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Gene flow, genetic diversity and differentiation of myocilin gene among Patients with Adult - Onset Primary OpenAngle Glaucoma who are indegens of Rivers State, Nigeria

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ABSTRACT
Gene flow, genetic diversity and differentiation of myocilin gene were assessed among patients with Adult - Onset Primary OpenAngle Glaucoma in Rivers State, Nigeria. Blood samples were collected from patients and control groups from which DNA was extracted and subjected polymerase chain (PCR) using myocilin gene primers. PCR amplicons were sequenced and the sequences subjected to bioinformatics analysis. The results revealed thatnumber of segregating sites (S) was 177.00 among the control while a higher value of 194.000 was observed among the patients. Haplotype diversity (Hd) of 0.778 was observed among the control group but a higher value of 0.917 was recorded among the patients. Average number of differences (k) in the myocilin nucleotides was 36.178 among the control group while it was 47.028 among the patient groups. Furthermore, nucleotide diversity (Pi) of 0.103 was recorded among the control groups and 0.135 was reported among the patients. Average gene diversity within population (Hs) was 0.842. In addition, the measures of genetics differentiation based on haplotype statistics (Hst) was -0.008. Furthermore, global genetic differentiation (Kst) was 0.014. This value was highly significant. Also, the measure genetic differentiation based on nucleotide statistics (Z) was 83.226 while the nearest neighbour statistic (Snn) was 0.546. The Chi square value was 11.479. However, the value (0.4040) was not significantly different (p>0.05). These results have provided basic information needed to generate genetic markers for typing or detecting POAG in humans but subject to further verification.

Keywords: *Gene flow, Genetic diversity, Glaucoma, Myocilin*

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I. Introduction

The problems associated with Adult - Onset Primary OpenAngle Glaucomacontinue to draw the attention of ophthalmologists and medical scientists worldwide. According to WHO (2020), visual impairment and blindness constitute major public health and social problems worldwide with serious economic implications, often leading to social dependence, poor quality of life, lack of access to education; and loss of productivity and income. In developing countries, like Nigeria, loss of vision is worsened by the poor quality of rehabilitative and supportive service due to a dearth of technology. The World Health Organization (WHO) estimates that 2.2 billion people in the world have vision impairment and blindness; and 90% of the world's blind live in developing countries out of which about 1.2 million people live in Nigeria (WHO, 2019). It has also been reported that 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 (WHO, 2019). Glaucoma is also the leading cause of irreversible blindness in Nigeria – being responsible for 15-20% of blindness in Nigeria (Abdull*et al*., 2009). The Africa region has the highest incidence and prevalence of glaucoma.

Primary open angle glaucoma disproportionately affects individuals of African ancestry and is the most common cause of permanent blindness in Africa (Liu, 2011). Most patients in Africa have poor or inadequate knowledge of glaucoma, and therefore present very late for clinical evaluation and treatment. In addition, there

is often reluctance in the acceptance of medical or surgical interventions among African populations (Bowman *et al*., 2010). Biomarkers are biological molecules found in blood, various body fluids, or tissues that are pathognomonic for specific disease entities or normal physiologic processes. There is an increased need to obtain biomarkers which would serve the following functions: markers for early detection of disease, those that will predict severity of disease, and those that will predict the rate of disease progression, and markers that will serve as predictors of response to treatment. Identifying biomolecular and biochemical events (biomarkers) responsible for adult-onset POAG is highly desirable for early detection and management of the disease condition to avoid inevitable vision loss with its associated effect negative effect on quality of life(Wiggs, 2007). Hence, this study on gene flow, genetic diversity and differentiation of myocilin gene were assessed among patients with Adult - Onset Primary OpenAngle Glaucoma in Rivers State, Nigeria was carried out to detect likely biomarkers responsible for adult-onset POAG.

