PREVALENCE OF MYOCILIN GENE MUTATION IN ADULT-ONSET PRIMARY OPEN ANGLE GLAUCOMA AND NON-GLAUCOMA SUBJECTS WHO ARE INDIGENES OF RIVERS STATE, NIGERIA

Abstract

Background: Glaucoma is the leading cause of irreversible blindness incapacitating over 80 million people worldwide. Several pathogenetic mechanisms have been postulated to explain the optic nerve damage that occur in POAG among which genetic predisposition is prominent. Gene-Linkage-based studies have identified genes associated with POAG: Myocilin, Optineurin, WDR36, Tank-Binding Kinase (TBK1) and APbb-2.**Objective:**To investigate the prevalence of myocilin gene mutation in adult-onset POAG patients and non-glaucoma subjects who are indigenes of Rivers State.**Methodology:**In this comparative cross-sectional study, 393 POAG patients attending the Glaucoma Clinic of UPTH, were comparedwith 393 age and sex- matched phenotypically normal participants.Clinical assessment combined with findings from clinical records were used.Venous blood was obtained for genomic analyses. Extracted DNA were sequencedwith specific primers for myocilin and polymerase chain reaction.Zymo-Bead Genomic DNA kit protocol was used to detect allelic differences.**Results:**Total of 786participants participated in the study. The mean age was 59.8 ± 11.8 years. The prevalence of myocilin gene mutation (MYOC)in the study population was 5.3%, in thePOAG group was 8.4%, and 2.3% in the non-glaucoma group. This observed difference was statistically significant (p=0.001). Location of the mutant myocilin gene was in GLC1A 171638779, 171638703, 171638610 and171638608. **Conclusion:**Mutations in myocilin gene are associated with adult-onset POAG in Rivers State. It's relevance as a biomarker for diagnosis of adult-onset POAGneeds further investigations.

Keywords: Prevalence, Myocilin gene mutation, adult-onset primary open angle glaucoma, Rivers State indigenes

Introduction

Glaucoma is aprominent cause of irreversible blindness worldwide and in Nigeria - accounting for 15 - 20% of blindness in Nigeria (WHO, 2020; Kyariet al., 2009; Ashaye 2010). Individuals of African ancestry are more affected by primary open angle glaucoma than their Caucasian counterparts (possibly due to genetic predisposition), and are more likely to have worse progression and prognosis(Allingham et 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 and their mutations contributing in varying proportions to Primary Open Angle Glaucoma.These include the myocilin, optineurin, WDR36, threonine protein binding kinase-1(TANK-1) and amyloid-β a4 precursor protein-binding family B member 2 (APbb-2) (Allingham et al., 2009; Monemiet al., 2005;Liu et al., 2013).

The major genetic etiopathogenetic component of POAG among sub-Saharan African populations continue to draw the interest of several researchers. However, some evidences have emanated which strongly link mutations in myocilin and APbb-2genes.

Myocilin is a glycoprotein composed of 504 amino acidwith a molecular weight of 55-57 kDa (Stone, et al., 1997). It is found in the ciliary body, Golgi apparatus of corneal fibroblasts, Schlemm's canal endothelial cells, trabecular meshwork, lamina cribrosa, retina, optic nerve,

aqueous and vitreous humor of the eye. Outside the eye, expression of myocilin is found in the skeletal muscle, heart, breast, small intestine, prostate, testis, colon, stomach, thyroid, trachea, bone marrow, and brain. Myocilin has also been detected in Schwann cells, myelin sheath of peripheral nerves, renal podocytes, mesangial cells, intervertebral disks, and plasma (Kubota et al.,1997).The precise function of myocilin is unknown. However, scientific research has linked it to other proteins, making it part of a protein complex. For instance, the isoform of the cytochrome P450 protein, has shown interaction with myocilin. Many scientists believe that myocilin has a role in cytoskeletal function (Fingert, 2011).

