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Analysis of CYP_1B_1 Gene Mutations Spectrum in Families with Open-Angle Glaucoma in Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors MEK and AJU conceived, designed the research, performed laboratory work and wrote the first draft of manuscript. Authors RD and EE clinically characterized participants and collected samples. Authors OUU, AJU, EVI, EEE, OME, EO and MOO analyzed the data using statistical and bioinformatic tools. Authors SOA, NME, NE and NME managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Primary open-angle glaucoma is one of the commonest causes of blindness due to optic nerves damage because of elevated intraocular pressure and it is the commonest form of glaucoma. We determined the frequency of CYP_1B_1 gene mutations spectrum among families with open-angle glaucoma in Calabar.
Methods: Ophthalmologists examined the recruited subjects to establish the diagnosis on patients.

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Ninety-six (96) subjects were recruited, comprising 15 primary open-angle glaucoma patients, 11 primary childhood glaucoma patients, 17 parents and 53 healthy unrelated, aged-matched controls were selected from individuals attending the eye clinic. 2-3 ml of blood was collected from each participant, DNA extracted, PCRs and bidirectional sequencing performed on all subjects for CYP_1B_1 mutation on exon 3. The nucleotide sequences of the CYP_1B_1 gene were edited from chromatograms using the Bio edit software. Multiple sequence alignment and pairwise comparison of CYP_1B_1 gene was carried out on MEGA 6.06 software. GORIV software was used in determining the secondary structure for each individual with and without the CYP_1B_1 gene mutation. Phyre2 software was utilized for predicting the 3D tertiary structure for individuals. Statistical analyses were performed using SPSS version 20.0 software. Significant level was set at $P<0.05$.

Results: CYP_1B_1 gene were amplified and sequenced from all recruited participants for this study. The mean age of primary childhood glaucoma was 98.36±12.43 months and 56.44±7.1 years for POAG, while the mean age at disease presentation of 43.42±1.4 months and 52.33±7.4 years for primary childhood glaucoma and POAG, respectively. The mean intraocular pressure (IOP) in right/left eye was 24/23 mmHg and 32.1/31 mmHg for primary childhood glaucoma and POAG, respectively. All cases of glaucoma were bilateral. Two non-synonymous mutations (g.291G>C, g344C>T) and 3 synonymous mutations (g.1T>C, g.1T>G, g.46T>C) of CYP_1B_1 gene mutations spectrum were observed in primary childhood glaucoma, POAG and parents. The g.291G>C (p.Q97H) recorded the frequency of 36.4%, 33.3% and 29.4% for primary childhood glaucoma, POAG patients and parents respectively. Eight different nucleotides deletions including g.2-4delCTC, g.2delC, g.317delT, g.535delG, g.378-380delATG, and others were detected. The deletion g.370-380delATG frequency was 27.3%, 20.5%, and 41.2% for primary childhood glaucoma, POAG patients and parents respectively. The CYP_1B_1 gene mutations spectrums were not detected in all controls.

Conclusion: This research identified CYP_1B_1 gene mutations spectrum, which were detected in primary childhood glaucoma, POAG cases and parents but not observed in controls. It suggests molecular genetics etiology of the disease in Calabar population, but further studies are required. This will forms baseline information for further molecular studies among glaucoma patients in Nigeria.

Keywords: Open-angle glaucoma; CYP_1B_1 gene; mutations; patients; Calabar.