II. Materials And Methods

Study Area

This study was carried out in communities in Rivers State and the Glaucoma Unit of University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. Rivers State is one of the thirty-six states that make up the Federal Republic of Nigeria, located in the oil-rich region of the Niger Delta, in Southern Nigeria. It is made up of 23 local government areas (LGAs): -Abua-Odual, Ahoada -East, AhoadaWest, Akuku -Toru, Andoni, Asari -Toru, Bonny, Degema, Eleme, Emohua, Etche, Gokana, Ikwerre, Khana, Obio/Akpor, Ogba/Egbema/Ndoni, Ogu/Bolo, Okrika, Omuma, Opobo/Nkoro, Oyigbo, Port Harcourt and Tai.

Study Design

This is a comparative cross-sectional study of the prevalence of mutations in myocilin gene (genetic biomarker) among established adult-onset primary open angle glaucoma patients who are indigenes of Rivers State and their age and sex-matched non-glaucoma subjects also indigenes of the State.

Study population

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.

The study population consisted of adults 40years or older (≥40 years) who were indigenes of Rivers State. Inhabitants of Rivers State reside in both riverine and upland communities. There are many ethnic groups in Rivers State and these include the following: Ikwerre, Ogoni, Kalabari, Ekpeye, Etche, Igbani, Andoni, Okrika, Ogba, Abua, Odual, and others.

There are two groups of study participants in this work. Group one consisted of established cases of adult-onset POAG cases. These were recruited from the Glaucoma Clinic of University of Port Harcourt Teaching Hospital through a simple random sampling technique. Using the Glaucoma Register of the year 2021 as the sampling frame, all glaucoma patients who met the inclusion criteria were recruited into the study. The second groups of study participants were recruited from the 23 LGAs in Rivers State through a multi-stage random sampling technique.

Blood collection, DNA Extraction, Polymerase Chain Reaction, Sequencing and data analysis

The procedures for blood collection, DNA extraction, polymerase chain reaction, sequencing and data analysis have been reported in Onua and Agaviezor (2023). Sequences of Homo sapiens chromosome 1 myocilin (GLC1A) gene complete cds was downloaded from Genbank and was used to compare the sequences generated from this study

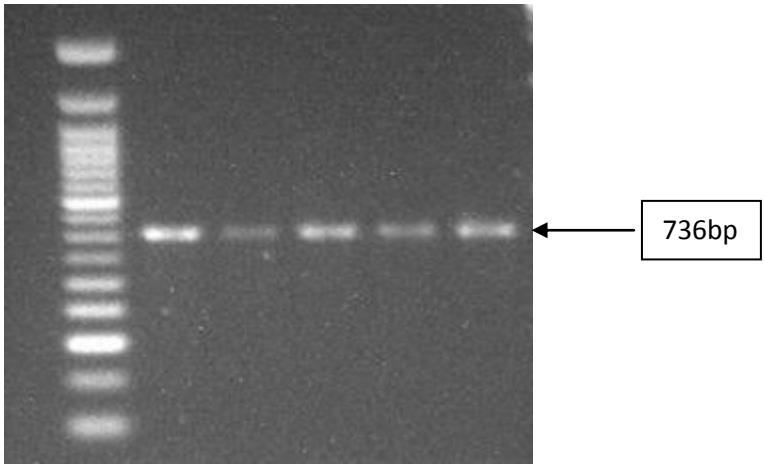

Figure 1: Gel Electrophoresis Showing Polymerase Chain Reaction 736bp amplicons of myocilin gene

Ethical Considerations

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.

III. Results

Figure 2 shows the evolutionary history of patients, respondents and that of Homo sapiens chromosome 1 myocilin (GLC1A) gene complete cds inferred using the UPGMA method. Two clades were identified. The patients were completely separated from the control and that of the Homo sapiens chromosome 1 myocilin (GLC1A) gene complete cds. This result indicates that nucleotide sequences of patients of POAG in the University of Port Harcourt are significantly different from those of the control.