Myocilin was first identified as a glaucoma gene in 1997 and its mutations are the most common cause of glaucoma with a known molecularly defined basis (Fingert, 2011). Myocilin protein is produced by many healthy ocular tissues and secreted into the aqueous humor. However, accumulation of abnormal myocilin protein produced by myocilin mutations is toxic to the trabecular meshwork resulting in poor egress of aqueous humor and elevation in IOP often leading to glaucomatous damage of the optic nerve head (Kwon et al., 2009).

Significant prevalence of mutation in myocilin gene mutation could be used as a biomarker in the screening and detection of the population for POAG; hence this work on prevalence of myocilin gene mutation in adult-onset primary open angle glaucoma and non-glaucoma subjects who are indigenes of rivers state, Nigeria.

Materials and Methods

This was a comparative cross-sectional study of 393 knownAdult-onset Primary Open Angle glaucoma patients attending the Glaucoma Clinic of the University of Port Harcourt Teaching Hospital with 393 age and sex- matched phenotypically normal indigenes of Rivers State,were recruited via multi-stage sampling technique from different parts of the State. The study was conductedbetween January 2021 and November 2022. Clinical assessment combined with findings from clinical records were used. Subjects with history of eye surgery prior to diagnosis of glaucoma, cases of secondary glaucoma, angle -closure glaucoma, history of ocular trauma, or history of significant use of systemic or ocular glucocorticoids for a period exceeding 6 months were excluded from the study.

Ethical Statement:

Ethical approval to conduct this study was obtained from the Ethics Committee of University of Port Harcourt. This study adhered to the tenets of the Declaration of Helsinki on study involving human subjects.Study participants' informed consents were obtained. Participation was absolutely voluntary. Participants were free to opt out at any stage of the study without victimization. All information obtained from the participants of this study was treated with utmost confidentiality. No personal identification (names, clinic number) was stored electronically. There was no health risk to the participants of this study.Benefits to the participants included free reading glasses, ocular assessment and counseling.

Calculation of Sample Size:

Minimum sample size estimation formula for comparing two proportions, is that by Lwanga, SK.et al., (1999):

$$\mathbf{n} = \frac{(\boldsymbol{Z\alpha/_2 + Z_{1-\beta}})^2}{(\boldsymbol{P1-P2})^2}\{P1(1-P1) + P2(1-P2)\}$$

Where:

- n = minimum sample size in the 2 groups
- $Z_{\alpha/2}$ =standard normal deviate corresponding to 5% level of significance = 1.96
- $Z_{1-\beta}$ = standard normal deviate corresponding to a power of 80% = 0.84
- P1 = 4.4% = 0.044
- P2 = 1% = 0.01
- P1 – P2 = the smallest difference between the two groups of scientific or clinical importance which the study would not want to miss

Substituting the values of $Z_{\alpha/2,}$ $Z_{1-\beta}$, P1 and P2 in the formula;

$$n = \frac{(1.96+0.84))^2}{(0.044-0.01)^2}\ \{0.044(1-0.044) + 0.01(1-0.01)\}$$

$$n = \frac{(2,8)^2}{(0.034)^2}\ \{0.044(0.956) + 0.01(0.99)\}$$

$$n = \frac{7.84}{0.001156}\ \{0.042064 + 0.0099\}$$

$$n = 6{,}782.01\ X\ 0.051964$$

$$n = 352.4 \approx 353$$

An adjustment is made for non-response rate of 10%. Non-Response Rate (NRR) accounts for households that could be either absent, not accessible, refuse to be surveyed or any other reason that prevent survey team from surveying a selected household.

Final Sample Size (N) = $\frac{n}{1-non-response\ rate} = \frac{n}{1-10\%} = \frac{n}{1-0.1} = \frac{353}{0.9}$ =392.2

≈ 393 persons in each group.

Eligibility Criteria:

Inclusion Criteria

Patients diagnosed with primary open angle glaucoma who are indigenes of Rivers State.

Exclusion Criteria

Subjects with a history of eye surgery prior to diagnosis of glaucoma, cases of secondary glaucoma, angle -closure glaucoma, a history of ocular trauma, or a history of significant use of systemic or ocular glucocorticoids for a period exceeding 6 months (to avoid steroid-responders to increase in intraocular pressure.