1. INTRODUCTION

Glaucoma is a complex heterogeneous group of optic neuropathy, characterized by elevated intra-ocular pressure, which results in progressive damage of retinal ganglion cell, causing vision loss and irreversible blindness [1]. It is one of the most frequent causes of irreversible blindness globally, due to late diagnosis or resistance to therapy [1]. Open-angle glaucoma is classified as primary when no anatomical identifiable underlying cause of the events that led to outflow obstruction and intraocular pressure (IOP) elevation can be found. The etiology is generally regarded as an abnormality in the trabecular meshwork and genes mutations are responsible for the fraction of families with primary open-angle glaucoma [2, 3]. Primary open-angle glaucoma (POAG) is the most common causes of blindness and one of the common form of glaucoma, representing half of all cases of glaucoma [1,2]. Currently, some genes have been associated with POAG namely; CYP_1B_1, myocilin, optineurin and WDR 36 (WD repeat domain 36 gene) and these genes had been identified in some populations [2-7]. Positive family history of glaucoma, outflow facility, optic nerve head and intraocular pressure are known to be important risk factors and are believed to be inherited [8, 9]. CYP_1B_1 mutations have been documented in families with both primary childhood glaucoma and POAG coexisted, and sporadic cases of POAG in various populations [2,6,]. The pathogenesis of glaucoma is multifactorial [10] and sometimes obscure or unknown [11,12]. POAG have been associated with risk factors like central corneal thickness, structure of the optic nerve head, age, genetic factors, race, and intraocular inflammation. A high prevalence of POAG cases are observed among Africans [13]. In Northwest Nigeria, precisely Dambatta district, POAG amounted for 15% of blindness and 70% of individual visually impaired [14]. In Southwestern Nigeria, glaucoma recorded a blindness rate of 11.1% [15]. Genetic screening through targeted gene finding may be helpful in early disease detection, treatment and prognostication of

disease [16]. The human cytochrome $P450_1B_1$ (CYP_1B_1) gene displays a high degree of allelic heterogeneity, and more than 70 different mutations of this gene causal to glaucoma have been identified and reported [2,4,6,7,17,18].

Childhood glaucoma is a heterogeneous group of eye disorders that shares the same final pathways of ocular hypertension, intraocular pressure that damage optic structures [19,20]. Primary childhood glaucoma refers to two major groups of paediatric glaucoma namely; primary congenital glaucoma (PCG) and juvenile open-angle glaucoma (JOAG) [21]. These glaucoma sometimes are genetic in origin and could be linked with systemic diseases and other ocular defects [22]. PCG is characterized by elevated intraocular pressure (IOP) because of an obstruction of aqueous outflow from the anterior segment of the eye, due anatomical defects in the trabecular meshwork [23]. Children affected with the disease typically present with the following triad of symptoms like photophobia, epiphora, corneal clouding, enlargement of globe or cornea and the occurrence of this eye disorder is both sporadic and familial cases [24].

Primary congenital glaucoma is subdivided based on age at onset into: neonatal or new born: 0-1 month, infantile: >1-24 months and late-onset or late-recognized: of more than two years. The second category of primary childhood glaucoma called JOAG has no ocular enlargement, no congenital ocular anomalies or syndromes and an open angle with normal appearance [20].

A population based research accounted for 23% of primary childhood glaucoma in Florida and Caribbean Islands [19], 2.29% per 100,000 residents in Minnesota [25] and 43% in Calabar, Cross River State, Nigeria [26]. The primary childhood glaucomas are chronic, life long and difficult to treat. Glaucoma may eventually lead to blindness if not properly treated with appropriate surgical methods. Molecular genetic screenings and other medical diagnosis are very relevant in the characterization, management and treatment of glaucoma [6,26].

Genetic studies are used to identify, confirm, treat and prognosticate diseases [16,27,28]. The CYP_1B_1 gene display a high degree of allelic heterogeneity in some populations investigated and different alterations of this gene are associated with the molecular etiology of glaucomas [6,24,28-34]. The frequency of CYP_1B_1 gene mutations in different populations varies from 20% to 70% globally, and the common mutations are clustered on specific haplotype background [31,33,34]. In different populations, various molecular genetics studies had been conducted concerning the association of CYP_1B_1 gene mutations with open-angle glaucoma [4,6,7,34]. There is no documented research on CYP_1B_1 gene mutations spectrum in families with open-angle glaucoma in Calabar, Nigeria. Thus, this research seeks to investigate the frequency of CYP_1B_1 gene mutation spectrum in families with open-angle glaucoma in Calabar, Nigeria. This clinical and molecular-genetic screening approach will aid ophthalmologists in decisive counseling, treatment and management of this eye disorder.