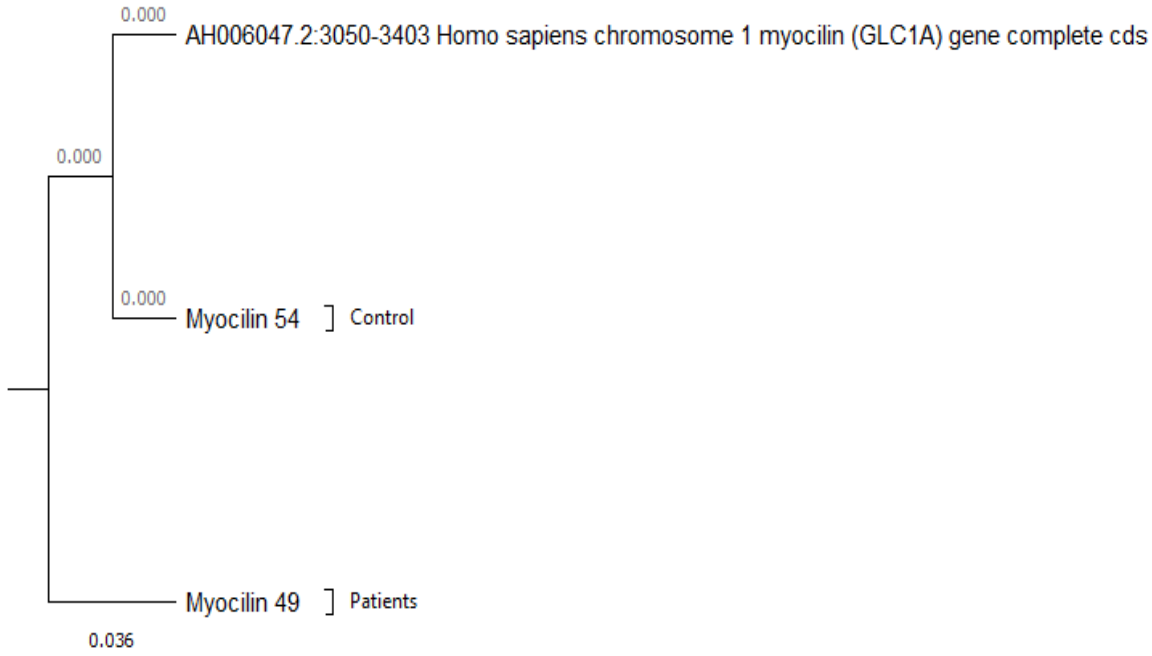

Figure 2: The evolutionary history of patients, respondents and that of Homo sapiens chromosome 1 myocilin (GLC1A) gene complete cds inferred using the UPGMA method

Table 1 shows haplotype and nucleotide diversities in myocilin gene in control and patient groups. While the number of segregating sites (S) was 177.00 among the control, a higher value of 194.000 was observed among the patients. In addition, 1.000 haplotype was observed among the control while 4.000 were recorded for the patients. Haplotype diversity (Hd) of 0.778 was observed among the control group but a higher value of 0.917 was recorded among the patients. Average number of differences (k) in the myocilin nucleotides was 36.178 among the control group while it was 47.028 among the patient groups. Furthermore, nucleotide diversity (Pi) of 0.103 was recorded among the control groups and 0.135 was reported among the patients.

Table 1: Haplotype and nucleotide diversities in myocilin gene in control and patient groups

Parameters	Control	Patients
Number of segregating sites, S:	177.000	194.000
Number of haplotypes, h:	1.000	4.000
Haplotype diversity, Hd:	0.778	0.917
Average number of differences, K:	36.178	47.028
Nucleotide diversity, Pi:	0.103	0.135

Table 2 shows the genetic differentiation estimates of myocilin gene among patients and control groups. The average gene diversity within population (Hs) was 0.842. In addition, the measures of genetics differentiation based on haplotype statistics (Hst) was -0.008. Furthermore, global genetic differentiation (Kst) was 0.014. This value was highly significant. Also, the measure genetic differentiation based on nucleotide statistics (Z) was 83.226 while the nearest neighbour statistic (Snn) was 0.546. The Chi square value was 11.479. However, the value (0.4040) was not significantly different (p>0.05).