Sample and Data Analysis

Blood Sample Collection for Genetic Diagnostics

The cubital fossa of every study participant was prepared for phlebotomy. A nurse performed the phlebotomy. After informing the participant of the procedure and obtaining consent, a tourniquet was applied to the participant's upper arm above the antecubital fossa, cleansed with an antiseptic solution (name) and three milliliters of peripheral venous blood were collected with a sterile needle and syringe and preserved in a specimen bottle containing an anticoagulant -

ethylene-diamine-tetra-acetate (EDTA) for genetic analysis. The specimen bottles were correctly labelled with the participant's serial number.

Extraction of Deoxyribonucleic Acid (DNA) from the Blood Samples

- DNA was extracted from the blood samples of study participants using Zymo-Bead Genomic DNA kit protocol. The procedure was as follows:
- 50µl of blood sample was put in a clean, grease-free test tube.
- To the above, 10µl Zymo-Bead slurry was added; that is re-suspended by vortexing.
- 200µl of Genomic Lysis Buffer was then added and mixed by inversion and incubated at room temperature for 5 minutes. The content of the test tube was centrifuged at 1,500×g for 1 minute and then carefully removed.
- 20µl of Genomic Lysis Buffer was then added to the above. The mixture was re-suspended by pipetting up and down and centrifuged at 1,500×g for 1 minute. The supernatant was then discarded.
- 200µl of DNA Pre-Wash Buffer was added to the supernatant. The pellet was re-suspended and then centrifuged at 1,500×g for 1 minute. The supernatant was discarded.
- 500µl of g-DNA Wash Buffer was added to the supernatant above, to resuspend the pellet and then centrifuged at 1,500×g for 1 minute. The supernatant was discarded and recentrifuged briefly to remove any residual wash buffer.
- 35 µl of Elution Buffer was added. The pellet was resuspended by pipetting up and down, and then centrifuged at 10,000×g for 1 minute.

- The supernatant was then collected. The supernatant contains purified DNA that was used immediately or stored at -20^{0}C for later use.

Gel Electrophoresis

The quality of DNA, the products of PCR were assessed using gel electrophoresis using a Portable Gel hood built in blue LED (470nm) by Royal Biotech/Biolympics (www.royalbiotech.com) 1.5% agarose gel at a constant voltage and 1X TBE for approximately 1 hour. They were visualized by ethidium bromide staining and photographed under ultraviolet light. The ladder used is 1kb base pair ladder from thermos-scientific.

POLYMERASE CHAIN REACTION

Genomic DNA was extracted from the venous blood of all participants. Linkage analysis was performed with five microsatellite markers around the MYOC gene (Myo-IFa, Myo-IRa, Myo-2F, Myo-2R, Myo-3Fa and Myo-3Ra). Mutation screening of all coding exons of MYOC was performed by direct sequencing of PCR-amplified DNA fragments and restriction fragment length polymorphism (RFLP) analysis.

Polymerase chain reaction was carried out using specific primers [Table 1]. A solution of DNA polymerase, dNTPs, reaction buffer and water assembled into 1.8 ml microcentrifuge tube. The premixed PCR Master Mix contained: 10× PCR buffer, 25mM Mgcl2, 5pMol forward primer, 5pMol reverse primer, DMSO, 2.5Mm DNTPs, Taq 5u/ul and10ng/µl DNA. A total of 25µL of the Master Mix was utilized. The amount of each reagent added to the Master Mix at optimal concentrations for effective amplification of DNA templates by PCR is presented in Table 2.