2. MATERIALS AND METHODS

2.1 Subjects Recruitment and DNA Analysis

The research adhered strictly to the tenets of the declaration of Helsinki by obtaining full approval from the Institutional Review Board of the hospitals (Certificate number: UCTH/HREC/33/400 and CRSMH/CGS&E-H/018/Vol.1/40) before subjects were recruited. Ophthalmic examinations were performed on recruited subjects to establish the diagnosis for primary childhood glaucomas (primary congenital glaucoma and juvenile open-angle glaucoma) and primary open-angle glaucoma by more than one ophthalmologist after obtaining informed consent from each participant. Patients included in the research underwent a complete ophthalmic examination: an anterior segment examination, determination of uncorrected and best-corrected visual acuity, gonioscopy, a fundus examination with a cup-disc ratio evaluations and a tonometric curve and visual field examination. At the end of the examination, recruited subjects were placed into 1 of 3 groups namely: (1) those with healthy eyes (not affected by glaucoma), (2) those with ocular hypertension or suspected glaucoma (intra-ocular pressure greater than 21mmHg and no visual field defects) and (3) those affected by glaucomas (primary childhood glaucoma and POAG). Patients were defined as affected when they showed at least two of the following criteria: glaucomatous visual field defects (based on the corrected-pattern SD and the mean deviation perimetric indexes), ocular hypertension (intra-ocular pressure greater than 21 mmHg), and optic nerve head glaucomatous changes (cup-disc ratio greater than 0.7 or notches). Samples collections were carried out

between 2014 – 2016 in three different hospitals namely; University of Calabar Teaching Hospital, Elim Eye Hospital, and College of Health Technology Eye Clinic, all in Calabar, Cross River State, Nigeria. A total of 96 participants, comprising 15 primary open-angle glaucoma patients, 11 primary childhood glaucoma patients (five primary congenital glaucoma and seven Juvenile open-angle glaucoma), 17 parents of the glaucoma patients and 53 healthy unrelated, aged-matched controls were recruited for this study. The control participants were of two groups namely: 30 children control and 21 adult control group. They were controls for primary childhood glaucoma (primary congenital glaucoma and JOAG) and adult open-angle glaucoma, respectively. All participants recruited as controls were individuals attending the eye clinic for general eye examinations that has no glaucoma or history of glaucoma. Demographic details and clinical variables of patients, parents and relatives were collected as interview initiated and secondary data from patient files.

Venous blood samples of 2–3mls were collected from each glaucoma patients, parent and controls. The blood samples were stored in EDTA bottles neatly labeled and kept at a temperature of -20°C until it was transported to International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria where the genetic analysis was performed.

2.2 Genomic DNA Extraction and PCR

DNA extractions, PCRs were carried out at the Department of Virology and Molecular Diagnostic Units, IITA, Nigeria. DNA sequencing was performed at the DNA Facility Laboratory, Iowa State University, Ames, USA. DNA was extracted from EDTA whole blood samples in a similar fashion as previously documented [35] but with few modifications. 150 µl of whole blood was put into 1.5ml of sterile Eppendorf tubes placed on rack neatly labeled and 350 µl of extraction buffer was added to the tubes. 40 µl of Sodium dodecyl sulphate was added to the tubes and then inverted four times before incubated in a water bath at 65^0C for 10 minutes. Then 160µl of Potassium acetate was added to the tubes. The tubes were inverted three times and centrifuged at 10,000 rcf for 10 minutes. 400µl of supernatant was carefully transferred into new Eppendorf tubes and 200µl of cold isopropanol was then added. The tubes were inverted gently for six times to precipitate DNA. The tubes were then kept in freezer at -20°C for 30 minutes. The tubes were centrifuged at 10,000 rcf for 10 minutes to sediment the DNA. The supernatant was carefully decanted in order to ensure that pellet was not disturbed and 500 µl of cold ethanol was added to the pellet to wash the DNA and centrifuged again at 10,000 rcf for 10 minutes. The ethanol was decanted and the DNA was air dried at room temperature until no traces of ethanol was observed in the tubes. The modifications were: 1% monothioglycerol was added to the extraction buffer instead of mercapto-ethanol and the tubes were kept in the freezer (– 20°C) for 10-20 minutes after the addition of cold isopropanol. PCR amplification were performed in 50 µl cocktail containing 4 µl of genomic DNA, 10 µl of PCR buffer, 3 µl of $MgCl_2$, 1.0 µl of dNTPs, 1.0 µl of each primer (forward and reverse primer), 29.76µl of nuclease free water, and 0.24 µl of Taq DNA polymerase. The primers sequences utilized were previously documented [36] for targeted CYP_1B_1 gene on exon 3 and intron-exon boundary and the pair of primer is as follows: GL3-F1- CTCACTTGCTTTTCTCTCTCC and GL3-R1- CATCACTCTGCTGGTCAGGT.

