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Two Novel Single Nucleotide Polymorphisms in Myocilin Gene among Patients with Adult-Onset Primary Open Angle Glaucoma Indigenes of Rivers State, Nigeria

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Abstract
BACKGROUND: *Glaucoma is an optic neuropathy with characteristic visual field defects resulting from the gradual retinal ganglion cell death. It is the second commonest cause of blindness and the leading cause of irreversible blindness in the world. Several pathogenetic theories have been postulated but the genetic factor is gaining much ground. Mutation in myocilin gene is one of the culprits.* ***Objective:****To investigate the presence of myocilin gene mutation in adult-onset primary POAG subjectsof Rivers State.*
Methodology:*This was a case-control study. Three hundred and ninety-three adult-onset POAG patients attending the Glaucoma Clinic at the University of Port Harcourt Teaching Hospital were compared with 393 age and sex matched phenotypically normal non-glaucoma indigenes of Rivers State,between May and December 2021. Venous blood samples were obtained for genomic analysis from the study population. DNA wasextracted; amplified; with specific primers for myocilin using polymerase chain reaction. Single Nucleotide Polymorphism (SNPs) were detected after sequencing. Bioinformatic analyses were done with SMART software. SPSS Version 25 was employed for demographic and inferential statistics.* ***Results:*** *A total of 786participants aged ≥40 years were recruited. Mean age of the study population was 59.8 ± 11.8 years. The prevalence of mutation in the myocilin gene among POAG group was 8.4%. Four SNPs with missense mutations were identified and 2 of the SNPs are novel. The chromosomal locations of the SNPs in mutant myocilin gene were 171638779, 171638703, 171638610 and 171638608 in chromosome 1-GLC1A. Thymine replaced adenine in the novel variants with consequent missense mutation in the protein molecule - from anserine to valine which were registered with theNCBI-New York on 26/10/21(GenBank: OK632693.1).****Conclusion****: Two novel mutation locations (SNPs) were observed in chromosome 1: 171638779 and chromosome 1: 171638703. We recommend further investigations into their pathogenetic and clinical peculiarities among Africans.*

Keywords: *Myocilin gene mutation, Novel Single Nucleotide Polymorphisms, Adult-onset Primary Open Angle Glaucoma, Rivers State.*

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I. Introduction

Glaucoma is the second commonest cause of blindness after cataract and a leading cause of irreversible blindness; accounting for 0.3% of blindness in the world (Bourneet al., 2017; WHO, 2020). In Nigeria, it isthe leading cause of irreversible blindness and responsible for 15-20% of blindness (Abdullet al., 2009).

Several pathogenetic mechanisms have been postulated to explain the optic nerve damage that occurs in primary open -angle glaucoma. However, no single mechanism can adequately explain the great variations in susceptibility to damage and the patterns of damage seen in this disease. The etiology of POAG is likely to be multifactorial. Genetic, mechanical, vascular and other interwoven factors are said to influence individual susceptibility to optic nerve damage (Bowling, 2016).

Genetical predisposition has been shown to play an important role in the pathogenesis of POAG (Fan et al., 2010; Fingert, 2011). Many gene linkage-based studies have identified several genes with varying contributions to glaucoma including mutations in the myocilin,optineurin, WD repeat-containing protein 36, threonine protein binding kinase-1 (TANK-1), Amyloid beta precursor protein binding family B member 2 (Appbb2) genes(Allingham et al., 2009; Monemiet al., 2005; Liu et al., 2013).

The major genetic etiopathogenetic component of POAG among sub-Saharan African populations are still investigated, however, some recent studies have identified mutations inAPPbb -2 and myocilin proteins as culprits in adult onset POAG among individuals of African descent(Liu et al., 2013).

In the human genome, the simplest form of mutation is base changes, where one base is converted to another. Larger mutations include insertion of whole new sequences, often due to movements of transposable elements in the DNA or to chromosome changes such as inversions or translocations (https://medlineplus.gov/genetics/chromosome).

However, not all mutations cause change in the amino acid coded for (these are called silent or synonymous mutations). Mutations that do cause substitution sequence in amino acid are referred to as replacement mutations (missense mutations)(https://medlineplus.gov/genetics/chromosome). Some missense mutations have very large effects, while others have minimal or no effect (https://medlineplus.gov/genetics/chromosome).

