EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 01
97-100
Year: 2017

MUTATIONS OF THE HUMAN CYP1B1 GENE IN PATIENTS WITH PRIMARY CHILDHOOD GLAUCOMA'S IN NIGERIA.

***Kooffreh Mary[1], Duke Roseline[2], Umoyen Anthony John[1] and Ozoje Michael[1,3]**

[1]University of Calabar, Faculty of Biological Sciences, Department of Genetics and Biotechnology, Calabar, Cross River State.
[2]University of Calabar Teaching Hospital, Calabar Children's' Eye Center, Department of Ophthalmology, Calabar.
[3]Federal University of Agriculture, Department of Animal Science and Genetics, Abeokuta, Nigeria.

***Corresponding Author: Kooffreh Mary**
University of Calabar, Faculty of Biological Sciences, Department of Genetics and Biotechnology, Calabar, Cross River State.

Article Received on 10/10/2016 **Article Revised on 01/12/2016** **Article Accepted on 22/12/2016**

ABSTRACT
Purpose: One of the primary molecular defect underlying the majority of Childhood Glaucoma cases has been identified as mutations in the cytochrome $P450_1B_1$ (CYP_1B_1) gene. There are no documented molecular studies on the genetic basis of chlidhood glaucoma in our population. Our aim is to identify any mutations present in exon 3 of the CYP_1B_1 gene which has been implicated in the pathogenesis of congenital glaucoma in diagnosed cases of primary childhood glaucoma and their families in other populations. **Methods:** This study included 11 children with primary childhood glaucoma, 30 controls from whole genomic DNA was extracted and PCRs were carried out. The amplicons were sequenced for mutations in exon 3. The nucleotide sequences of the CYP_1B_1 candidate gene on exon 3 and the exon-intron boundary were decoded from the chromatograms (bidirectional sequences) using Bioedit software and aligned manually. Multiple sequence alignment was determined using CLUSTAL W. Statistical analyses were performed using SPSS version 18 software. Significance was set at $P<0.05$. **Results:** The g.291G>C mutation which is a substitution of guanine by cytosine that resulted in an amino acid substitution of glutamine by histidine at position 97((p.Q97H)) was observed in 4(36.36%) out of the 11 patients. Three of these patients had primary congenital glaucoma, only 1 patient had juvenile open angle glaucoma. The following four single nucleotide deletions g.317delG, g.324delG, g.327delG, g.535delG (single deletion of guanine) were observed in 3(27.27%) out of the 11 patients, all three cases were all males with primary congenital glaucoma. **Conclusions:** This results form baseline information for further molecular studies among Nigerian patients with Primary childhood glaucoma.

KEYWORDS: Primary childhood glaucoma, children, CYP1B1 gene, Calabar.

INTRODUCTION
Childhood glaucoma refers to two main categories of pediatric glaucomas.[1] They are: Primary congenital glaucoma (PCG) which has isolated angle anomalies, presence or absence of mild congenital iris anomalies and usually with ocular enlargement. It is Subcategories based on age at onset into: Neonatal or newborn: 0–1 month, Infantile; > 1–24 months and Late-onset or late-recognized of more than 2 years. The second category under primary childhood glaucoma is Juvenile open-angle glaucoma (JOAG) in which there is no ocular enlargement, no congenital ocular anomalies or syndromes and an open angle with normal appearance.

From population based studies in Cross River State, Nigeria, causes accounting for 19% was the second important cause of blindness and severe visual impairment in children. Glaucoma was responsible in 7 (43%) of this.[2] The Primary childhood glaucomas are chronic, life long and difficult to treat. They may eventually lead to blindness if not adequately treated with appropriate surgical methods.

The presentation of primary congenital glaucoma is that of the classic triad of symptoms namely epiphora photophobia and blepharospasm.[3]

The human cytochrome $P450_1B_1$ (CYP_1B_1) gene exhibits a high degree of allelic heterogeneity, and more than seventy different mutations of this gene implicated in childhood glaucoma have been identified and reported.[5,6,] The frequency of CYP_1B_1 gene mutations in populations varies widely from 14% to 70% worldwide, and the common mutations are strongly clustered on specific haplotype backgrounds, irrespective of their geographic locations. In different populations, several molecular genetics studies had been conducted

concerning the association of CYP_1B_1 gene polymorphism with congenital glaucoma.

There is no documented molecular research on the genetic polymorphism of CYP_1B_1 gene that could be associated with childhood glaucoma in Nigeria. Hence the need to investigate the mutations on exon 3 that present in children with childhood glaucoma in the Calabar children's Hospital, Calabar.

