CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1464615

Available online at: http://www.iajps.com Research Article

AN ASSESSMENT OF NON-SYNDRAOMIC CCONGENITAL CATARACT LINKAGE IN THE SUBJECTS SELECTED AT ALLIED HOSPITAL, FAISALABAD

[1]Dr. Muhammed Hanif, [2]Dr. Ahsan Ali, [3]Dr. Shafaq Javed
[1]BHU Thatha Khairo Matmal, Pindi Bhattian, Hafizabad
[2]MO Bhu Kot Naou Bahar Nankana Sahb
[3]Jinnah Hospital Lahore

Abstract:
Purpose: *Through homozygosity mapping and linkage analysis, our aim is find a cataract locus which affects a family cataract locus which affects a family. An identification of a CATI family was done from Mayo Hospital, Lahore in March 2016. This family have autosomal recessive non-syndromic congenital cataract (ARCC). This family have a common practice of consanguineous manages in the family and their four affected members. By using the standard phenol chloroform method, we extracted DNA. To observe linkage with 12 reported loci of ARCC, genotyping was done with 28 sets of microsatellite markers. On SSR markers, the DNA obtained was passed. To find the pattern of banding, native polyacrylamide gel electrophoresis was carried out. The result indicates that there was no linkage of kindred with all known reported loci of ARCC. After the study was performed it is deducted that might be presence of a novel gene participation in the cause of autosomal recessive congenital cataract. Using 392 sets of microsatellite markers or whole exam sequencing, in order to point out the causative gene, it is therefore needed to carry out genome wide association scan.*

Keywords: *Genome wide association scan, Linkage, Microsatellite markers.*

Corresponding author:
Dr. Muhammed Hanif,
BHU Thatha Khairo Matmal,
Pindi Bhattian,
Hafizabad

QR code

Please cite this article in press Muhammed Hanif et al., ***An Assessment of Non-Syndraomic Ccongenital Cataract Linkage in the Subjects Selected At Allied Hospital, Faisalabad.,*** *Indo Am. J. P. Sci, 2018; 05(10).*

INTRODUCTION:

All over the world, the most common cause of childhood blindness is a cataract. A cataract is an opacification of lens. Congenital cataract is a Mendelian disease. This disease has an estimated frequency of about 1per 4000 live births [1]. With approximate occurrence of about 1 – 15 per 10,000 live births, congenital can be either syndromic or non-syndromic [2 – 4]. There is a great variation in the structure and acuteness of congenital cataract. Mainly the nuclear, cortical, polar, sub-capsular parts of lens are affected by it. There is a less chance that whole less is affected by this variation [5]. About 30 genes have been reported and identified through linkage analysis up till now. The selection of most of these genes is done from crystalline family. The crystallin family includes alpha crystallin (CRYBB1, CRYBB2, CRYBB3, CRYBB1, CRYBA3 and CRYBA4) and gamma crystallin (CRYGA, CRYGC, CRYGD and CRYGS) [6-7]. Remaining changes in gene sequences are found in genes encoding major intrinsic proteins, gap junction proteins, transmebrane protein 114, lens intrinsic membrane protein 2, heat shock transcription factors and paired like home domain transcription factor 3. Thirty-eight autosomal dominant and12 autosomal recessive loci have been surveyed up till now. Eight changes in gene sequence have been reported from the 12 mapped autosomal recessive loci, [8 – 18]. One of the reliable methods is linkage analysis. This method not only allows us to map novel locations but also help us in positional cloning of previously known positional cloning of previously known loci [19 – 20]. In societies where consanguineous marriages are practiced commonly, this method becomes helpful. According to a survey, 60% of marriages are within the families in these societies [21].

MATERIAL & METHODS:

Family enrolment and clinical evaluation. The registration of the family was done from Mayo Hospital, Lahore in March 2016. This study was approved by institutional review board. From the 9 participating subjects, we obtain a written and signed agreement. These subjects are with the study being performed in accordance with the tenets of the members of the family, we obtained a detailed medical history.