Some forms of POAG are inherited by a primary point defect in a single gene (SNP). These cases of glaucoma have a simple genetic basis and are inherited in Mendelian patterns - as an autosomal dominant trait. Examples of SNPs in POAG are myocilin (MYOC), optineurin (OPTN), and TANK binding kinase 1 (TBK1). Each of these is capable of causing open angle glaucoma with little or no influence from other genes or environmental factors (Fingert, 2011; Miller et al., 2016).

However, there are other complex genetic cases of glaucoma caused by the combined action of many genetic and environmental risk factors. Such genetic factors increase the risk for developing glaucoma, but each are incapable of causing disease in isolation (Miller et al., 2016).

The identification of genetic etiology of POAG and its possible use as molecular marker will meet the much desirable goalof reliable screening tool, early diagnosis, efficient treatment modality and better visual prognosis for those at risk in the population.Achieving this goal will be an exciting event in sub-Saharan Africa where about 90% of POAG patients resides.

This study investigates the presence of myocilin gene mutation in adult-onset primary POAG subjects, indigenes of Rivers State, Nigeria.

II. Materials and Methods

This was a case-control study. Three hundred and ninety-three adult-onset POAG patients attending the Glaucoma Clinic at the University of Port Harcourt Teaching Hospital were compared with 393 age and sex matched phenotypically normal non-glaucoma indigenes of Rivers State, Nigeria between May and December 2021. Venous blood samples from were obtained for genomic analysis from the study participants. DNA wasextracted; amplified; with specific primers for myocilin using polymerase chain reaction. Single Nucleotide Polymorphism (SNPs) were detected after sequencing. Bioinformatic analyses were done with SMART software for protein domain structure prediction and MEGAX for evolutionary genetic analyses. SPSS Version 25 was employed for demographic and inferential statistics.

III. Results

Table 1: Sociodemographic characteristics of study subjects

Variables	Distribution in Adult onset POAG cases n=393		Distribution in Normal subjects n=393		Total	(%)	Chi-Square Value	p-Value
	(n)	(%)	(n)	(%)				
Gender								
Male	197	(25.1)	196	(24.9)	393	(50)		
Female	196	(24.9)	197	(25.1)	393	(50)		
Total	**393**	**(50)**	**393**	**(50)**	**786**	**(100)**		
Age Group (Years)								
40-49	91	(11.6)	91	(11.6)				
50-59	108	(13.7)	108	(13.7)				
60-69	117	(14.9)	117	(14.9)				
70-79	48	(6.1)	48	(6.1)				
80-89	29	(3.7)	29	(3,7)				
Total	393	(50)	393	(50)	786	(100)		
Mean age = 59.8±11.8		**Age Range 40 to 86 years**					**0.000**	**1.000**

The male to female ratio was 1:1, with a mean age in both groups of 59.8 ± 11.8 years, and an age range of 40 to 86 years. The modal age was 60-69 accounting for 14.9% of the study population in each of the two groups. The difference in the ages of the participants in the two groups was not statistically significant (p=1.000) [Table 1].