METHODS

A total of 11 children with primary childhood glaucoma were recruited into the study from the Children Eye Clinic, University of Calabar Teaching Hospital, Calabar. Thirty children attending the pediatric clinic (in the same hospital) for general eye examinations who did not have glaucoma or any other eye disease were recruited as control subjects. Ethical approval was granted by the University of Calabar Ethical Review Committee, University of Calabar, Calabar. The parents or guardians of the children gave informed consent on behalf of very young children. Two - three mls of blood was collected from each child into EDTA bottles and kept in freezers of-20˚C for further analysis. DNA was extracted as previously reported by Kooffreh et al 2012 but with modifications, 1% monothioglycerol was added to the extraction buffer instead of mecarpto-ethanol and the tubes were kept in the freezer (-20˚C) for 10-20 minutes after the addition of cold isopropanol (they are usually kept in the fridge). PCR amplifications were performed in 50µl cocktail containing 4µl of template DNA, 10µl of PCR buffer, 3µl of $Mgcl_2$, 1.0µl of dNTPs, 1.0µl of primer each, 29.76µl of nuclease free water and 0.24µl of taq DNA polymerase. The primers sequence are as follows:

GL3-F1- CTCACTTGCTTTTCTCTCTCC
GL3-R1- CATCACTCTGCTGGTCAGGT Alfadhli et al, 2006

Cycling conditions

Initial denaturation step at 95°C for 3 minutes. Then 35 cycles of denaturation at 95°C for 1 minute, annealing at 58 to 62°C for one minute, and elongation at 72°C for one minute. Then a final extension step of 10 minutes at 72°C. About 5µl of the PCR products was checked on agarose gel electrophoresis of PCR amplicon of F and R primers from exon 3. The protocol for purifying the amplicons was carried out according to Bejjani et al., (1998) protocol. 75µl of ethanol (95%) was added to eppendorf tubes containing 30µl of the PCR amplicons and inverted 3-5 times. The tubes were then transfered into -20^{o}C freezer for 1 hour. The tubes were centrifuged at 12,000 rcf for 10 minutes. The supernatant was decanted gently and 500µl of cold 70% ethanol was added, centrifuged again at 12,000rcf for 5 minutes. Then the alcohol was decanted and tubes air dried at room temperature until no traces of alcohol was seen. The PCR products were transported to DNA Laboratory, Iowa State University, Ames, Iowa State, USA where bidirectional sequencing was performed on all PCR products. The nucleotide sequences of the CYP_1B_1 candidate gene targeted on exon 3 and the exon-intron boundary were decodes from the chromatograms (bidirectional sequences) using Bioedit software and aligned manually. The nucleotide sequences of the CYP_1B_1 gene from patients were compared with the reference sequences in the NCBI gene bank to query for similarity and homology on the database using the link: http://blast.ncbi.nlm.nih.gov/Blast.cgi. Statistical Analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. Quantitative and clinical variables were compared using simple percentage and chi-square (X^2) test.

RESULTS

Fig 1 shows the PCR amplification of the CYP1B1 gene.The socio-demographic variables among childhood glaucoma patients, and control subjects were represented and compared for significant differences in Table 1. The patient population consist of 5 primary congenital glaucoma (PCG) and 6 juvenile open angle glaucoma (JOAG). The PCG cases consist 3(27.3%) males and 2 (18.2%) females while JOAG cases consist of 4(36.3%) males and 2 (18.2%) females. There were no significant differences between gender in the study population. The controls were children between the ages of 6-17 years with 14(46.7%) males and 16(53.3%) females and a mean age of 10.80±1.92.

Table 2 presents the CYP1B1 mutation in the patient population. These mutations were absent in the control subjects. The g.291G>C mutation which is a substitution of guanine by cytosine that resulted in an amino acid substitution of glutamine by histidine at position 97((p.Q97H)) was observed in 4(36.36%) out of the 11 patients. Three of these patients had primary congenital glaucoma, only 1 patient had juvenile open angle glaucoma. The following four single nucleotide deletions g.317delG, g.324delG, g.327delG, g.535delG (single deletion of guanine) were observed in 3(27.27%) out of the 11 patients, all three cases were all males with primary congenital glaucoma. Figure 2 is the chromatogram of the nucleotide substitution of guanine by cytosine at position 291 on exon 3 of the CYP1B1 gene.

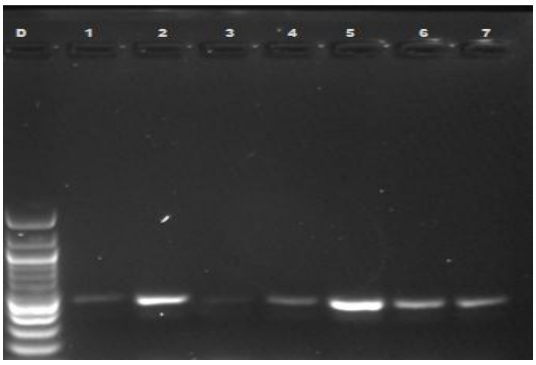


Figure 1. Agarose gel showing PCR amplification of the CYP1B1 gene.