2.3 Cycling Conditions

Initial denaturation at 95°C for three minutes. Then 35 cycles of denaturation at 95°C for one minute, annealing at 58 to 62°C for one minute, and elongation at 72°C for one minute, then a final extension steps of 10 minutes at 72°C. 5µl of the amplicons was checked on agarose gel electrophoresis for PCR amplification of forward and reverse primers in exon 3. The amplicons were purified using the documented protocols [37]. Seventy-five micro liters of 95% ethanol was added to eppendorf tubes containing 30µl of PCR amplicons and inverted 3-5 times. The tubes were then transferred into -20^0C freezer for one hour. Then the tubes were centrifuged at 12000 rcf for 10 minutes. The supernatant was decanted gently and 500µl of cold 70% ethanol was added, centrifuged again at 12000 rcf for 5 minutes. Then the alcohol was decanted and tubes air dried at room temperature until no traces of alcohol were observed. The purified amplicons were re-suspended in double distilled water and stored in the freezer until packaging and transportation to DNA Facility Laboratory, Iowa State University, USA for bidirectional sequencing of all PCR products.

2.4 Bioinformatics and Mutational Analysis of Sequenced CYP_1B_1 Gene

The amplicons were screened for CYP_1B_1 gene mutation on exon 3 and intron-exon boundary

using ABI 3730 XL sequence (Applied Biosystems, USA). The nucleotide sequences of the CYP_1B_1 gene were edited from the chromatograms using Bioedit software [38]. Multiple sequence alignment was performed using CLUSTAL W in MEGA 6.06 software [39]. The nucleotide sequence of the targeted gene from patients, parents of glaucoma patients and controls were compared with the published CYP_1B_1 sequence by blasting NCBI gene bank to query for similarity on the database. The GORIV software was used in determining the secondary structure for individual with CYP_1B_1 gene mutation and those without the gene mutation. The Phyre^2 software was used for predicting of 3D tertiary structure for individual with CYP_1B_1 gene mutations and cases without the gene mutation. The extracted nucleotide sequence was first transformed into amino acids sequence using the GENSCAN software before pasting the translated amino acids sequence on FASTA format into the window of the Phyre^2 for prediction of tertiary protein structure. The tertiary structures were displayed and viewed using RasMol software version 2.7.5.2.

2.5 Statistical Analysis of Socio-demographic and Clinical Variables

The statistical analyses of socio-demographic and clinical data were carried out using Statistical Package for Social Sciences, (SPSS) version 20.0. Quantitative and Clinical variable were compare using chi-square (X^2) and simple percentage.