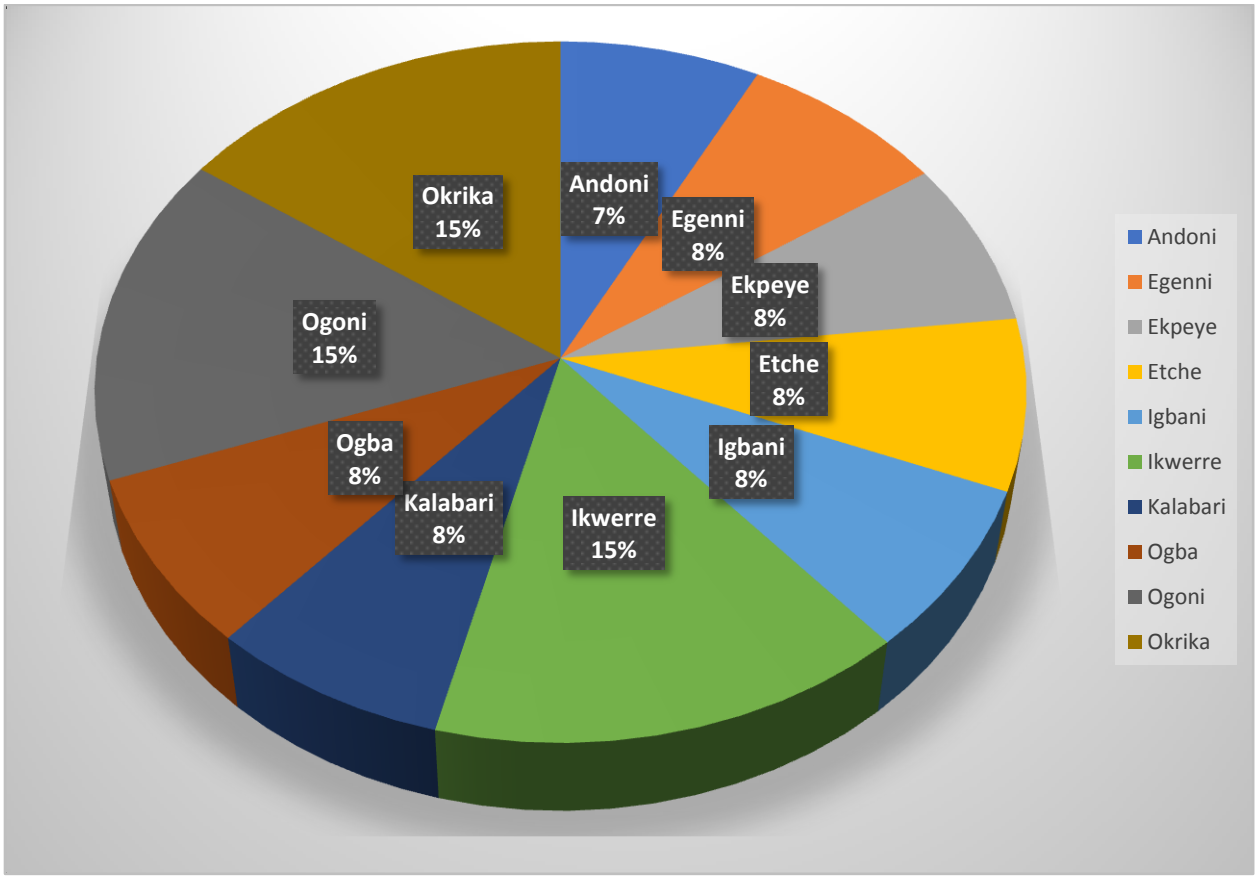

Figure 1: Ethnicity of the population studied

The participants of this study were from the following ethnic groups in Rivers State: Andoni, Ekpeye, Engenni, Etche, Igbani, Ikwerre, Kalabari, Ogba, Okrika, and Ogoni with equal number of participants represented from each participating LGA [Figure 1].

Table 2: Family History of Glaucoma of the Study Population

Positive Family History of Glaucoma	**N**	**(%)**	**N**	**(%)**	**Total**	**(%)**		
First Degree Relative	326	(41.5)	18	(2.3)	344	(43.8)		
Second Degree Relative	120	(15.3)	12	(1.5)	132	(16.8)		
Unaware	67	(8.5)	375	(47.7)	442	(56.2)		
Total	**393**	**(50.0)**	**393**	**(50.0)**	**786**	**(100)**	**T-test**	**p-value**
							573.993	**0.000**

Three hundred and twenty-six (n=326; 41.5%) of the subjects with adult-onset POAG had a positive family history of glaucoma in first degree relatives, while 15.3% had positive family history of glaucoma among second degree relatives; 8.5% were unaware of any family history of glaucoma among first- or second-degree relatives [Table 2]. Eighteen (n=18; 2.3%) of the respondents in the non-glaucoma group had a positive family history of glaucoma in first degree relatives and 1.5% had a positive family history of glaucoma among second degree relatives; 47.7% were unaware of any incident of glaucoma among their first- or second-degree relatives [Table 2].

Table3: Mutation Analysis of Single Nucleotide Polymorphisms (SNPs) in Myocilin Gene among the Study Population

S/N	Position in Genome	Mutation	POAG patients N (%)	Non-Glaucoma Subjects N (%)	Allelic Frequency Aden (%)	Thym (%	Consequences	Impact	Feature Type	Remark
1	Chrom 1: 171638779	A>T	13(3.3)	-	0.79	0.21	Missense Variant	Moderate	Transcript	Novel
2	Chrom 1: 171638703	A>T	6 (1.5)	-	0.74	0.26	Intron Variant	Moderate	Transcript	Novel
3	Chrom 1: 171638610	A>T	10 (2.5)	-	0.84	0.16	3 prime UTR variant	Moderate	Transcript	
4	Chrom 1: 171638608	G>A	5 (1.2)	9 (2.3)	0.88	0.12	Synonymous Variant	Low	Transcript	
	Total		**34(8.4)**	**9 (2.3)**			**p-value= 0.042**			