LINKAGE ANALYSIS:

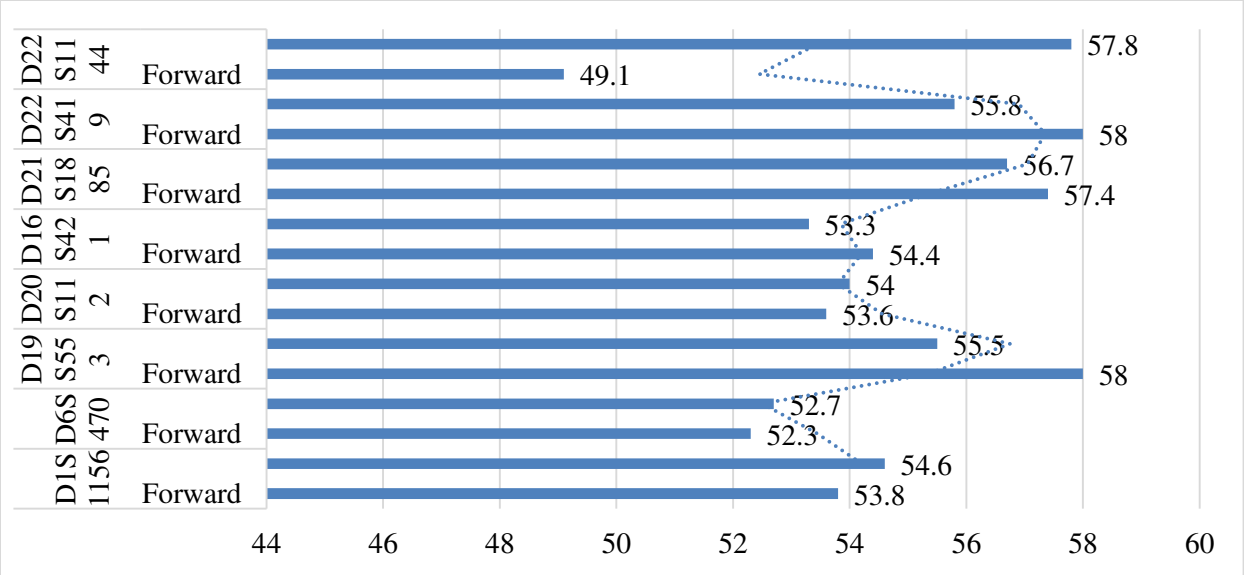
From each of the family members, blood sample withdrawn was 5ml. According to the standard method, extraction of genomic DNA was done [22] and we performed agarose gel electrophoresis. By starting the construction of master mixture, PCR was performed. 2mi of template DNA (80ng), 2-5ml of 10x PCR template DNA (80ng), 2-5mi of 10x PCR buffer, 1.0mi of DNTPS (100mm), 2-5mi $MgCl_2$ (1.5mm), 1.25mi of both forward and reverse primer (10 pic moles) each,13-9mi of water and 0-5mi of Tag (0-5U) DNA polymerase (Tag Gold, ABI) are included in the reaction mixture. In a Bio-Rad thermal cycler, amplification was done. In Table 1, there is a list of sequence of some primers used excessively, along with their Tm. With an initial inactivation step at 95^0C for about 5 minutes, the conditions were followed by 35 cycle's denaturation at 95^0 C for 45sec, and annealing at 58^0C for 45sec with a final extension at 72^0C for 10minutes. On 8% non-denaturing polyacrylamide gel, the product of PCR was passed. On the gel documentation system, the pattern of banding was observed. In the end, haplotypes were constructed.