Table 1: Primer sequences for myocilin gene polymerase chain reaction

Primer Name	Primer Sequence	Amplified Sequence Length
Myo-1Fa	5'-CCTCACGTGGCCACCTCTGTC-3'	554 bp
Myo-1Ra	5'-GGTTTCCAGCTGGTCCCGCTC-3'	554 bp
Myo-2F	5'-GCCGGCAGCCTATTTAAATGTC-3'	404 bp
Myo-2R	5'-CCTGCTCTGACAAGGGAACAG-3'	404 bp
Myo-3Fa	5'-GCTGTCACATCTACTGGCTCTG–3'	736 bp
Myo-3Ra	5'-GTCATAAGCAAAGTTGACGGTAGC-3'	736 bp

Cocktail mix of DNA for PCR

A solution of DNA polymerase, dNTPs, reaction buffer and water assembled into 1.8 ml microcentrifuge tube. The premixed PCR Master Mix contained: 10× PCR buffer, 25mM Mgcl2, 5pMol forward primer, 5pMol reverse primer, DMSO, 2.5Mm DNTPs, Taq 5u/ul and10ng/µl DNA [Table 2]. A total of 25 µL of the Master Mix was utilized. The amount of each reagent added to the Master Mix at optimal concentrations for effective amplification of DNA templates by PCR is presented in table 2.

Table 2: Cocktail mix of DNA for PCR

10× PCR buffer	2.5
25mM Mgcl2	1.0
5pMol forward primer	1.0
5pMol reverse primer	1.0
DMSO	1.0
2.5Mm DNTPs	2.0
Taq 5u/ul	0.1
10ng/µl DNA	3.0
H_2O	13.4
TOTAL	25µL

The above cocktail mix of DNA and conditions for the PCR were utilized.

Touchdown PCR protocol used for the primers

This is a method for increasing specificity of PCR reactions. In this procedure, 9 cycling program was used initially and then 35 cycling programs for the annealing temperature and gradually reduced at 1°C /every second cycle. The initial annealing temperature was 94˚C and final temperature was 72˚C [Table 3].

Table 3: Touchdown PCR Condition

	9 Cycle			35 Cycles				
Initial den.	Den.	Ann. Temp.	extension	Den.	Ann. Temp.	extension	Final extension	Hold Temp.
94˚C	94˚C	65˚C	72˚C	94˚C	55˚C	72˚C	72˚C	10˚c
5min	15sec	20sec	30sec	15sec	20sec	30sec	7min	∞

DNA Sequencing

The polymerase chain reaction (PCR) products, free of contaminating bands due to non-specific amplification, was sent to the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria for bi-directional sequencing using an ABI Prism 3500 DNA sequencer with dye-termination chemistry. A 96 well plate was used for cycle sequencing and the products were purified using Ethanol/EDTA precipitation method. 25ng of the PCR product was used to perform cycle sequencing.

Nucleotide changes was detected by identifying double peaks in the chromatogram due to heterozygosity of the DNA sample analyzed and confirmed by sequencing from the opposite direction. In addition, the sequences were analyzed using Pairwise BLAST to determine if there were any changes from the normal sequence available in the database.

Restriction Enzyme Digestion

Mutations identified by DNA sequencing was screened in additional POAG patients and control samples by PCR amplifying the suspected region of genomic DNA [Table 4]. The PCR products were digested with appropriate restriction enzymes that distinguished between the mutant and normal alleles (Table 3) under the conditions described by the manufacturer (New England BioLabs, Beverly, MA) in a total reaction volume of 20 mls. Similarly, single nucleotide polymorphisms in the promoter region (pSNP) and coding sequence (cSNP) were screened in both patient and control samples using *Ava* I and *Bsm*A I restriction enzymes. DNA fragments in the digest was separated by electrophoresis in 6% polyacrylamide gels, stained with ethidium bromide and visualized using an UV transilluminator. Alleles were scored based on the DNA band patterns in the gel.

Mutation Screening by Allele Specific Restriction Digestion

Several restriction endonucleases were utilized to digest the nucleic acids and detect specific sequences of nucleotides in a DNA strand, thereby enabling the recognition of point mutations in DNA and eliminates the need for subcloning and sequencing [Table 4].

Detection of Mutations/Single Nucleotide Polymorphism (SNPs)

Bioedit was used to trim and edit the sequences. Bioedit and Clustal X were used for Single Nucleotide Polymorphisms (SNPs) detection using reference sequences downloaded from genbank (https://www.ncbi.nlm.nih.gov/genbank/). Ensembl were used to predict the effect of the Single Nucleotide Polymorphisms (SNPs).