Table 2: Genetic Differentiation Estimates of myocilin gene among patients and control groups

Parameters	Values
Average gene diversity within population (Hs)	0.842
Measures of genetics differentiation based on haplotype statistics (Hst)	-0.008
Global genetic differentiation (Kst)	0.014*
Measure genetic differentiation based on nucleotide statistics (Z)	83.226
Nearest neighbour statistic (Snn)	0.546
Chi2:	11.479
P-value of Chi2	0.4040 ns

ns, not significant; *, 0.01<P<0.05; **, 0.001<P<0.01; ***, P<0.001

Figure 3 shows the network of haplotypes of myocilin gene in patients, control and Homo sapian. Haplotype 1 was only found in the Homo sapien nucleotide sequence downloaded from Genbank (NCBI website) used for this analysis. However, haplotype 2 was found in the control, patients, and human normal nucleotide. The control group contributed the largest volume of this haplotype. Haplotypes 3, 4 and 5 were peculiar to the patients only. These special haplotypes could be genetic markers for typing or detecting POAG in humans but this assertion is subject to further verification using a larger population size and among more ethnic groups.

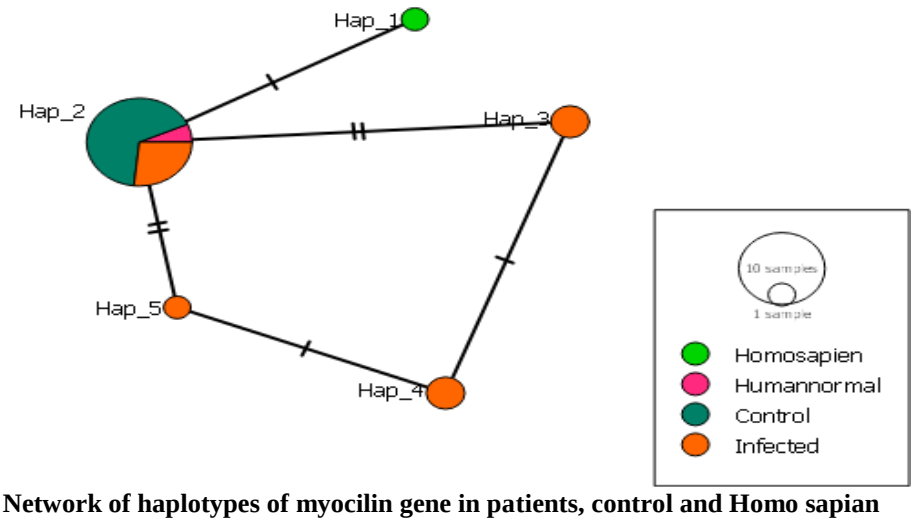

Figure 3. Network of haplotypes of myocilin gene in patients, control and Homo sapian

Table 3 shows gene flow estimates of myocilin gene among patients and control groups. Haplotype data information revealed a Gst value of 0.184 while the Nm value was 1.110. In addition, the sequence data information revealed a Delta St value of 0.001 and a Gamma St value of 0.312 while the Nm value was 0.550. However, the value of Nst and Nm according to Lynch and Crease 1990 (with Jukes and Cantor correction) were 0.396 and 0.380 respectively. The value of Fst and Nm according to Hudson, Slatkin and Maddison 1992 are 0.395 and 0.380.