3. RESULTS

Exon 3 and the exon-intron boundary of CYP_1B_1 gene were amplified and sequenced from all 5 primary congenital glaucoma, 7 juvenile open-angle glaucoma (JOAG), 15 primary open-angle glaucoma (POAG), 17 parents, and 53 unrelated aged-matched controls recruited in three hospitals, Calabar, Nigeria. Table 1 summarizes the socio-demographic distribution of primary congenital glaucoma, juvenile open-angle glaucoma cases and the children control group with males having the frequency of 27.3%, 36.3% and 46.7%, respectively. The mean age of primary childhood glaucoma was 98.36±12.43 months and10.80±1.92 years for children control group. Table 2 shows demographic variables of primary open-angle glaucoma patients and adult control group. Males (66.7%) and females (33.3%) have a mean age of 56.44±7.1 years for primary open-angle glaucoma patients, while the mean age of unrelated adult control group was 48.1±3.6 years. There were no significant differences in gender, ethnicity between patients and controls. Table 3 shows clinical variables in primary childhood glaucoma and primary open-angle glaucoma patients with a mean age at disease presentation of 43.42±1.4 months and 52.33±7.4 years, respectively. The mean intraocular pressure (IOP) in left/right eye was 23/24 mmHg and 31/32.1 mmHg for primary childhood glaucoma and primary open-angle glaucoma, respectively. All cases of glaucoma were bilateral.

Table 1. The socio-demographic distribution of primary childhood glaucoma cases

Variables	Primary congenital glaucoma age <1-3 years	Juvenile open-angle glaucoma >3 years	Children control n=30	X^2	df	P-value
Gender	Male 3(27.3%) Female 2(18.2%)	Male 4(36.3%) Female 2(18.2%)	Male 14(46.7%) Female 16(53.3%)	0.34	1	0.61
Ethnicity	Bekwarra 1(9.1%) Boki 0(0.00%) Efiks 2(18.2%) Ibibio 3(27.3%) Igbo 0(0.00%)	0(0.00%) 1(9.1%) 1(9.1%) 2(18.2%) 1(9.1%)	5(16.7%) 4(13.3%) 9(30.0%) 10(33.3%) 2(6.7%)	0.27	4	0.32
Positive family history of glaucoma	Father 1(9.1%) Mother 2(18.2%)	1(9.1%) 2(11.2%)	_ _	1.09	2	0.81
Clinical suspect of glaucoma	98.36±12.43 (months)			0.130	1	0.401
Mean age (months/year)			10.80±1.92 years			

Table 2. Demographic variables of primary open-angle glaucoma patients

Variables	POAG (n=15)	Adult controls (n=23)	X^2	Df	p-value
Gender	Males 10 (66.7%) Females 5(33.3%)	12(57.1%) 9(42.9%)	0.36	1	0.57
Ethnicity	Efik 3(20.0%) Ibibio 3(20.0%) Ibo 6(40.0%) Yoruba 1(6.7%) Anang 2(13.3%)		0.87	4	0.15
Mean age	56.44±7.10 (years)	48.1±3.6 (years)			

Table 3. Clinical variables in primary childhood glaucoma and POAG cases

Type of glaucoma	Blindness (%)	Means age at disease presentation	Mean IOP (mmHg) RE/LE	Mean C/D (mm) RE/LE	Laterality
PCG	0.00	43.42±1.4 (mth)	23/24	0.5/0.4	B
POAG	66.7	52.33±7.4 (yrs)	31/32.3	0.9/0.8	B

IOP: Intraocular pressure, C/D: Cup to disc ratio, RE: Right eye, LE: Left eye, B: Bilateral cases, PCG: Primary childhood glaucoma, POAG: Primary open-angle glaucoma, Mth: Months, Yrs: Years

Figure 1 displays the results of the PCR amplification of the CYP_1B_1 gene that was sequenced and Figure 2 shows the chromatogram of the nucleotide substitution of guanine by cytosine at position 291 on exon 3. Figure 3 displays 3D tertiary structures of protein (amino acids) in individual that has no CYP_1B_1 mutation and individual that has CYP_1B_1 gene mutations. Table 4 summarizes CYP_1B_1 gene mutation spectrum (missense mutations and deletions) in primary childhood glaucoma (primary congenital glaucoma and juvenile open-angle glaucoma), primary open-angle glaucoma and parents with the frequency of 36.4%, 33.3% and 29.4% for g.291G>C (p.Q97H) non-synonymous missense mutation, respectively. The deletion g.370-380delATG frequency was 27.3%, 20.5%, and 41.2% for primary childhood glaucoma, primary open-angle glaucoma patients and parents, respectively. The CYP_1B_1 gene mutation spectrum was not detected in controls.