Table 4:Mutation Screening by Allele Specific Restriction Digestion

Nucleotide change	Amino acid change	Location	Sequence of primer pairs (5' to 3') and PCR condition	Length of PCR product (bp)
144 G->T	Gln48His	Exon 1	CTTCTGTGCACGTTGCTGC A CTGGTCCAAGGTCAATTGG T 94 °C 30 s, 52 °C 30 s, 72 °C 60 s for 30 cycles using 1 mM $MgCl_2$	313
1109 C->T	Pro370Leu	Exon 3	ATACTGCCTAGGCCACTGG A CAATGTCCGTGTAGCCACC 94 °C 30 s, 58 °C 30 s, 72 °C 60 s for 35 cycles using 1 mM $MgCl_2$	198

Data Analysis

The data obtained were entered into Microsoft Excel sheet, cleansed and later exported to IBM Statistical Package for Social Sciences (SPSS) version 25 software (SPSS) Inc; Chicago, IL, USA for statistical analysis. Relevant data were presented in tables and charts. Statistical significance was performed using Chi square and statistical significance was set at $p \leq 0.05$. Zymo-Bead Genomic DNA kit protocol (YeaStar Genomic DNA Kit-D2002)(https://www.zymoresearch.com)was used to detect allelic differences.

Results:

This study participants were 786 subjects; there were 393 adult-onset primary open angle glaucoma (POAG) patients and 386 non-glaucoma subjects, all indigenes of Rivers State. Fifty percent (n=393) of the study subjects were establishedadult-onsetPOAG patients while 50% (n=393) were age and sex-matched non-glaucoma phenotypically normal subjects. 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 5]. The participants of this study were from the following ethnic groups in Rivers State, Nigeria: Andoni, Ekpeye, Engenni, Etche, Igbani, Ikwerre, Kalabari, Ogba, Okrika, and Ogoni with equal number of participants represented from each participating Local Government Area.

Table 5: Age-Gender characteristics of the study population

Variables	Distribution in Adult onset POAG cases n=393 (n)	(%)	Distribution in Normal subjects n=393 (n)	(%)	Total	(%)	Chi-Square Value	p-Value
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				

Educational Status of the study subjects

Over 13% of study participants in the POAG group had tertiary education compared to 4% in the non-glaucoma group; 23.9% of the study participants in the POAG group had secondary education compared to 7.4% in the non-glaucoma group. Only 1.5% of study participants in the POAG group had no formal education compared to 25.2% in the non-glaucoma group. The difference in the distribution of the study participants according to their educational status was statistically significant (p=0.000) [Table 6].

Table 6: Educational Status of the study subjects

Variables(Educational Status)	Distribution in Adult onset POAG cases n=393 (n)	(%)	Distribution in Normal subjects n=393 (n)	(%)	Total	(%)	Chi-Square Value	p-Value
Tertiary	106	(13.5)	32	(4.1)	138	(17.6)	274.84	0.000
Secondary	188	(23.9)	58	(7.4)	246	(31.3)		
Primary	87	(11.1)	105	(13.3)	192	(24.4)		
No Formal Educ	12	(1.5)	198	(25.2)	210	(26.7)		
Total	**393**	**(50)**	**393**	**(50)**	**786**	**(100)**		

Occupational Status of the Adult-onset POAG Patients

Majority of the study participants in the adult-onset POAG group (30.0%) were retired civil servants;23% were engaged in public service, 21% were engaged in diverse businesses and trading activities; 8% were artisans, 6% (fishing), 5% farming, 4% were full time housewives and 3% were unemployed [Figure 1].

