-2 pp- 45-55.

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.

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 frequencie 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 ± 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 Filed Defect (VFD). Optic disc asymmetry ≥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 Tropgan laboratory. Extracted DNA was kept in at least 5 aliquots and stored at – 80^{o}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^{0}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	Controls	Total Number of Participants (%)
	% (№)	% (№)	
GENDER			
Male	50.9 (84)	51.4 (89)	173 (51.2)
Female	49.1 (81)	48.6 (84)	165 (48.8)
AGE GROUP			
<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)
ETHNICITY			
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)
TOTAL	**100 (165)**	**100 (173)**	**338 (100)**

The POAG cases had a mean age of 51.3± 17.9 years, while the mean age for the controls was 55.3±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.

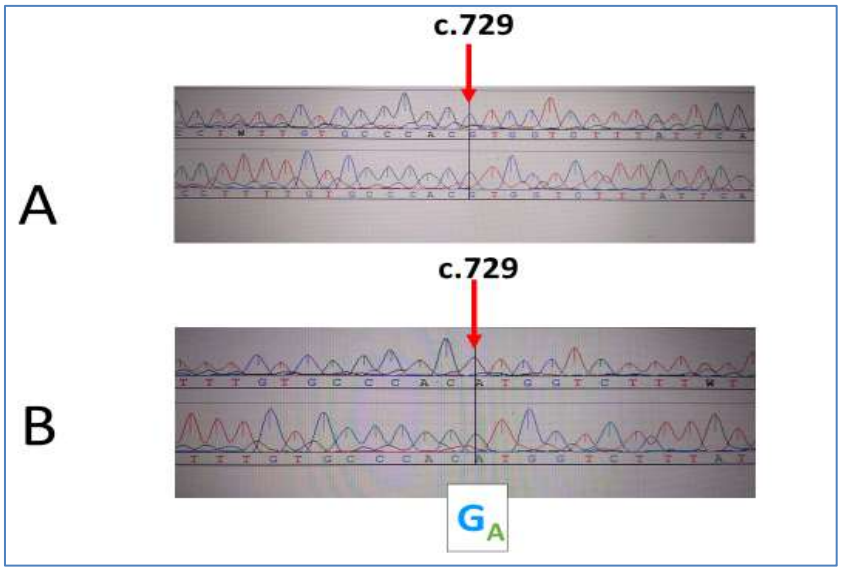

Fig-1: Mutation analysis of MYOC in 45 patients. MYO mutation c.729G>A. *Genomic sequence of the MYOC gene from normal individuals A to mutated individual B at position 729, heterozygous c.729G>A, (Thr474Thr).

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.

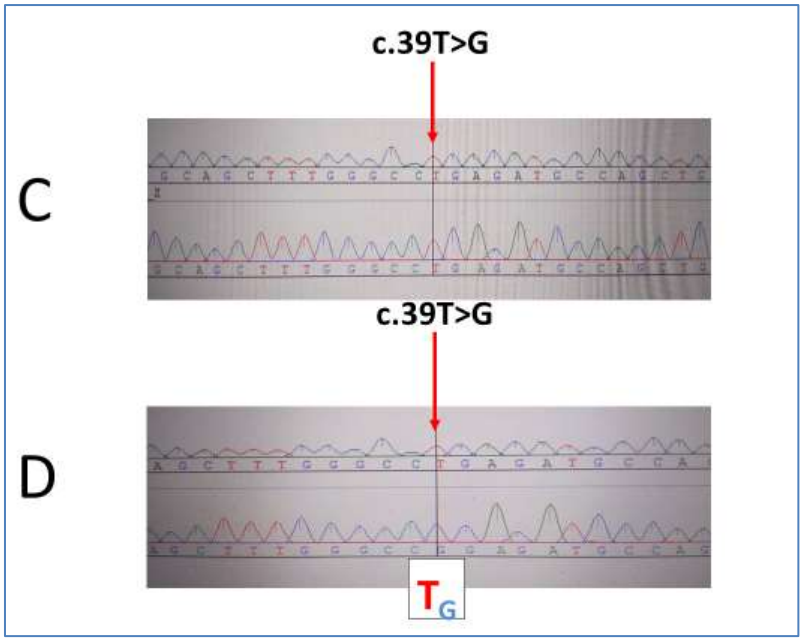

Fig-2: Mutation analysis of MYOC in 4 patients. MYO mutation c.39T>G *Genomic sequence of the MYOC gene from normal individuals C to mutated individual D at position 39, heterozygous c.39T>G, (Leu158Arg)