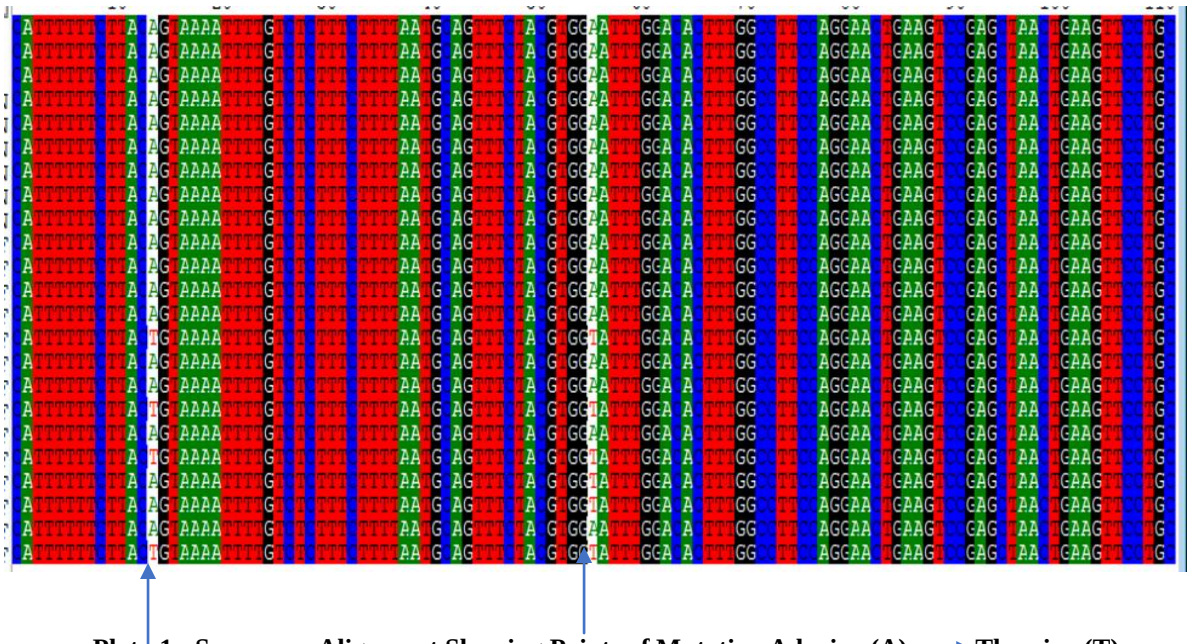

Plate-1: Sequences Alignment Showing Points of Mutation Adenine (A) ⟶ Thymine (T)

IV. Discussion

This study sets out to investigate the presence of myocilin gene mutation in adult-onset primary POAG subjects, indigenes of Rivers State. There were 393 cases of adult-onset POAG patients and 393 phenotypically normal non-glaucoma indigenes of Rivers State in the control group.

The age-gender distribution of the study participants is depicted in table 1. The mean ageof the study population in both groups was 59.8 ± 11.8 years. Adult-onset primary open-angle glaucoma occurs from the age of 40 years (Allen et al., 2015; Fan et al., 2010; Kyari et al., 2015; Awoyesuku et al., 2012). Working independently and in different periods of time, Murdoch et al., in a study among 1563 people of Hausa/Fulani ethnic extraction of Nigeria; reported that POAG was more prevalent in individuals aged 45 years and older (Murdoch et al., 2001) while Adeoye in South Western region of Nigeria observed that POAG was more prevalent in individuals aged 50 years and older; and that POAG accounted for 11.1% of blindness in Nigeria (Adeoye, 2001). These studies of Kyari et al., 2015; Awoyesuku et al., 2012; Murdoch et al., 2001 and Adeoye, 2001 were population-based studies with large sample sizes, independently done at different periods in similar socio-cultural background and similar results, thus lending credence to the assertion that adult-onset POAG occurs at age 40 years and older.

Corroborating with the findings of this work are the works of Leske et al., in the Barbados Eye Study which observed that adult-onset POAG was predominately in populations 45 years and older and that POAG significantly increases with age in all populations. Murdoch et al., postulated that older black populations may exhibit a tendency to present with more advanced POAG at diagnosis, including severe optic nerve cupping and extensive visual field loss. This position, however, needs further investigation in our locality in the Niger-Delta region of Nigeria.