Legend
Lane D is the 1kb DNA ladder
Lane 1-7 are the PCR products.

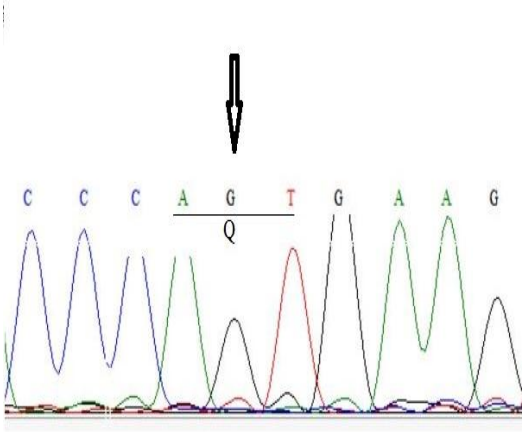
TABLE 1: Socio-demographic distribution in primary childhood glaucoma cases

Variables		Primary childhood glaucoma cases n=11		controls n=30	X^2	df	p-value
		Primary	Juvenile				
		congenital	open angle				
		glaucoma	glaucoma				
Age		<1-3years	>3yrs	6-17yrs			
Gender	males	3(27.3%)	4(36.3%)	14(46.7%)	0.36	1	0.54
	females	2(18.2%)	2(18.2%)	16(53.3%)			
Mean age		98.36±12.43		10.80±1.92			
		months		years			

TABLE 2: Prevalence of CYP_1B_1 gene mutations in primary childhood glaucoma cases

Cases	CYP1B1 Mutations		Gender		
			Males	Females	Total
Primary congenital glaucoma	g.291G>C		2	1	3
Juvenile open angle glaucoma	g.291G>C		1	0	1
		Total	3(27.27%)	1(9.09%)	4(36.36%)
Primary congenital glaucoma	g.317delG, g.324delG, g.327delG, g.535delG		3	0	3
Juvenile open angle glaucoma	g.317delG, g.324delG, g.327delG, g.535delG		0	0	0
		Total	3(27.27%)	0(0.00%)	3(27.27%)

Individual 1



Individual 11

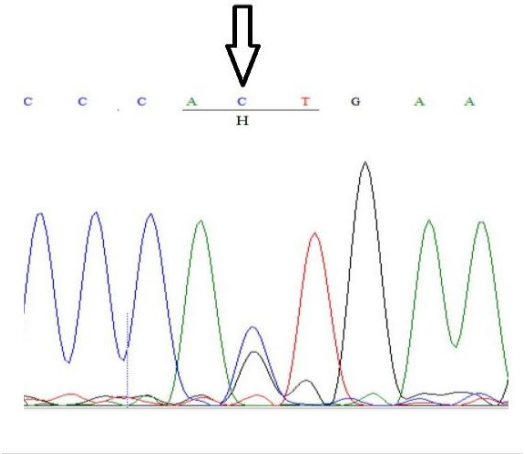


Figure 2 g.219G>C missense mutation on exon 3 of the CYP1B1 gene.

Legend
- Individual 1 has no mutation
- Individual 11 shows the G>C substitution
- The arrow indicates the G>C substitution
- the codons are underlined and H represents Histidine while Q represents Glutamine.

DISCUSSION

In Calabar, a Nigerian population with primary childhood, we identified g.291G>C mutation in CYP1B1 leading to the substitution of glutamine by histidine (P.Q97H) on exon 3. This mutation was not present in the unmatched healthy controls. CYP1B1 is one of the candidate gene believed to participate in trabecular meshwork development of the eye that functions as a filter for drainage of the anterior chamber fluid.[5,7] Mutations in the human CYP1B1 gene were documented where deleterious mutations resulted in abnormal development of the trabecular meshwork in the eyes[8] resulting in an increased intraocular pressure[10] of the eye.[9] A G-C transition at the position 364 on exon 3 which resulted in an amino acid substitution of methionine by valine was observed among Indonesian and European primary congenital glaucoma cases[10] but our study reports a G-C substitution at position 291 in exon 3 resulting in the substitution of glutamine by histidine with a frequency of 36.36%.