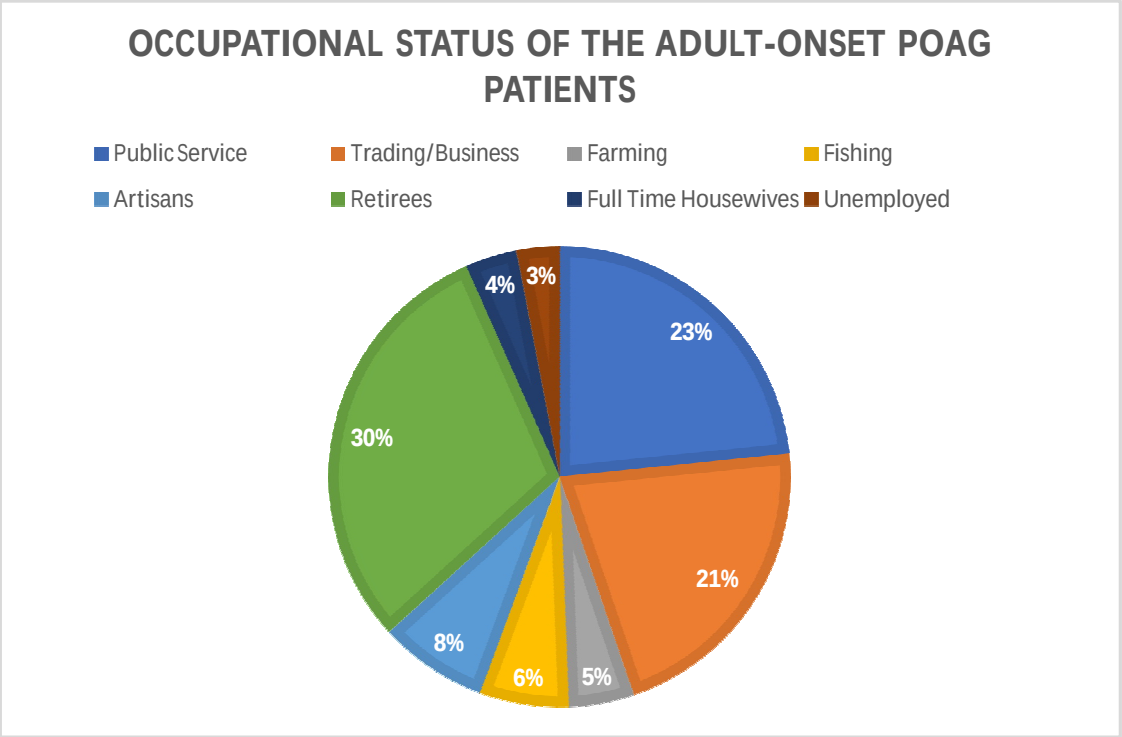

Figure 1: Occupational Status of the Adult-onset POAG Patients

Occupational Status of the phenotypically normal non-glaucoma Subjects

In the phenotypically normal non-glaucoma group,22% were engaged in fishing, 20% in farming, 14% were retired public servants; 13% were public servants;13% were unemployed, 8% were full time housewives; 6% were engaged in diverse businesses and trading activities while 4% were artisans[Figure 2].