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		(%)
ETHNICITY								
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)
TOTAL	**4**	***8**	**25**		***8**	**20**	**49**	**14.5 (49/338)**

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	
SEX							
Male	1	7	17		2	10	28 (57.1)
Female	3	1	8		6	10	21 (42.9)
SUB-TOTAL	**4**	***8**	**25**		***8**	**20**	**49**
AGE GROUP IN YEARS							
<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)
SUB-TOTAL	**4**	***8**	**25**		***8**	**20**	**49**

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 Ala474Thr) 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]. Zambians, like other African groups, have a high prevalence of POAG compared to Caucasians. However, Zambians had a high percentage of MYOC mutations in this study and this could be the explanation of the high prevalence of POAG in the Zambian population [39].

CONCLUSION

A significant number of black Zambians (14.5%) carry MYOC mutations. This finding represents a high frequency. The Leu158Arg may represent novel glaucoma, causing missense mutation. This study has probably helped to establish the contribution of MYOC mutations to POAG in the Zambian population, which is a sub-Saharan population. It can also serve as a basis upon which future works can be developed in genetic screening in Zambia and identify novel mutations. The observed presence of significant burden of genetically associated POAG in the study population, suggest the presence of an unexplored public health challenge in Zambia. This burden may not be a random phenomenon which requires exploring 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.

ACKNOWLEDGEMENTS

The authors wish thanks the Tropical Gastroenterology and Nutrition Group (TROPGAN) team led by Prof. Paul Kelly for allowing us to use the laboratory facilities. The entire TROPGAN team (Prof. Kelly, Dr. Mpala Mwanza-Lisulo, Dr. Caroline Chisenga, Ms. Ellen Besa and Ms. Kanekwa Zyambo) made us feel at home in their laboratory.

We further wish to thank the School of Veterinary Medicine team led by Dr. Muso Munyeme for allowing me to use the lab facilities. The entire team (Dr. Musso Munyeme, Mr. Joseph Ndebe, Mr. Penjani Kapila, Mr. Propher Chimpala and Mr. Joseph Mwanza) made us complete the study. We will be failing in our duties if we did not acknowledge the support we received from the Ministry of Health (MoH) headquarters at Ndeke House. MoH granted us the authority to conduct the study at the University Teaching Hospitals – Eye Hospital and Kitwe Teaching Eye Hospital. We also appreciate the Lusaka Eye Hospital for allowing us to conduct the study at their facility.

REFERENCES

1. Quigley, H.A. (1996). Number of people with glaucoma worldwide. *Br J Ophthalmol*, 80:389-93. [PMID: 8695555]
2. Weinreb RN, Khaw, P.T. (2004). Primary open-angle glaucoma. Lancet 363(9422): 1711-1720.
3. Sommer, A., Tielsch, J. M., Katz, J., Quigley, H. A., Gottsch, J. D., Javitt, J., & Singh, K. (1991). Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans: the Baltimore Eye Survey. *Archives of ophthalmology*, *109*(8), 1090-1095.
4. Klein, B. E., Klein, R., Sponsel, W. E., Franke, T., Cantor, L. B., Martone, J., & Menage, M. J. (1992). Prevalence of glaucoma: the Beaver Dam eye study. *Ophthalmology*, *99*(10), 1499-1504.
5. Faiq, M., Sharma, R., Dada, R., Mohanty, K., Saluja, D., & Dada, T. (2013). Genetic,