Four single nucleotide deletions: g317delG; g324delG; g327delG; g.535del.G; were observed three patients in our study. The g.535del.G mutation was reported among Portuguese children[11], Tunisian individuals[12], Brazilians[13] and in Moroccans.[14] Primary childhood

glaucoma in children accounts for about 25% of paediatric glaucoma and causes high percentage of childhood blindness globally.[17,18,19,20] A number of researches on the CYP1B1 gene mutations reveal a high level of heterogeneity with respect to the mutational spectrum and the causative involvement of candidate genes.[14,15,21,22]

CONCLUSION

A substitution of guanine by cytosine at position 291(g.291G>C) and single nucleotide deletions of guanine at four positions (g317delG; g324delG; g327delG; g.4340delG; g.535del.G) of the CYP1B1 gene were observed among childhood glaucoma patients in Calabar. The frequency of the CYP1B1 mutations observed for primary childhood glaucoma in this study provides baseline information for further studies on the prevalence of these mutations among Nigerian childhood glaucoma patients

REFERENCES

1. Sarfarazi M, Stoilov I, Sehenkman JB. Genetics and biochemistry of primary congenital glaucoma. Ophthalmology clinical North America, 2003; 16(4): 543-554.
2. Stoilov I, Akarsu A.N, Sarfarazi M. Identification of three different truncating mutations in cytochrome P450, B, (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to GLC3A on chromosome 2p21. Human mol. Genet, 1997; 6(4): 641-647.
3. Pirro G.H, Ching-Yu C, Henriet S, Stuart M et al. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. Nature Genetics, 2014, 46: 1126-1130. dio: 10.1038/ng. 3087
4. Sitorus R, Ardjo S.M, Lorenz B, Preising M. CYP1B1 gene analysis in primary congenital glaucoma in Indonesian and European patients. J Med Genet, 2003; 40: eq doi: 10. 1136/jmg. 40./.eq
5. Cardoso M.S, Anjos R, Vieira L, Ferreira C, Xavier A, Brito C. CYP1B1 gene analysis and phenotypic correlation in Portuguese children with primary congenital glaucoma. Eur. Jophthalmol, 2015; 25(6): 474-477. doi: 10. 5301/ejo. 5000618
6. Yosra B, Salim B.Y, Nesrine A, Rim K, Ryme K, Moncef K, Sonia A. CYP1B1 gene mutations causing primary congenital glaucoma in Tunisia. Annals of human genetics, 2014; 78: 255-263.
7. Stoilov I.R, Costa V.P, Vasconcellos J.P, et al. Molecular genetics of primary congenital glaucoma in Brazil. Invest. Ophthalmol Vis. Sci., 2002; 43: 1820-1827.
8. Belmouden A, Melki R, Hamdani M, Zaghloul K, Amraoui A, Nadifi S, Akhayat O, Garchon H.J.A novel Frameshift founder mutation in the cytochrome P4501B1(CYP1B1) glaucoma in Morocco. Clinical Genetics, 2002; 62: 334-339.
9. Bejjani B.A, Lewis R.A, Tomey K.F, Anderson K.L, Dueker D.K, Jabak M, Astle W.F, Otterud B, Leppert M, Lupski J.R. mutations in CYP1B1, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaaucoma in Saudi Arabia. American J. Human Genetics, 1998: 62: 325-333.
10. Kooffreh M.E, Anumudu C.I, Akpan E.E, Ikpeme E.V, Kumar P.L.A study of the M235T variant of the angiotensinogen gene and hypertension in a sample population of Calabar and Uyo, Nigeria. The Egyptian Journal of Medical Genetics, 2013, 14: 13-19.
11. Colomb E, Kaplan J, Garchon H. Novel cytochrome P4501B1(CYP1B1) mutations in patients with primary congenital glaucoma in France. Human, 2003; 22: 496.
12. Moore D.B, Tomkins O, Ben-zion I. A review of primary congenital glaucoma in the developing world. Surv ophthalmol, 2013; 58: 278-285.
13. Liu Y, Allingham R.R. Molecular genetics in glaucoma. Exp. Eye Res., 2011; 93: 331-339.
14. Vasiliou V, Gonzalez F.J. Role of CYP1B1 in glaucoma. Annu Rev. Pharmacol. Toxicol, 2008; 48: 333-358.
15. Chakrabartis S, Kaur K, Kaur I, Mandal A.K, Parikh R.S, Thomas R, Majumder P.P. Globally CYP1B1 mutations in primary congenital glaucoma are strongly structured by geographic and haplotype backgrounds. Invest. Ophthalmol Vis Sci., 2006; 47: 43–47.
16. Dimasi D.P, Hewitt A.W, Straga T, Pater J, Mackinnon J.R, Elder J.E, Casey T, Mackey D.A, Craig J.E. Prevalence of CYP1B1 mutations in Australian patients with primary congenital glaucoma clinical Genetics, 2007; 72: 255-260.