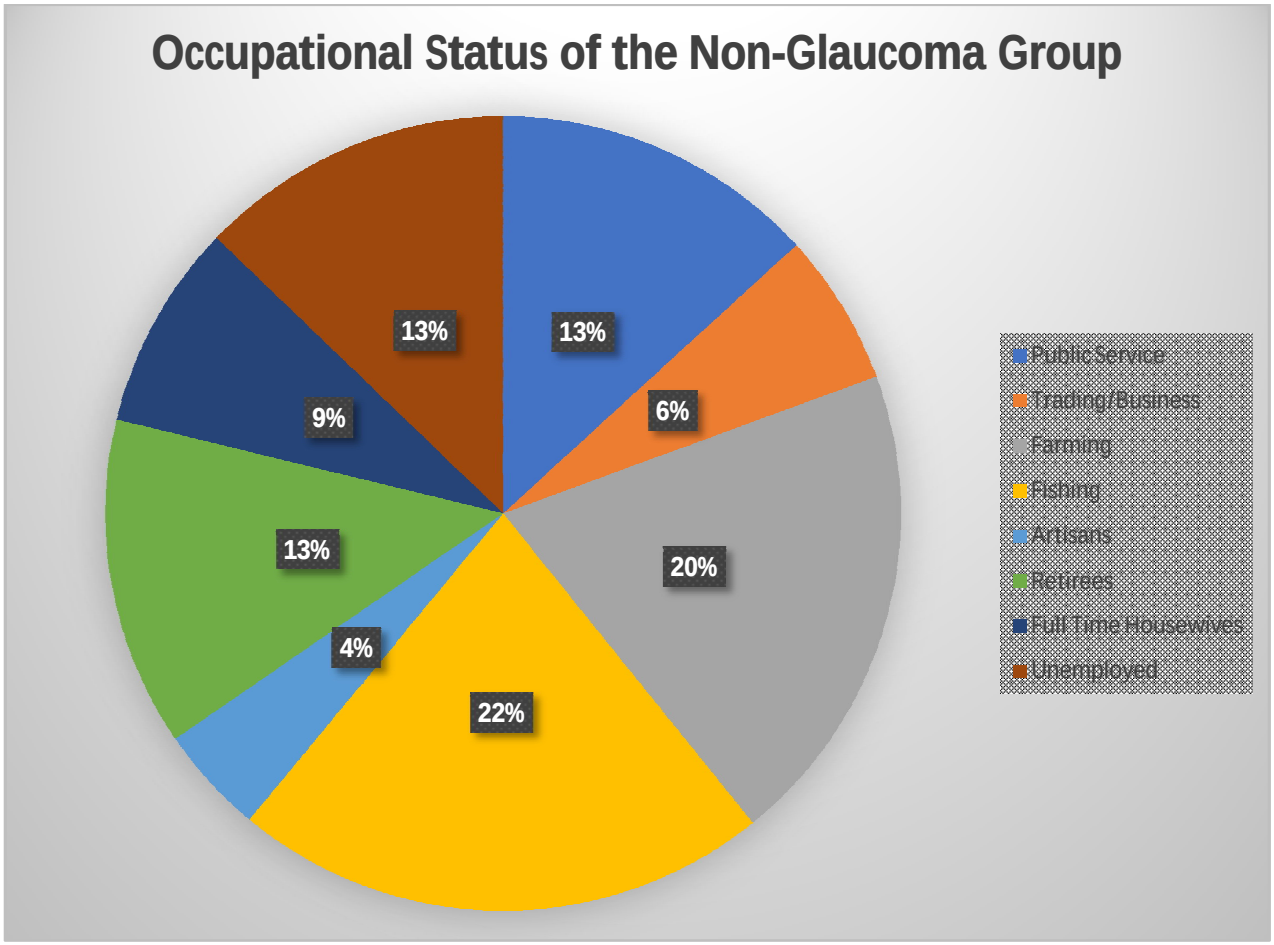

Figure 2: Occupational Status of the Adult-onset POAG Patients

Prevalence of mutation in the myocilin gene among in the two groups

Prevalence of Myocilin gene mutation on the total population studied was 5.3%; 8.4% among adult-onset POAG group and 2.3% in the phenotypical, normal non-glaucoma group [Table 7]. The difference in the prevalence in myocilin gene mutation in the groups was statistically significant (p=0.001).

Table 7: Prevalence of mutation in the myocilin gene among in the two groups

	Mutation in Myocilin gene PRESENT	**Mutation in Myocilin gene ABSENT**	**TOTAL**	**Prevalence**
POAG patients Group	33	360	393	8.4%
Non-Glaucoma Group	9	384	393	2.3%
TOTAL	42	744	786	5.3%
Pearson Chi-Square Test =14.488 p-value = 0.001				

Discussion

This study sets out to study the prevalence of myocilin mutant gene among adult-onset primary open angle glaucoma (POAG) patients and non-glaucoma phenotypically normal subjects, who are indigenes of Rivers State.