biochemical and clinical insights into primary congenital glaucoma. *Journal of current glaucoma practice*, *7*(2), 66.
6. Muma, M. K. I., Bailey, R., & Michelo, C. (2018). Primary open angle glaucoma as seen at the University Teaching Hospital, Lusaka, Zambia. *The Journal of Ophthalmology of Eastern, Central and Southern Africa*, *22*(2).
7. Weih, L. M., Nanjan, M., McCarty, C. A., & Taylor, H. R. (2001). Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology*, *108*(11), 1966-1972.
8. Muma, K. I., Mboni, C., Mwale, C., Sibande–Muma, K., Nyalazi, J. I., Zulu, G., ... & Michelo, C. (2020). Juvenile-onset Open-Angle Glaucoma at the University Teaching Hospitals-Eye Hospital, Lusaka Zambia. *Medical Journal of Zambia*, *47*(2), 112-124.
9. Allingham, R. R., Liu, Y., & Rhee, D. J. (2009). The genetics of primary open-angle glaucoma: a review. *Experimental eye research*, *88*(4), 837-844.
10. Hewitt, A. W., Mackey, D. A., & Craig, J. E. (2008). Myocilin allele-specific glaucoma phenotype database. *Human mutation*, *29*(2), 207-211.
11. Gong, G., Kosoko-Lasaki, O., Haynatzki, G. R., & Wilson, M. R. (2004). Genetic dissection of myocilin glaucoma. *Human molecular genetics*, *13*(suppl_1), R91-R102.
12. Thylefors, B., Négrel, A. D., Pararajasegaram, R., & Dadzie, K. Y. (1995). Global data on blindness. *Bulletin of the world health organization*, *73*(1), 115.
13. Leske, M. C., Connell, A. M. S., Schachat, A. P., & Hyman, L. (1994). The Barbados Eye Study: prevalence of open angle glaucoma. *Archives of ophthalmology*, *112*(6), 821-829.
14. Wiggs, J. L., Damji, K. F., Haines, J. L., Pericak-Vance, M. A., & Allingham, R. R. (1996). The distinction between juvenile and adult-onset primary open-angle glaucoma. *American journal of human genetics*, *58*(1), 243.
15. Stone, E. M., Fingert, J. H., Alward, W. L., Nguyen, T. D., Polansky, J. R., Sunden, S. L., ... & Sheffield, V. C. (1997). Identification of a gene that causes primary open angle glaucoma. *Science*, *275*(5300), 668-670.
16. Rezaie, T., Child, A., Hitchings, R., Brice, G., Miller, L., Coca-Prados, M., ... & Sarfarazi, M. (2002). Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science*, *295*(5557), 1077-1079.
17. Monemi, S., Spaeth, G., DaSilva, A., Popinchalk, S., Ilitchev, E., Liebmann, J., ... & Sarfarazi, M. (2005). Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22. 1. *Human molecular genetics*, *14*(6), 725-733.
18. Fan, B. J., Wang, D. Y., Lam, D. S. C., & Pang, C. P. (2006). Gene mapping for primary open angle glaucoma. *Clinical biochemistry*, *39*(3), 249-258.
19. Hewitt, A. W., Sharma, S., Burdon, K. P., Wang, J. J., Baird, P. N., Dimasi, D. P., ... & Craig, J. E. (2008). Ancestral LOXL1 variants are associated with pseudoexfoliation in Caucasian Australians but with markedly lower penetrance than in Nordic people. *Human molecular genetics*, *17*(5), 710-716.
20. Fingert, J. H., Stone, E. M., Sheffield, V. C., & Alward, W. L. (2002). Myocilin glaucoma. *Survey of ophthalmology*, *47*(6), 547-561.
21. Jacobson, N., Andrews, M., Shepard, A. R., Nishimura, D., Searby, C., Fingert, J. H., ... & Sheffield, V. C. (2001). Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. *Human molecular genetics*, *10*(2), 117-125.
22. Zhou, Z., & Vollrath, D. (1999). A cellular assay distinguishes normal and mutant TIGR/myocilin protein. *Human molecular genetics*, *8*(12), 2221-2228.
23. Liu, Y., & Allingham, R. R. (2011). Molecular genetics in glaucoma. *Experimental eye research*, *93*(4), 331-339.
24. Fingert, J. H., Robin, A. L., Stone, J. L., Roos, B. R., Davis, L. K., Scheetz, T. E., ... & Stone, E. M. (2011). Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. *Human molecular genetics*, *20*(12), 2482-2494.
25. He, Y., Leung, K. W., Zhuo, Y. H., & Ge, J. (2009). Pro370Leu mutant myocilin impairs mitochondrial functions in human trabecular meshwork cells. *Molecular vision*, *15*, 815.
26. Ennis, S., Gibson, J., Griffiths, H., Bunyan, D., Cree, A. J., Robinson, D., ... & Lotery, A. (2010). Prevalence of myocilin gene mutations in a novel UK cohort of POAG patients. *Eye*, *24*(2), 328-333.
27. Fingert, J. H., Héon, E., Liebmann, J. M., Yamamoto, T., Craig, J. E., Rait, J., ... & Stoned, E. M. (1999). Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Human molecular genetics*, *8*(5), 899-905.
28. Liu, W., Liu, Y., Challa, P., Herndon, L. W., Wiggs, J. L., Girkin, C. A., ... & Hauser, M. A. (2012). Low prevalence of myocilin mutations in an African American population with primary open-angle glaucoma. *Molecular vision*, *18*, 2241.
29. Melki, R., Idhajji, A., Driouiche, S., Hassani, M. I., Boukabboucha, A., Akhayat, O., ... & Belmouden,