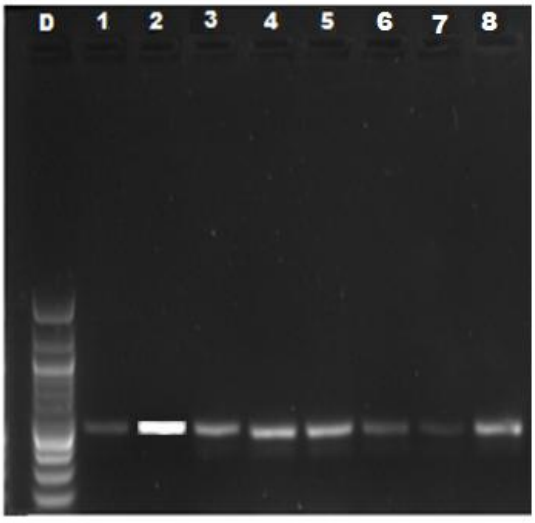


Figure 1: Agarose gel electrophoresis displaying amplicons of CYP1B1 gene after PCR amplification. Lane D is the 1kb DNA ladder and 1-8 contain the amplicons.

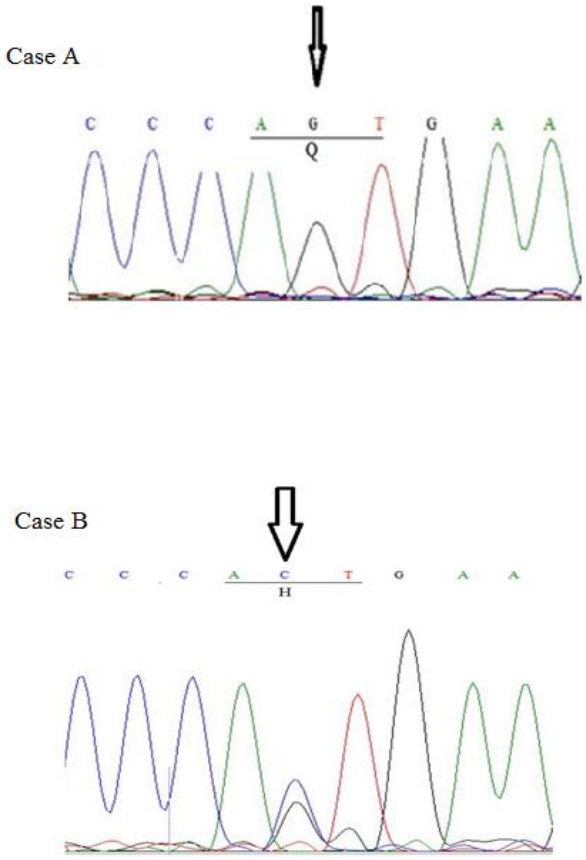
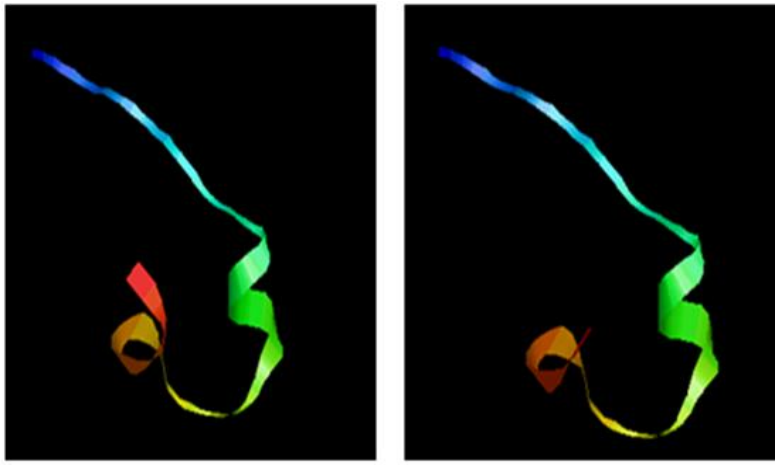