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). The age range of our study participants was 40 to 86 years with a mean age of 59.8 ± 11.8 years in both groups. The modal age group (60-69) accounted for 14.9%. This observation compares well with the study of Kyari

et al., in the Nigerian National Blindness and Visual Impairment Survey of 2005-2007. In their study, a sample of 13,591 people aged ≥40 years, representative Nigerian-nationals: the mean age of the subjects with POAG was 66.2 ± 12.3 years. 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 among individuals aged 45 years and older (Murdoch et al., 2001) while Adeoye in South Western region of Nigeria observed that POAG was more prevalent among 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 giving credence to the assertion that adult-onset POAG occurring at ≥40 years.

Corroborating with our findings are the works of Leske et al., in the Barbados Eye Study which observed that adult-onset POAG was predominately among populations 45 years of age and older and that POAG significantly increases with age in all populations. It was also 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 Niger-Delta region, Nigeria (Leske et al., 2008)

In this research, we recruited equal participants aged 40 years and older from both sexes and age-matched population 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.

Concerning the occupational distribution of the study population, majority of the study participants (21.8%) were retired civil servants with different levels of educational status. There was a statistically significant difference in the distribution of the participants in this study among the various occupational status (p=0.000). The modal class of the study participants in our study was 60-69 years; most of them- retirees (15.1%). The distribution of the proportion of retired civil servants in the study population is in tandem that the retirement age from civil service in Nigeria is from 65 years (Federal Republic of Nigeria. Public Service Rules 2008); and that adult-onset POAG occur from 40 years and older.

The prevalence of mutation in myocilin gene in the study population was 5.3%. Among Adult-onset POAG group, the prevalence of myocilin gene mutation was 8.4% and among the control group - 2.3%. This observed difference was statistically significant (p=0.001).

Chi-Square Goodness of Fit Test was performed to determine if this observed difference in the prevalence was due to chance or not. Chi-Square Goodness of Fit Test establishes whether there is a discrepancy between the observed values and those expected of the model. The proportions did differ by the prevalence of mutations in myocilin gene between the POAG patients' group and the non-glaucoma group. This observed difference was statistically significant (p=0.001), implying that mutations in myocilin gene is likely to be associated with adult onset POAG.

Our findings in this study 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 population was larger than the study population of Challa et al., and probably could be responsible for their lower yield of the prevalence of mutations in myocilin gene among adult-onset POAG patients.

Our study corroborates with the findings of Fingert et al., in Iowa, United States of America where the prevalence of myocilin gene among African-American glaucoma population ranged between 2.8% and 5% among adult-onset POAG patients (Fingert, 2011). In the study, 1,703 glaucoma patients from Australia, Japan, Canada, and the USA (Caucasians from Iowa and African Americans from New York City) were examined. This was a mixed- population cross sectional study with limited power, though with large number of study participants.

In another study, Fingert et al., observed a statistically significant association between mutation in myocilin gene and POAG by screening 330 POAG patients and 471 controls for myocilin mutations; they found mutations of myocilin in 3.9% of POAG patients and in 0.2% of the control subjects (Fingert et al., 1999).

Also, Ennis et al., (2014) in Southern England identified an overall prevalence of 2.2% in Caucasian POAG patients. However, earlier studies by Fingert et al., in the year 1999 observed a prevalence of 2–4% of unrelated glaucoma patients. (Frequency of myocilin mutations in unrelated glaucoma patients (i.e., 4.3% in patients from Iowa, USA; 2.6 % in African Americans from New York; 2.8% in Japanese patients; 3.0% in Canadian patients; and 2.8% in Australian patients). Similar to other studies, our present finding was the disease-causing mutations detected in this population tended to occur in exon 3.

Our findings in this work are in agreement with the postulation that, mutations in myocilin gene are associated with adult-onset POAG and that the identified mutations are possible biogenetic markers among population with high risk of developing adult-onset Primary Open Angle Glaucoma.

Conclusion: Mutations in myocilin gene are associated with adult-onset POAG in Rivers State.It's relevance as a biomarker for diagnosis of adult-onset POAGneeds further investigations.

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Conflict of Interest There are no conflict of interest.

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