ejpmr, 2019,6(2), 16-19

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

RETINOBLASTOMA SUSCEPTIBILITY GENE (RB1) POLYMORPHISM AMONG CHILDREN IN CALABAR, NIGERIA

Mary E. Kooffreh*[1], Roseline Duke[2], Nkoyo Ephraim[1] and Anthony Umoyen[1]

[1]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.
[2]University of Calabar Teaching Hospital, Calabar, Nigeria.

***Corresponding Author: Mary E. Kooffreh**
Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.

Article Received on 20/11/2018 **Article Revised on 11/12/2018** **Article Accepted on 02/01/2019**

ABSTRACT

Retinoblastoma is an eye disease causing blindness and death in children. The screening of the RB1 gene in retinoblastoma children, relatives and controls lead to the documentation of more than 900 mutations in different populations, which are important for genetic counselling, characterization of phenotypic-genotypic relationships and clinical management of the disease. There is no information on the RB1 gene polymorphism among retinoblastoma children in Calabar, Nigeria. This study investigated the genetic polymorphism of RB1 susceptibility gene and its involvement in the molecular pathogenesis of retinoblastoma in Calabar. This research was carried out in the University of Calabar Teaching Hospital (UCTH) Calabar, Nigeria. Blood was collected from 9 clinically diagnosed retinoblastoma children, 30 children and 23 adults as controls. DNA was extracted from all the blood samples, PCR was performed yielding a 485bp amplicon that was digested using the M1u CI restriction enzyme. The digested fragments were visualized in 1.5% agarose and the genotype and allele frequency was determined. The genotype frequencies among the retinoblastoma children were 0(0%), 3(33.33%) and 6(66.67%) and in the controls were 0(0%), 13(24.53%) and 40(75.47%) for GG, GA and AA respectively. The G allele was low (0.17and 0.12 in patients and controls). There were no significant differences in the genotypic proportions of the RB1 gene polymorphism among patients and controls. The RB1 gene polymorphism may not be directly involved in the development of retinoblastoma however this requires further investigation.

KEYWORDS: Retinoblastoma, Children, Gene Polymorphism, RB1, Calabar.

BACKGROUND

Retinoblastoma has been reported to be the most common intraocular childhood disorder that can be fatal with poor management. The retinoblastoma susceptibility gene (RB1) is a tumor suppressor gene located on chromosome 13q14 (Kadam-pai *et al*., 2003; Shield and Shield, 2004).The screening of the RB1 gene in retinoblastoma children, relatives and controls lead to the documentation of more than 900 mutations in different populations (Wilson, 2001,Valverde *et al*., 2005; Owoeye *et al*., 2006; Abouzeid *et al*., 2007; Kanber *et al*., 2009; Eloy *et al*., 2016) which are important for genetic counselling, characterization of phenotypic-genotypic relationships and clinical management of the disease. With hopes of treatment of eye diseases based on gene replacement (gene therapy) or manipulation of the pathophysiological mechanisms that underlie the disease, an accurate molecular genetic diagnosis is inevitable (Manjandavida *et al*, 2018). During counselling parents will be informed of the risk of transmitting the disease to their children. This will influence decisions taken by parents. A SNP A-G dialleleic polymorphism at position 153,104 in intron 18 of the RB1 has restriction sites for Tsp 5091. No study on the RB1 gene has been reported among retinoblastoma cases in Calabar. This is a pilot study to determine genotype and allele frequencies of this polymorphism among retinoblastoma children and controls in Calabar. This will serve as baseline information for subsequent genetic studies.