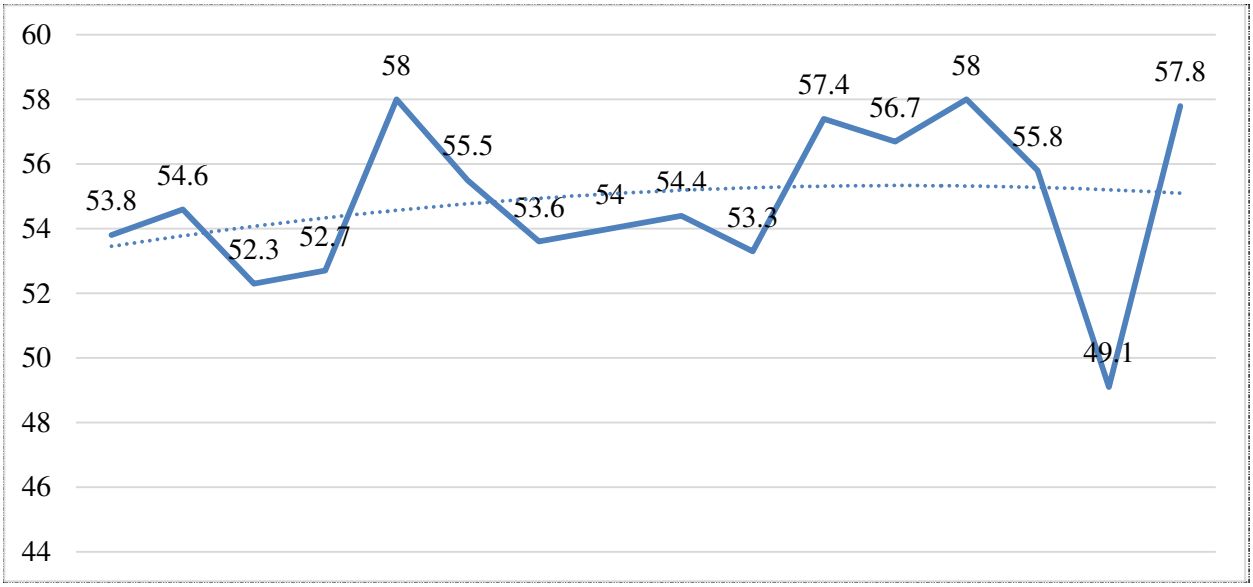
RESULTS:

After screening 12 reported loci of ARCC with the CAAT family was shown by the result. No linkage was seen in this study. All reported loci were excluded. Therefore, by using genome wide scan or exam sequencing, further work is required. This will result in recognizing the position of the particular locus and the possible causative gene present within that area.

Table: Sequence of some commonly used primers along with their melting temperature

Oligo nam	Primer	Orientation 5' - - - -3'	Tm
D1S1156	**Forward**	GCAACAGAAGGAGACTCTG	53.8
	Reverse	TGAAGCCTCGGTCATAGAG	54.6
D6S470	**Forward**	AAGCGATCTCACCATATACAC	52.3
	Reverse	ACACTGCAAAACGATTACCA	52.7
D19S553	**Forward**	CATGCCTCTAGTCCCAGCT	58
	Reverse	GACAAATGCCAGAAAGCCTG	55.5
D20S112	**Forward**	ATGGGTGTGCCAAATCTC	53.6
	Reverse	TTCTTGTAAGTCAGACAGCATCA	54
D16S421	**Forward**	ACATGAACCGATTGGACTGA	54.4
	Reverse	CCGTTCCCTATATTTCCTGG	53.3
D21S1885	**Forward**	AGCATGGCACTGGCATC	57.4
	Reverse	AGGACAAGTTTGGCCCC	56.7
D22S419	**Forward**	GGCTCAGGGACTCTGGA	58
	Reverse	GGCCAATCGGTAGGTCA	55.8
D22S1144	**Forward**	GCTGAAATTGCCAAAGTTTA	49.1
	Reverse	GAGCCTCTGGTCCTCTGT	57.8





DISCUSSION:

Therefore, for the screening of genome of this family, there is a requirement of father study. By using 392 highly polymorphic microsatellite markers or by whole exam sequencing, screening of genome of this family can be done. The gene which showed partial linkage is CRYBB3 and screened by using closely spaced markers. While doing fine mapping of CRYBB3 gene, no complete linkage was confirmed with this gene. Only one mutation has been reported in CRYBB3 in a 2onular form of cataract according to our present information. This mutation is reported in a family with autosomal recessive mode. Of inheritance with the change at position number 493 in exon 6 with a changes of G to C nucleotide, [23] genes encoding B crystallin have been observed with many polymorphisms. Most of these genes are with the autosomal dominant mode of inheritance. Previous work indicated that in position of CRYBB2 gene, three differ families were linked to 22qll region. Inspite having different ethnic background, these genes have the some mutation [24 – 26].

