

ANALYSIS OF CYP₁B₁ GENE MUTATIONS SPECTRUM IN FAMILIES WITH OPEN-ANGLE GLAUCOMA IN CALABAR, NIGERIA

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 CYP₁B₁ gene mutations spectrum among families with open-angle glaucoma in Calabar.

Methods: Ophthalmologists examined the recruited subjects to establish the diagnosis on patients. 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 mls of blood was collected from each participant, DNA extracted, PCRs and bidirectional sequencing performed on all subjects for CYP₁B₁ mutation on exon 3. The nucleotide sequences of the CYP1B1 gene were edited from chromatograms using the Bioedit software. Multiple sequence alignment and pairwise comparison of CYP1B1 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 CYP1B1 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: CYP1B1 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 left/right eye was 23/24 mmHg and 31/32.1 mmHg for primary childhood glaucoma and POAG, respectively. All cases of glaucoma were bilateral. Two non-synonymous mutations (g.291G>C, g.344C>T) and 3 synonymous mutations (g.1T>C, g.1T>G, g.46T>C) of CYP1B1 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 CYP1B1 gene mutations spectrums were not detected in all controls.

Conclusion: This research identified CYP1B1 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; CYP₁B₁ gene; mutations; patients; Calabar.

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; CYP₁B₁, 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]. CYP₁B₁ 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 P450B1 (CYP₁B₁) 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 CYP₁B₁ 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 CYP₁B₁ 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 CYP₁B₁ gene mutations with open-angle glaucoma [4, 6, 7, 34]. There is no documented research on CYP₁B₁ gene mutations spectrum in families with open-angle glaucoma in Calabar, Nigeria. Thus, this research seeks to investigate the frequency of CYP₁B₁ 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.

MATERIALS AND METHODS

Subjects recruitment and DNA analysis

The research adhered strictly to the tenets of the declaration of Helsinki by obtaining full approval of the Institutional Review Board from hospitals where 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. Samples collections were carried out between 2014 - 2016 in the Department of Ophthalmology, 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.

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 in a similar fashion as previously documented [35] but with few modifications, 1% monothioglycerol was added to the extraction buffer instead of mecaptop-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 50µl cocktail containing 4µl of genomic DNA, 10µl of PCR buffer, 3µl of MgCl₂, 1.0µl of dNTPs, 1.0µl of each primer (forward and reverse primer), 29.76µl of nuclease free water, and 0.24µl of Taq DNA polymerase. The primers sequences utilized were previously documented [36] for targeted CYP₁B₁ gene on exon 3 and intron-exon boundary and the pair of primer is as follows: GL3-F1- CTCACTTGCTTTTCTCTCTCC and GL3-R1- CATCACTCTGCTGGTCAGGT.

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°C 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.

Bioinformatics and mutational analysis of sequenced CYP1B1 gene

The amplicons were screened for CYP1B1 gene mutation on exon 3 and intron-exon boundary using ABI 3730XL sequence (Applied Biosystems, USA). The nucleotide sequences of the CYP1B1 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 CYP1B1 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 CYP1B1 gene mutation and those without the gene mutation. The Phyre² software was used for predicting of 3D tertiary structure for individual with CYP1B1 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 Phyre² for prediction of tertiary protein structure. The tertiary structures were displayed and viewed using RasMol software version 2.7.5.2.

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 (X²) and simple percentage.

RESULTS

Exon 3 and the exon-intron boundary of CYP1B1 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 and 10.80±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

191 pressure (IOP) in left/right eye was 23/24 mmHg and 31/32.1 mmHg for primary childhood
192 glaucoma and primary open-angle glaucoma, respectively. All cases of glaucoma were
193 bilateral.

194 Figure 1 displays the results of the PCR amplification of the CYP1B1 gene that was
195 sequenced and figure 2 shows the chromatogram of the nucleotide substitution of guanine by
196 cytosine at position 291 on exon 3. Figure 3 displays 3D tertiary structures of protein (amino
197 acids) in individual that has no CYP1B1 mutation and individual that has CYP1B1 gene
198 mutations. Table 4 summarizes CYP1B1 gene mutation spectrum (missense mutations and
199 deletions) in primary childhood glaucoma (primary congenital glaucoma and juvenile open-
200 angle glaucoma), primary open-angle glaucoma and parents with the frequency of 36.4%,
201 33.3% and 29.4% for g.291G>C (p.Q97H) non-synonymous missense mutation, respectively.
202 The deletion g.370-380delATG frequency was 27.3%, 20.5%, and 41.2% for primary
203 childhood glaucoma, primary open-angle glaucoma patients and parents, respectively. The
204 CYP1B1 gene mutation spectrum was not detected in controls.