METHODOLOGY: A total of 62 subjects comprising of 9 established retinoblastoma patients that were children and 53 controls. The control group consist of 30 children and 23 adults. The research was carried out at the pediatric Ophthalmology and Strabismus unit, Department of Ophthalmology, University of Calabar Teaching Hospital (UCTH) and Elim Eye Hospital, Calabar, both in Calabar, Cross River State, Nigeria. Ethical approval was obtained in accordance with the Helsinki declaration, from the Health Research Ethics Committee (HREC), University of Calabar Teaching Hospital (UCTH), Calabar. Data was collected on family history of retinoblastoma, detailed medical history, age at disease presentation, age at first contact in the clinic or hospital, and other socio-demographic data such as ethnicity and gender. Informed consent were duly signed or thumb printed by patients, control subjects and parent

or guardian for children below the age of expressing and taking decisions on their own after carefully explaining the research to the participants. The blood samples were collected from clinically diagnosed retinoblastoma patients, relatives and control subjects without the diseases. Molecular studies were carried out in the molecular diagnostic unit, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. DNA was extracted from all the blood samples. DNA were extracted from the blood samples according to the protocols of Kooffreh *et al*., (2013) with little modifications. A set of primers was used to amplified the retinoblastoma susceptibility gene (RB_1) on exon 19 and its flanking intronic sequences. The forward primer was 5^1 – AGGCAGTAATCCCCAGGAAAAGCC – 3^1 and the reverse primer was 5^1 – CACAGAGATATTAAGTGACTTGCCC – 3^1 (Kadam – pai *et*. al., 2003).

PCR amplification was performed in 50µl cocktail containing 4µl of template DNA, 10µl of PCR buffer, 3µl of $Mgcl_2$, 1.0µl of $dNTP_3$, 1.0µl of each primer (forward and reverse primer), 29.7µl of nuclease free water and 0.24µl of Taq DNA polymerase. The cycling conditions were 94^0c for 7 mins, followed by 35 cycles of denaturation at 94^0c for one minute, annealing at 56^0c for 1 min. The first extension at 72^0c for 2 mins, ending with a single 3- mins final extension step at 72^0c. M1u CI enzyme, (New England Biolabs, USA) which is an isochizomer of the Tsp5091 was used to digest the 485bp PCR product. Digested fragments were resolved and visualised using 1.5% agarose gel electrophoresis. The socio-demographic variables, clinical data was computed and analyzed using statistical package for social sciences (SPSS/PASW) version 20.0. Demographic and clinical variables were compared chi-square (x^2) test. Simple percentage calculation was used to determine the sex ratio of the disease. Allele and genotype frequencies, Significance levels of comparison were determined at 0.005 level of significance.

RESULTS AND DISCUSSION

Our study population consist of seventy-three subjects comprising nine established retinoblastoma patients, eleven parents and fifty-three ethnically unrelated controls. The controls subjects included 30 children and 23 adults with a mean age of 10.8+1.92 and 22.3 + 4.8 years respectively. In the children sub-control population, 53.3% and 46.6% were males and females respectively, while in the adult sub-control population, 39.1% and 60.8% were males and females respectively. Retinoblastoma patients comprised of six males (66.7%) and three females (33.3%) respectively (Table 1). The Efiks and the Ibibios have the highest frequency among the control population because they are the major ethnic groups residing within Calabar. The primers amplified a 485bp product of the RB1 gene (plate 1). The SNP being investigated is an A→G transition at nucleotide 153104 in intron 18, this affects one of the Tsp 5091 restriction sites in the amplified fragment (Kadam pai *et al*, 2003).. G is considered the variant allele and it shows three fragments, an uninterrupted 237bp, 210bp and 38bp. The wild type A shows 210bp, 38bp and the 237 completely cut into 157bp and 80bp fragments. The AG heterozygotes usually have five bands 237bp, 210bp, 157bp, 80bp, 38bp but the 38bp was not visualized in the gel for all genotypes (plate 2). The frequency of these three genotypes GG, GA, AA were observed in the study population and documented in Table 2. Genotype frequency calculated for our population showed the AG as 33.33% and AA as 66.67 in patients, AG as 24.53% and AA as 75.47% in controls respectively). The GG genotype was not observed in our sample population. However the genotype frequencies observed in the patient and control population did not differ significantly from what would be expected if the population was in the Hardy-Weinberg equilibrium. The frequency of the G allele which is an indicator of the RB1 SNP was low (0.17 in patients and 0.12 in controls) in our study population and this is similar to documented research by Kadam pai *et al* (2003) who also documented frequencies less than 0.18 in Asian populations. The genotype and allele frequency of RB1 gene polymorphism among patients and control in the study population was similar indicating that the SNP may not be directly responsible for disease manifestation and may be acting in concert with other mutations to cause disease in our population. This study however provides baseline information for subsequent molecular research in retinoblastoma patients in Calabar.

