

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

Azubuike A. Onua¹, Brilliant O. Agaviezor² and Chinyere N. Pedro-Egbe¹

¹Department of Ophthalmology, University of Port Harcourt, Port Harcourt, Nigeria

²Department of Animal Science & Genetics, University of Port Harcourt, Port Harcourt, Nigeria

Corresponding author: Onua A. A., E-mail: azubuike.onua@uniport.edu.ng,+2348037206138

Background: Glaucoma is a heterogenous optic neuropathy with characteristic visual field defects resulting from the gradual retinal ganglion cell death and the second commonest cause of blindness worldwide¹⁻⁵. Several pathogenetic theories have been postulated but the genetic factor is gaining more acceptance. Gene-Linkage-based studies have identified myocilin gene mutation to be associated with open-angle glaucoma⁶⁻¹⁰.

Objective: To investigate the presence of myocilin gene mutation in adult-onset primary POAG subjects of Rivers State.

Materials and Methods: This was a case-control study of the prevalence of mutations in myocilin gene among established adult-onset primary open angle glaucoma patients and their age and sex-matched non-glaucoma phenotypically normal subjects who are indigenes of Rivers State, recruited from the 23 LGAs in Rivers State through a multi-stage random sampling technique.

Sample size was determined from the formula for comparing two proportions¹¹:

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

- Where: n = minimum sample size
- $Z_{\alpha/2}$ = standard normal deviate (5% level of significance = 1.96)

- $Z_{1-\alpha}$ = standard normal deviate corresponding to a power of 80% = 0.84
- P1 = 4.4% = 0.044 (prevalence of myocilin mutation among patients with adult-onset glaucoma in Ghana was 4.4%¹²)
- P2 = 1% = 0.01 (prevalence of myocilin mutation in the general non-glaucoma population was 1%¹³)
- P1 – P2 = the smallest difference between two groups

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

$$n = 352.4 \approx 53$$

An adjustment for non-response rate of 10% $392.2 \approx 393$ persons in each group

Venous blood samples from 786 consenting study participants were obtained for genomic analysis. DNA was extracted; amplified; with specific primers for myocilin using polymerase chain reaction. Bioinformatic analyses were done with Simple Modular Architecture Research Tool (SMART) software for protein domain structure prediction and Molecular Evolutionary Genetics Analysis (MEGAX) for evolutionary genetic analyses. Statistical Package for Social Sciences (SPSS) Version 25 was employed for demographic and inferential statistics.

Results: A total of 786 participants aged e"40 years were recruited. Mean age of the study population was 59.8 ± 11.8 years. Four single nucleotide polymorphisms (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 (Table 1 and Figure 1). Thymine replaced adenine in the novel variants.

Table 1: 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= 0.000			

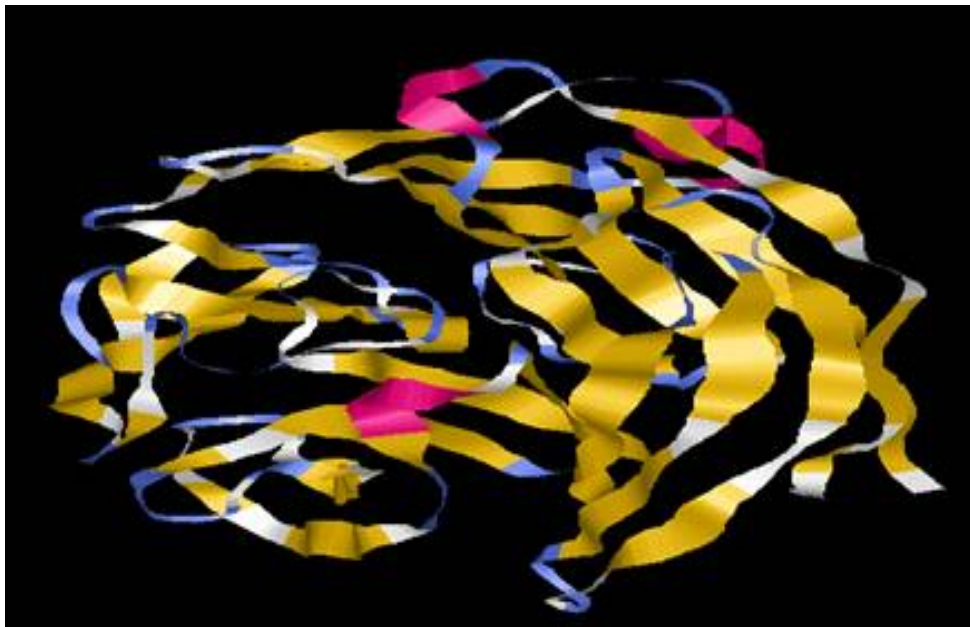


Plate 1: Mutant Myocilin Molecule-Representative of subjects with adult-onset POAG

Discussion: The chromosomal location of the mutant myocilin gene that is associated with adult onset POAG was in chromosome 1-GLC1A. This is in tandem with the work of Stone *et al*¹⁴. In our study, we found 4 single nucleotide polymorphisms associated with the mutations in the myocilin gene among adult-onset primary open angle glaucoma subjects. Our findings compare well with the works of Nazir in Pakistan who find novel SNP rs879255525 in myocilin mutant gene⁸.

Conclusion: Two novel mutations in the myocilin gene among adult-onset POAG subjects have been identified on chromosome 1: GLC1A 171638779 and 171638703. This needs further investigation among African populations.

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

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- ### Factors Affecting Intraocular Pressure in Normal Subjects and Glaucoma Patients: Evidence from Abakaliki
- Ireka OJ¹, Ogbonnaya CE¹, Obinna Arinze C¹, Aniemeka DO¹, Ginger-Eke HA¹, Ezisi CN¹, Chuka-Okosa CM²*
- ¹Department of Ophthalmology, Alex Ekwueme Federal University Teaching Abakaliki, Ebonyi, Nigeria
²Department of Ophthalmology, University of Nigeria Teaching Hospital Ituku Ozalla, Enugu, Nigeria
- Corresponding author:** Onyekachi Jane Ireka,
Email: onyireka@gmail.com; +234 806 4094 382
- Background:** Intraocular pressure (IOP) is affected by factors like age, gender, body mass index (BMI), and blood pressure among others.^{1,2} Studies have investigated the relationship between IOP and age, gender, BMI, and refractive error.³⁻⁵ There has been conflicting results as some studies have reported a correlation between IOP and age, gender, BMI, and refractive error^{3,5} while results from other studies found no such association.^{4,6} Therefore, the aim of this study was to determine the relationship between IOP and age, gender, body mass index and refractive status in the primary open angle glaucoma (POAG) and non-glaucomatous eyes.
- Patients and Methods:** A case-controlled study involving consecutive newly diagnosed POAG and non-glaucomatous patients conducted in the eye clinic of Alex Ekwueme Federal University Teaching Hospital, Abakaliki.