Figure 2: g.219G>C missense mutation on exon 3 of the CYP1B1 gene. Case A on the upper panel (control) has no mutation while case B (patient) has mutation. The arrows indicate the G>C substitution and the codons underlined: Q and H represent Glutamine and Histidine respectively.



3a: Case without mutation 3b: Case with mutation

Figure 3: 3a represents 3D tertiary structures of protein for case without CYP1B1 gene mutations while 3b has CYP1B1 gene mutation.

Table 4. CYP_1B_1 Mutations spectrum among patients and parent

CYP_1B_1 mutations	% of PCG with mutations (n=11)	% of POAG with mutations n=15	% of parents with mutations n=17
g.291G>C	36.4	33.3	29.4
g.344C>T	27.3	26.7	17.6
g.1T>C	18.2	26.7	1.76
g.1T>G	9.1	13.3	0.0
g.46T>C	9.1	0.0	0.0
g.2del C	9.1	0.0	0.0
g.2-4delCTC	9.1	0.0	5.9
g.2-18delCTCACTTGCTTTTCTCT	18.2	0.0	0.0
g.317delT	9.1	0.0	0.0
g.535delG	9.1	6.7	0.0
g.324-380CTCGATTCTTGG…ATG	9.1	0.0	17.6
g.370-380delGCAGAGTGATG	18.2	33.3	35.3
g.378-380delATG	27.3	20.5	41.2

PCG: Primary childhood glaucoma, POAG: Primary open-angle glaucoma

4. DISCUSSION

Primary open-angle glaucoma and other forms of glaucoma represent significant public health problems in different populations [1-8,14,15,28-33]. The human cytochrome $P450_1B_1$ (CYP_1B_1) gene plays a functional role in the trabecular meshwork development of the eye [12], and mutations of this candidate gene is associated with molecular etiology of glaucoma as documented [4,6,7]. In our study in Calabar, we detected CYP_1B_1 gene mutations in primary congenital glaucoma, juvenile open-angle glaucoma (JOAG) and primary open-angle glaucoma (POAG) which correlated to clinical variables like increased IOP, cup/disc ratio considered as important risk factors. This result is in harmony with other documented findings [7, 40]. Analysis of CYP_1B_1 gene mutation spectrum in primary congenital glaucoma patients in Brazil were associated with severe, rapid disease manifestation and required more surgical operations to control intraocular pressure [41]. Previously, [36] documented that some PCG cases in Kuwait responded poorly to treatment, and surgery which correlate to CYP_1B_1 gene mutations. These findings were similar to our present study in Calabar. The damaging effects attributed to CYP_1B_1 gene mutation were associated with primary congenital glaucoma cases in Tunisia [30]. Previous documented studies have shown that CYP_1B_1 gene mutations affect highly conserved and functionally important regions of the gene, resulting in significant structural changes, reduced CYP_1B_1 activity associated to the disease etiology [42, 43,44]. In our present study, the 3D tertiary structure of protein (amino acids) observed in glaucoma patients having CYP_1B_1 mutations shows complex irregular structural changing regions when compare with cases without CYP_1B_1 mutations, suggesting damaging effects due to this gene mutations.