Table. 1: Socio-demographic of subjects.

Variables	Retinoblastoma (n=9)	Controls (n=53)		X^2	df	P-value
	Patients	Children	adults			
		(n=30)	(n=23)			
Gender	Males 6(66.7%)	16(53.3%)	9(39.1%)			
	Female 3(33.3%)	14(46.6%)	14(60.8%)	0.34	1	0.61
Ethnicity	Boki 1(11.1%)	3(10.0%)	3(13.0%)			
	Efiks 2(22.2%)	9(30.0%)	7(30.4%)			
	Ibibios 2(22.2%)	10(33.3%)	8(34.8%)			
	Ibos 3(33.3%)	6(20.0%)	4(17.3%)			
	Ijaws 1(11.1%)	2(6.6%)	1(4.3%)	0.19	4	0.29
Mean age	26.4 + 6.9	10.8+1.92	22.3 + 4.8			
(months/year)	(months)	(years)	(years)			

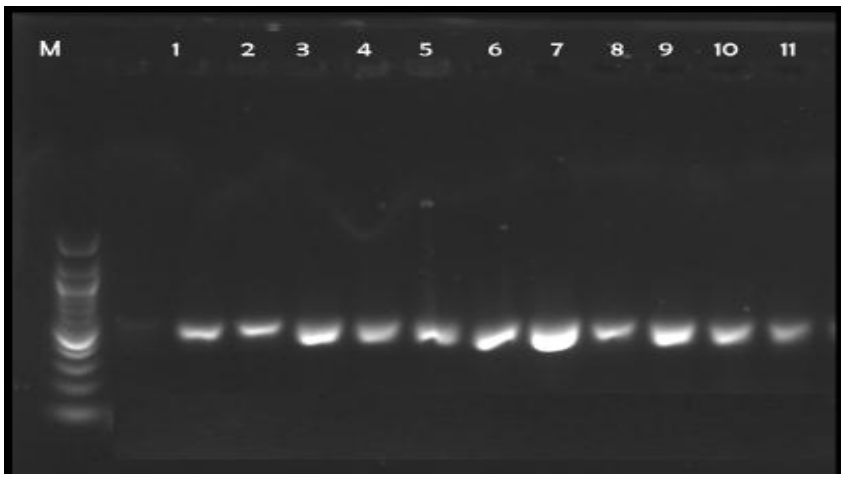

Plate. 1: Gel Electrophoresis showing 485bp PCR products after amplification of the retinoblastoma gene
Legend: M is the 100bp DNA ladder.

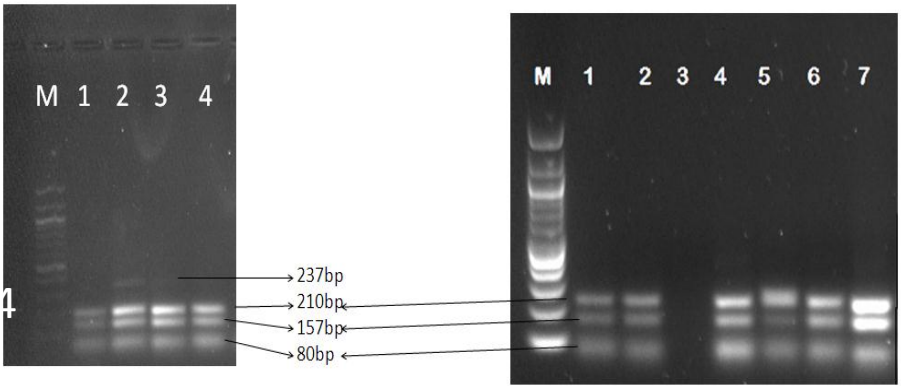

Plate. 2: Gel Electrophoresis showing 485bp PCR product digest by the MLU CI enzyme.
Legend: M is the 100bp DNA ladder
Lane 1 and other lanes on both gel represents the A/A genotype
Lane 2 on the 1st gel represents the A/G genotype
Lane 3 on the 2nd gel had to be digested again

Table. 2: Genotype and allele frequency of the RB1 gene polymorphism in patient and control population.