In this work, we recruited equal participants aged 40 years and older of both sexes and age-matched populations of the same ethnic and socio-cultural background. This was deliberate as the study-design from the onset was intended to eliminate influences from differences in age, sex and racial identities in the two groups.

Moreso, it was intended to make the comparative groups as similar in characteristic features as possible, thereby achieving some level of homogeneity.

The participants of this study were from the following ethnic groups in Rivers State with similar socio-cultural background: Andoni, Ekpeye, Engeni, Etche, Igbani, Ikwerre, Kalabari, Ogba, Okrika, and Ogoni [Figure 1]. We recruited equal number of participants in the various local government areas giving a good and wide-spread of the study population - both riverine and upland communities. Ikwerre, Ogoni and Okrika ethnic groups had 2 Local Government Areas each; randomly selected at the second stage of our multi-stage random selection of the study participants. Etche, Abua/Odual and Ogba-Ndoni-Egbema Local Government Areas had 31 study participants while the other 10 Local Government Areas had 30 study participants each (3 most populous Local Government Areas in the sampling frame according to the 2006 projected census (National Population Commission 2006).

Many studies have identified positive family history of primary open angle glaucoma in black populations, as strongly correlated with risk of developing glaucoma reflecting heritability and/or shared environmental factors (Tielsch et al., 1994; Agbeja-Baiyeroju et al., 2003; Leske et al., 2008). This study showed that, three hundred and twenty-six (n=326;83%) of the subjects with adult-onset POAG had positive family history of glaucoma among first degree relatives while 30.5% had positive family history of glaucoma among second degree relatives. Eighteen (n=18; 4.6%) of the respondents in the non-glaucoma group had positive family history of glaucoma (first degree relatives) while 3.1% had positive family history of glaucoma among second degree relatives. This difference in the presence of POAG along family linage was found to be statistically significant (p=0.000) [Table 2]. First degree relatives are members of the nuclear family, but without spouses: parents, full sibling or child. First degree relatives share approximately 50% of their genes while second degree relatives include an individual's grandparents, grandchildren, uncles, aunts, nephews, nieces, and half-siblings (Ginsburg, 2008).

However, Awoyesuku et al., in the Port Harcourt Hospital Glaucoma Study, found that 9.6% of glaucoma patients had a positive family history. The comparatively low proportion in Awoyesuku et al. study could be attributed to difficulties inherent in eliciting family medical histories. Information concerning family history may be incomplete, fraught with bias or lack of familiarity with glaucoma as a diagnosis and this could make this variable difficult to measure reliably (Banerjee et al., 2013). This difficulty in eliciting family medical history was also encountered in our study as 8.5% of the POAG group were unaware of any incident of glaucoma in their first- or second-degree relatives; compared to 47.7% in the non-glaucoma group.

In the Baltimore Eye Survey, previously diagnosed individuals reported POAG family history significantly more often than newly diagnosed individuals, suggesting that individuals are less aware of their family history at initial diagnosis (Tielsch et al., 1994). Recalling and eliciting family medical history (history of glaucoma in one's family) could also be a function of his/her educational background. Thisstudy observed that most of subject-participants had basic education (Primary and Secondary level education) unlike participants in the study of Awoyesuku et al.

The prevalence of mutation in myocilin gene in our study was 5.3%. The prevalence of mutation in myocilin gene in the POAG group was 8.4% and in the non-glaucoma phenotypically normal group was 2.3%. This difference was statistically significant (p=0.042) [Table 3], implying that mutations in myocilin gene is likely to be associated with adult onset POAG.

These findings are in tandem with the findings of Challa et al., in Accra, Ghana. Challa et al., studied 90 adult-onset POAG patients with 70 age-matched controls and found that the prevalence of mutation in myocilin gene was 4.4%. Also, in their study it was observed that four individuals with severe POAG were found to have mutations in exon 3-Asp380Asn and Arg342Lys mutations which were not detected in the controls (Challa et al., 2010).

Our study noted mutations in the myocilin gene in exon-3, anserine (ASN) was replaced by valine (VAL) and as a consequence, the amino acid adenine was replaced by thymine in the genomic sequence [Plate 1]. This alteration in the amino acid sequence of myocilin protein could be responsible for its altered physiological function. This assertion needs further investigations as gene variants (mutations) are known to prevent one or more proteins from working properly. On the novel mutation sites, adenine nitrogenous base was replaced by thymine with resultant change in the protein molecule from anserine to valine. This variant of myocilin gene had moderate impacts at the level of RNA transcription (nucleotides from DNA to form RNA strands with resultant missense mutation [Table 3]. However, further investigations need to be done to determine the role of these new genetic variants of myocilin protein on the pathogenesis and peculiarity on individuals of African descent.