REFERENCES:

1. Hussain R, Bittles AH. J Biosoc Sci 1998; 30: 261-275.
2. Miller SA, Dykes DD, Polesky HF. Nucleic Acids Res 1988; 16: 1215.
3. Riazuddin SA, Yasmeen A, Yao W, Sergeev YV, Zhang Q, et al.. Investigative Ophthalmology and Visual Science 2005; 46(6): 2100-2106.
4. Gill D, Klose R, Munier FL, McFadden M, Priston M, et al. Investigative Ophthalmology and Visual Science 2000; 41(1): 159-165.
5. Litt M, Carrero-Valenzuela R, LaMorticella DM, Schultz DW, Mitchell TN, et al. Human Molecular Genetics 1997; 6(5): 665- 668.
6. Vanita SV, Reis A, Jung M, Singh D, Sperling K, et al. Journal of Medical Genetic 2001; 38(6): 392-396.
7. Heon E, Paterson AD, Fraser M, Billingsley G, Priston M, et al. Am J Hum Genet 2001; 68: 772-777.
8. Pras E, Pras E, Bakhan T, Levy-Nissenbaum E, Lahat H, et al. Isr Med Assoc J 2001; 3: 559-562.
9. Pras E, Levy-Nissenbaum E, Bakhan T, Lahat H, Assia E, et al. E. Am J Hum Genet 2002; 70: 1363-1367.
10. Pras E, Raz J, Yahalom V, Frydman M, Garzozi HJ, et al. Invest Ophthalmol Vis Sci 2004; 45: 1940-1945.
11. Smaoui N, Beltaief O, BenHamed S, MRad R, Maazoul F, et al. Invest Ophthalmol Vis Sci 2004; 45: 2716-2721.
12. Riazuddin SA, Afshan Y, Wenliang Y, Yuri VS, Qingjiong Z, et al. Invest Ophthalmol Vis Sci 2005; 46: 6.
13. Butt T, Yao W, Kaul H, Siaodong J, Gradstein L, et al. Mol Vis 2007; 13: 1635- 1640.
14. Cohen D, Bar-Yosef U, Levy J, Gradstein L, Belfair N, et al. Investigative Ophthalmology & Visual Science 2007; 48(5): 2208-2213.
15. Ponnam SP, Ramesha K, Tejwani S, Ramamurthy B, Kannabiran CJ. Med Genet 2007; 44: 85.
16. Ramachandran RD, Perumalsamy V, Hejtmancik JF. Hum Genet 2007; 121: 475- 482.
17. Friedman TB, Griffith AJ. Annu Rev Genomics

Hum Genet 2003; 4: 341-402.
18. Woods CG, Cox J, Springell K, Hampshire DJ, Mohamed M D, et al. Am J Hum Genet 2006; 78(5): 889-896.
19. Rahi JS, Dezateux C. Invest Ophthalmol Vis Sci 2001; 42: 1444-1448.
20. Francis PJ, Berry V, Bhattacharya SS, Moore AT. J Med Genet 2000; 37(7): 481–488.
21. Apple DJ, Ram J, Foster A, Peng Q. Surv Ophthalmol 2000; 45: S195–S196.
22. Gilbert C, Foster A. Bull World Health Organ 2001; 79(3): 227–232.
23. Riazuddin S A, Yasmeen A, Yao W, Sergeev Y V, Zhang Q, et al. Investigative Ophthalmology and Visual Science 2005; 46(6): 2100-2106.
24. Liska F, Chylikova B, Martanek J, Kren V. Mol Vis 2008; 14: 823-828.
25. Zhang L, Fu S, Ou Y, Zhao T, Su Y, et al. Mol Vis 2009; 15: 276-282.
26. Pras E, Frydman M, Levy-Nissenbaum E, Bakhan T, Raz J, et al. Invest Ophthalmol Vis Sci 2000; 41: 3511-3515.