We detected g.291G>C non-synonymous missense mutation among primary CYP_1B_1 childhood glaucoma (primary congenital glaucoma and JOAG), primary open-angle glaucoma cases and parents in Calabar, resulting in a substitution of glutamine by histidine at position 97(p.Q97H). The same non-synonymous missense mutation was reported in Nigeria among primary childhood glaucoma cases [45]. The g.291G>C missense mutation in our study was associated with rapid disease manifestation and progression, but G>A transition of missense mutation on exon 3 (substitution of met364val) was reported among primary congenital glaucoma patients in Japan [46], Indonesians and Europeans [29]. Another non-synonymous missense mutation namely g.344C>T (p.T115M) was observed in different forms of glaucoma patients and parents at varying frequency in this present study at Calabar. In France, [31] documented the g.4547C>T and g.8167C>T missense mutations, while in Korea, [47] observed the g.55C>T (p.Q19X) non-synonymous missense mutations of CYPIBI gene known to cause primary congenital glaucoma in children. These researchers [31, 47] documented on the same C>T missense mutations but at different nucleotide site. In Auckland, New Zealand, 6.1% of primary open-angle glaucoma cases showed pathogenic CYP_1B_1 non-synonymous missense mutation for p.Ser6Gly and p.Val243Leu as

reported [6]. All the non-synonymous missense mutations were detected in primary congenital glaucoma, JOAG, POAG patients and some parents of glaucoma cases in our study; suggesting parent-of-origin effect in some families, and [48] documented a parent-of-origin effect of ocular disease among families in Germany. Synonymous mutations namely: g.1T>C, g.1T>G, g.46T>C were detected among glaucoma patients and some parent in this present study and to the best of our knowledge they were novel from the literature reviewed.

The g.535delG of CYP_1B_1 mutation was detected among some primary congenital glaucoma, JOAG and POAG patients in this study. This same g.535delG deletion was previously reported among primary congenital glaucoma patients in Portuguese [29], Brazil [49], Tunisia [30], Morocco [50] and in Nigeria among primary childhood glaucoma patient [45]. Also, eleven base nucleotides deletions namely: g.324-380delGCAGAGTGATG was detected among some primary congenital glaucoma, JOAG, POAG patients and their parents that were diagnosed with glaucoma. It was interesting to note that both glaucoma patients and their parents that had CYP_1B_1 gene mutations have severe clinical presentations with elevated intraocular pressure and responded poorly to treatment, especially when deletions and missense mutations were acting in consort to each other. These similar disease presentations corroborate to the report of [51] and [6] that documented multiallelic contribution of CYP_1B_1 gene mutations to open–angle glaucoma phenotypes. Therefore, we can suggest that the disease predisposition in these families may be familial, pointing to hereditary forms of glaucoma. To the best of our knowledge through literature search, deletions g.324-380delGCAGAGTGATG, g.317delT, g.2delC, g.2-4delCTC, g.2-18delCTCACTTGCTTTTCTCT are novel and were detected in some primary congenital glaucoma, JOAG and POAG cases, but not detected in controls. All primary congenital glaucoma, JOAG, POAG patients recruited for this research were bilateral and more males were presented with this eye disorder, similar to previously reported research in Nigeria [52], Iran [53] and Brazil [40] where more male cases had the disease. The human CYP_IB_I gene is known to be involved in trabecular meshwork development of the ocular structure, which function as a drainage pathways for aqueous humour [54,55] and mutations of this CYP_IB_I gene is associated with the molecular etiology of glaucoma in some populations [3,4,6-8,23,27-33, 44-47]. In this study, analysis of CYP_IB_I gene mutations spectrum in families with open-angle glaucoma showed reasonable role in the molecular pathogenesis of the disorder and correlated to clinical variables like increased intraocular pressure (IOP), cup/disc ratio considered as important risk factors [40, 7]. The CYP_IB_I gene mutations spectrum identified in families with open-angle glaucoma in this present study need further investigation in larger populations for significant conclusions to be drawn.

5. CONCLUSION

Analysis of CYP_1B_1 gene mutations identified two non-synonymous missense mutations (g.291G>C, g.344C>T) and deletions including g.370-380delGCAGAGTGATG, g.2delC, g.317delT among families with open-angle glaucoma patients and their parent, suggesting molecular etiology of the disease.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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