A. (2003). Mutational analysis of the Myocilin gene in patients with primary open-angle glaucoma in Morocco. *Ophthalmic genetics*, *24*(3), 153-160.

30. Challa, P., Herndon, L. W., Hauser, M. A., Broomer, B. W., Pericak-Vance, M. A., Ababio-Danso, B., & Allingham, R. R. (2002). Prevalence of myocilin mutations in adults with primary open-angle glaucoma in Ghana, West Africa. *Journal of glaucoma*, *11*(5), 416-420.
31. Whigham, B. T., Williams, S. E., Liu, Y., Rautenbach, R. M., Carmichael, T. R., Wheeler, J., ... & Allingham, R. R. (2011). Myocilin mutations in black South Africans with POAG. *Molecular vision*, *17*, 1064.
32. Jorde, L. B., Watkins, W. S., Bamshad, M. J., Dixon, M. E., Ricker, C. E., Seielstad, M. T., & Batzer, M. A. (2000). The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *The American Journal of Human Genetics*, *66*(3), 979-988.
33. Kwon, Y. H., Fingert, J. H., Kuehn, M. H., & Alward, W. L. (2009). Primary open-angle glaucoma. *New England Journal of Medicine*, *360*(11), 1113-1124.
34. Allingham, R. R., Liu, Y., & Rhee, D. J. (2009). The genetics of primary open-angle glaucoma: a review. *Experimental eye research*, *88*(4), 837-844.
35. Hulsman, C. A., de Jong, P. T., Lettink, M., Van Duijn, C. M., Hofman, A., & Bergen, A. A. (2002). Myocilin mutations in a population-based sample of cases with open-angle glaucoma: the Rotterdam Study. *Graefe's archive for clinical and experimental ophthalmology*, *240*(6), 468-474.
36. Kanagavalli, J., Krishnadas, S. R., Pandaranayaka, E., Krishnaswamy, S., & Sundaresan, P. (2003). Evaluation and understanding of myocilin mutations in Indian primary open angle glaucoma patients. *Mol Vis*, *9*, 606-14.
37. Faucher, M., Anctil, J. L., Rodrigue, M. A., Duchesne, A., Bergeron, D., Blondeau, P., ... & Raymond, V. (2002). Founder TIGR/myocilin mutations for glaucoma in the Quebec population. *Human molecular genetics*, *11*(18), 2077-2090.
38. Hoffman, E. A., Perkumas, K. M., Highstrom, L. M., & Stamer, W. D. (2009). Regulation of myocilin-associated exosome release from human trabecular meshwork cells. *Investigative ophthalmology & visual science*, *50*(3), 1313-1318.
39. Chamary, J. V., Parmley, J. L., & Hurst, L. D. (2006). Hearing silence: non-neutral evolution at synonymous sites in mammals. *Nature Reviews Genetics*, *7*(2), 98-108.