205 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		

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211 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)			

212 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

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

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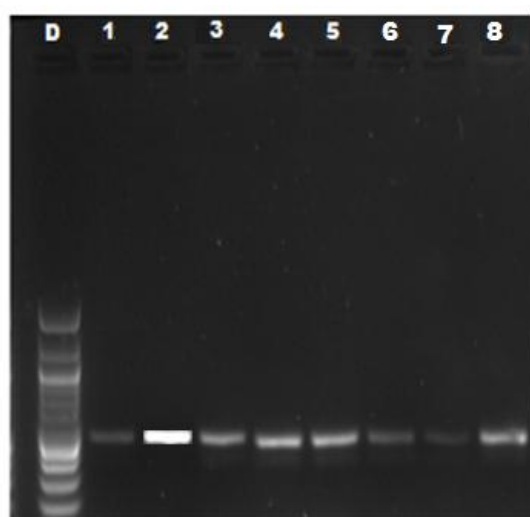


Figure 1: Agarose gel electrophoresis displaying amplicons of CYP1B1 gene after PCR amplification. Lane D is the 1kb DNA ladder and 1-8 contain the amplicons.

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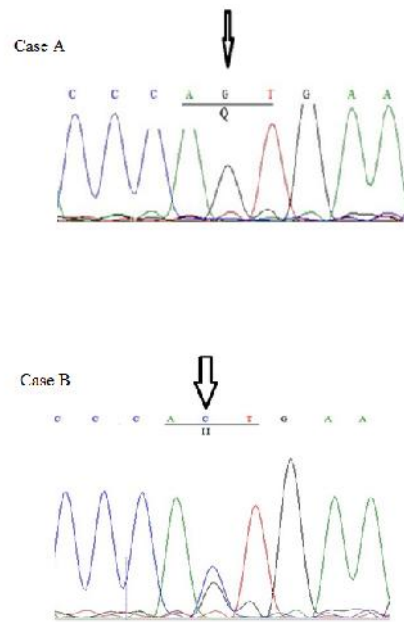


Figure 2: g.219G>C missense mutation on exon 3 of the CYP1B1 gene. Case A on the upper panel (control) has no mutation while case B (patient) has mutation. The arrows indicate the G>C substitution and the codons underlined: Q and L represent Glutamine and L-leucine respectively.

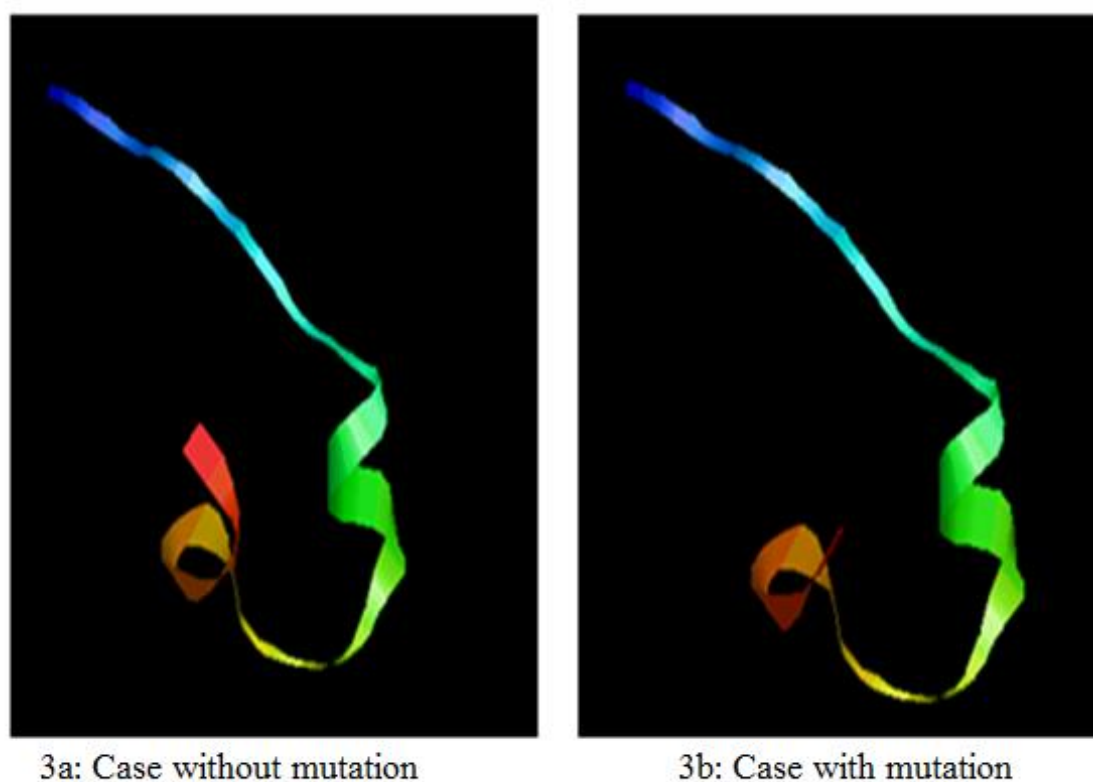


Figure 3: 3a represents 3D tertiary structures of protein for case without CYP1B1 gene mutations while 3b has CYP1B1 gene mutation.