Groups		**Allele frequency**		**Genotype frequency**						
				GG		**GA**		**AA**		**X^2**
	N	**G**	**A**	**Obs**	**Exp**	**Obs**	**Exp**	**Obs**	**Exp**	
Patients	9	0.17	0.83	0		3		6		
%				0	2.89	33.33	28.22	66.67	68.89	0.04
Controls	53	0.12	0.88	0		13		40		
%				0	1.44	24.53	21.12	75.47	77.44	0.02

CONCLUSION

The RB1 gene polymorphism was determined among 9 retinoblastoma children, 30 other children and 23 adults as controls. The genotype frequencies among the retinoblastoma children were 0(0%), 3(33.33%) and 6(66.67%) and in the controls were 0(0%), 13(24.53%) and 40(75.47%) for GG, GA and AA respectively. The G allele was low (0.17and 0.12 in patients and controls). The RB1 gene polymorphism may not be directly involved in the development of retinoblastoma in children in Calabar, however this requires further investigation.

REFERENCES

1. Abouzeid, H., Munier, F.L., Thonney, F., & Schorderet, D. F. Ten novel RBI gene mutations in patients with retinoblastoma. Molecular Vision, 2007; 19(13): 1740-1745.
2. Eloy, P., Dehainault, C., Sefta, M., Aerts, I., Doz, F.; Cssouk, D., Le Rouic, L. L., Stoppa-Lyonnet, D., Radvanyi, F., Millot, G.A., Gauthier-villars, M. & Houdayer, C. A parent-of-origin effect impacts the phenotype in low penetrance retinoblastoma families segregating the C.1981>T/P. Arg661 TrP Mutation of RBI. Plos Genetics, 2016; 12(2): 1-14.
3. Kadam-Pai, P., Su, X.Y., Miranda, J. J., Socmantri, A., Saha, N., Heng, C.K. & Lai, P.S. Ethnic

variations of a retinoblastoma susceptibility gene (RBI) polymorphism in eight Asian populations. Journal of Genetics, 2003; 82(1and2): 33-37.
4. Kanber, D., Benelava, T., Ammerpohl, O., Mitter, D., Richter, J., Siebert, R., Housthemke, B., Kohmann, D. & Buiting, K. The human retinoblastoma gene is imprinted. Plos Genetics, 2009; 5(12): 1-9.
5. Kooffreh ME, Anumudu CI, Akpan EE, Ikpeme EV, Kumar PL. 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 Human Genetics, 2013; 14: 13-19.
6. Manjandavida FP, Chahar S. The art of retinoblastoma management - Curable yet challenging. Kerala J Ophthalmol, 2018; 30: 17-27.
7. Owoeye, J. F. A., Afolayan, E. A.O. & Ademola-Popoola, D. S. Retinoblastoma – a clinico-pathological study in Ilorin, Nigeria. African Journal of Health Service, 2006; 13(1-2): 117-123.
8. Shields, C.L. & Shields, J. A. Diagnosis and management of retinoblastoma. Cancer Control, 2004; 11(5): 317-327.
9. Valverde, J. R., Alonso, Y., Palacios, I. & Pestana, A. RBI gene mutation up-date in meta-analysis based on 932 reported mutations available in a searchable database, 2005; 6(53): 1-9.
10. Wilson, M. W.; Rodriguez-Galindo, C.; Haik, B.G., Mushfeghi, D.M., Merchant, T.E. & Pratt, C.B. Multiagent chemotherapy as neoadjuventtreatment for multifocal intraocular retinoblastoma. Ophthalmology, 2001; 108(11): 2106-2114.