Table 3: Gene Flow Estimates of myocilin gene among patients and control groups

Haplotype Data Information (Nei 1973)		Sequence Data Information (Nei 1982)			Lynch and Crease 1990 (with Jukes and Cantor correction)		Hudson, Slatkin and Maddison 1992	
Genetic diversity statistics(Gst)	Gene flow (Nm)	DeltaSt:	GammaSt:	Gene flow (Nm)	Nst:	Gene flow (Nm)	Fst:	Gene flow (Nm)
0.184	1.110	0.001	0.312	0.550	0.396	0.380	0.395	0.380

IV. Discussion

Variation in nucleotide sequences of myocilin gene of patients of POAG in the University of Port Harcourt were observed and were significantly different from those of the control. This variation could be as a result of mutations or other disposing factors.Brown (2002) reported that homologous genes are ones that share a common evolutionary ancestor, revealed by sequence similarities between the genes. These similarities form the data on which molecular phylogenies are based and may be linked to the prevalence of Adult - Onset Primary OpenAngle Glaucoma among patients used in this study.

Varying values of haplotype and nucleotide diversities in myocilin gene were observed with higher values obtained among the patient groups than the in control. Haplotypes 3, 4 and 5 which was peculiar to only patients with Adult - Onset Primary OpenAngle Glaucoma may contain sequences that may be used as biomarker in typing Adult - Onset Primary OpenAngle Glaucoma. However, further screening needs to be carried out to verify this claim. The results in this study are similar to those reported by Nakajima *et al*. (2002) who in their work on the nucleotide diversity and haplotype structure of the human angiotensinogen gene in two populations reported Six (6) major haplotypes, indicating less allelic complexity than in many other genomic regions although the two populations studies were found to share all of the major AGT haplotypes.There were substantial differences in haplotype frequencies

Varying levels of gene flow and genetic differentiations of myocilin gene were observed across the control groups and patients of POAG in this study. However, the values were not significant. The non significant differentiation observed in this study can limit gene flow among among patients and control groups in the study area (Abdellaoui*et al*. 2014). The genetic statistic (GST) values reported in this study describe genetic differentiation among populations. The low GST value of myocilin gene shows that there is little variation that is proportioned among populations. The low genetic variation existing among populations could be attributed to the sample size and for the fact that the respondents are from only rivers state. The low genetic variation of myocilin gene existing among populations implies that there is a low differentiation of myocilin gene among the study populations both patients and control groups. Genetic diversity statistics, (GST) values reported in this study describes the proportion of genetic diversity existing among the populations. According to Nei (1973) GST is equivalent to FST when there are only two alleles at a locus, and, in the case of multiple alleles, GST is equivalent to the weighted average of FST for all alleles of the myocilin gene.

Many reasons could be attributed to the varying values of gene flow indices recorded in this study. Gene flow is a collective term that includes all mechanisms resulting in the movement of genes from one population to another. The low values of gene flow in this work could be as a result of spatial isolation of populations. Pluess and Stocklin (2004) reported that spatial isolation of populations could lead to low genetic diversity within the population.Gene flow generally occurs within a species but examples of interspecific gene flow are known. Gene flow can also be due to the movement of gamates, the extinction and recolonization of entire populations, or the movement of extranuclear segments of DNA. FST is the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance. The small F_{ST} values reported in this work implies that the allele frequencies within each population are similar; if it is large, it means that the allele frequencies are different.

In humans gene flow usually comes about through the actual migration of human populations, either voluntary or forced. Although gene flow does not change allele frequencies for a species as a whole, it can alter

allele frequencies in local populations.Zhang *et al*. (2010) has reported that low gene flow among small, isolated populations is proposed to result in inbreeding, loss of heterozygosity because of genetic drift and genetic differentiation among populations. The wealth of available genetic information is allowing the reconstruction of human demographic and adaptive history. Population genetics offers an alternative approach, complementary to clinical and epidemiological genetic studies, for the identification of disease risk alleles/genes, the characterization of their properties, and the understanding of the relative contributions of human genetic variation to rare, severe disorders and complex disease phenotypes.These results have provided basic information needed to generate genetic markers for typing or detecting POAG in humans but subject to further verification.

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Conflicts of Interest: There are no conflicts of interest.

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