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231 Table 4: CYP1B1 Mutations spectrum among patients and parent

CYP1B1 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
232	PCG: Primary childhood glaucoma, POAG: Primary open-angle glaucoma			
233	DISCUSSION			
234	Primary open-angle glaucoma and other forms of glaucoma represent significant public			
235	health problems in different populations [1-8, 14, 15, 28-33]. The human cytochrome P450 _{1B1}			
236	(CYP _{1B1}) gene plays a functional role in the trabecular meshwork development of the eye			
237	[12], and mutations of this candidate gene is associated with molecular etiology of glaucoma			
238	as documented [4, 6, 7]. In our study in Calabar, we detected CYP1B1 gene mutations in			
239	primary congenital glaucoma, juvenile open-angle glaucoma (JOAG) and primary open-angle			
240	glaucoma (POAG) which correlated to clinical variables like increased IOP, cup/disc ratio			
241	considered as important risk factors. This result is in harmony with other documented			
242	findings [7, 40]. Analysis of CYP1B1 gene mutation spectrum in primary congenital			
243	glaucoma patients in Brazil were associated with severe, rapid disease manifestation and			
244	required more surgical operations to control intraocular pressure [41]. Previously, [36]			
245	documented that some PCG cases in Kuwait responded poorly to treatment, and surgery			
246	which correlate to CYP1B1 gene mutations. These findings were similar to our present study			
247	in Calabar. The damaging effects attributed to CYP1B1 gene mutation were associated with			
248	primary congenital glaucoma cases in Tunisia [30]. Previous documented studies have shown			
249	that CYP1B1 gene mutations affect highly conserved and functionally important regions of			
250	the gene, resulting in significant structural changes, reduced CYP1B1 activity associated to			
251	the disease etiology [42, 43, 44]. In our present study, the 3D tertiary structure of protein			
252	(amino acids) observed in glaucoma patients having CYP1B1 mutations shows complex			
253	irregular structural changing regions when compare with cases without CYP1B1 mutations,			
254	suggesting damaging effects due to this gene mutations.			
255	We detected g.291G>C non-synonymous missense mutation among primary childhood			
256	glaucoma (primary congenital glaucoma and JOAG), primary open-angle glaucoma cases and			
257	parents in Calabar, resulting in a substitution of glutamine by histidine at position			
258	97(p.Q97H). The same non-synonymous missense mutation was reported in Nigeria among			
259	primary childhood glaucoma cases [45]. The g.291G>C missense mutation in our study was			
260	associated with rapid disease manifestation and progression, but G>A transition of CYP1B1			
261	missense mutation on exon 3 (substitution of met364val) was reported among primary			
262	congenital glaucoma patients in Japan [46], Indonesians and Europeans [29]. Another non-			
263	synonymous missense mutation namely g.344C>T (p.T115M) was observed in different			
264	forms of glaucoma patients and parents at varying frequency in this present study at Calabar.			
265	In France, [31] documented the g.4547C>T and g.8167C>T missense mutations, while in			
266	Korea, [47] observed the g.55C>T (p.Q19X) non-synonymous missense mutations of			
267	CYP1B1 gene known to cause primary congenital glaucoma in children. These researchers			
268	[31, 47] documented on the same C>T missense mutations but at different nucleotide site. In			
269	Auckland, New Zealand, 6.1% of primary open-angle glaucoma cases showed pathogenic			
270	CYP _{1B1} non-synonymous missense mutation for p.Ser6Gly and p.Val243Leu as reported [6].			
271	All the non-synonymous missense mutations were detected in primary congenital glaucoma,			
272	JOAG, POAG patients and some parents of glaucoma cases in our study; suggesting parent-			
273	of-origin effect in some families, and [48] documented a parent-of-origin effect of ocular			
274	disease among families in Germany. Synonymous mutations namely: g.1T>C, g.1T>G,			
275	g.46T>C were detected among glaucoma patients and some parent in this present study and			
276	to the best of our knowledge they were novel from the literature reviewed.			
277	The g.535delG of CYP1B1 mutation was detected among some primary congenital			
278	glaucoma, JOAG and POAG patients in this study. This same g.535delG deletion was			
279	previously reported among primary congenital glaucoma patients in Portuguese [29], Brazil			
280	[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 CYP1B1 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 CYP1B1 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.2-18delCTCACTTGCTTTCTCT 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 CYP1B1 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 CYP1B1 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 CYP1B1 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 CYP1B1 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.

Conclusion

Analysis of CYP1B1 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. However within families where mutations of CYP1B1 gene are found, a molecular genetic test may be very relevant in terms of pre symptomatic diagnosis for relatives who may benefit from early treatment. The information obtained in this study will form baseline for understanding the molecular-genetic etiology of the disease in Calabar, although further studies are needed.

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