In this study, the chromosomal location of the mutant myocilin gene was in chromosome 1-GLC1A. This agrees with the work of Stone et al. Stone et al., in 1997 first identified and reported the association of mutations in myocilin with POAG mapped to the GLC1A locus at 1q24.3-q25.2 - OMIM: 601652 (Hewitt et al., 2008).

In this work, we found 4 single nucleotide polymorphisms associated with mutations in the myocilin gene in the adult-onset primary open angle glaucoma subjects (chromosome 1: 171638779; chromosome 1: 171638703; chromosome 1: 171638610 chromosome 1: 171638608);on chromosome 1: 171638779;3% of the mutations occurred in POAG patients against 1.3% in non-glaucoma subjects.On chromosome 1: 171638703; 1.5% of mutations occurred among the POAG patients while 0.5% mutations were observed in non-glaucoma patients. Mutation on chromosome 1: 171638610 was only observed in 2.5% of POAG patientsand on chromosome 1: 171638608; 1% of the mutations in POAG patients whereas 0.5% of non-glaucoma subjects had mutations in this location. The observed differences in the mutation points in POAG patients and non-glaucoma subjects was statistically different (p=0.042)[Table 3].

Our work found that 13 (3.3%) individuals with adult onset POAG and 9 (2.3%) non-glaucoma subjects had novel missense mutations (SNPs) which wascoded on exon 2& partial coding sequence (CDS) respectively. These were observed in 4.7% of adult onset POAG subjects only [Table 3]. These findings compare well with the results in Pakistan that reported a novel SNP rs879255525 in a mutant myocilin gene associated with glaucoma that varied significantly between POAG patients and controls ($p<0.01$) (Nazir, S., 2018). In the study of Nazir et al., the change in the nucleotide sequence of rs74315341 resulted in the substitution of serine for arginine and the change in rs879255525 resulted in the substitution of asparagine for lysine. The study of Nazir et al. showed the replacement of guanine with thymidine. Nazir et al utilized a case-control epidemiological method with 100 patients and 100 controls subjects (40 males and 55 females had positive family histories of glaucoma, whereas none of the control subjects had a positive family history) unlike our study where 4.6% of the respondents in the control group had positive family history of glaucoma among first degree relatives and 3.1% among second degree relatives.

Our results are in tandem with the findings of Challa et al. in Ghana and Fingert et al., in the United States of America. Challa et al. in the Ghanaian study observed that 4 individuals with severe adult-onset POAG had novel missense mutations in exon 3.

Myocilin has three exons (the sequence of DNA present in messenger RNA, some of which encodes the amino acids of a protein) and contains two major homology regions, the N- and C-terminus (Aroca-Aguilar et al., 2005; Yang et al., 2015; Wang et al., 2018). Notably, majority of myocilin mutations are localized in exon 3, which encodes a 504-amino acid glycoprotein (Yang et al., 2015). This position supports the findings in our work. Over 278 different myocilin mutations have been reported, among which pathogenic mutations account for 37.77% (Aroca-Aguilar et al., 2005; Hewitt et al., 2008).

We demonstrated that the novel mutations in exon 2 of myocilin, located on chromosome 1: 171638779 and chromosome 1: 171638703 were significant among adult-on set POAG whereas Nazir et al., in Pakistan discovered that the mutant SNP on rs74315341 and rs879255525 polymorphic sites were associated with glaucoma onset, which is consistent with the role of rs74315341 in POAG development in an Australia, Brazilian and Caucasian populations (Nazir et al., 2018; Souzeau, et al., 2013; Povoa et al., 2006; Hewitt et al., 2007).

V. Conclusion:

Two novel mutations in the myocilin gene among adult-onset POAG subjects in Rivers State, Nigeria have been identified on chromosome 1: GLC1A 171638779 and 171638703and registered with the NCBI-New York on 26/10/21(GenBank: OK632693.1)(https://www.ncbi.nlm.nih.gov/nuccore/OK632693.1/#:~:text=An%20official%20website,USA.gov). This finding needs further investigation as to their clinical relevance in black African populations. There is a need forcollaborative studies between ophthalmologists and human genetic expertsin the area of epigenetics of glaucoma.

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Conflicts of Interest There are no conflicts of interest.

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