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AN ASSESSMENT OF NON-SYNDROMIC CONGENITAL CATARACT IN THE PATIENTS OF ARCC (AUTOSOMAL RECESSIVE NON-SYNDROMIC CONGENITAL CATARACT)

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Abstract:

Purpose: *Through homozygosity mapping and linkage analysis, our aim is finding a cataract locus which affects a family cataract locus which affects a family of province Punjab in Pakistan. An identification of a CATI family was done from Allied Hospital, Faisalabad (February to August 2017). This family have autosomal recessive non-syndromic congenital cataract (ARCC). This family have a common practice of consanguineous manages in the family and 4 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 the 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.*

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INTRODUCTION:

All over the world, the most common cause of childhood blindness is a cataract. A cataract is an opacification of the 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 the 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, transmembrane protein 114, lens intrinsic membrane protein 2, heat shock transcription factors and paired like home domain transcription factor 3. 38 autosomal dominant and 12 autosomal recessive loci have been surveyed up till now. 8 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 practised 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 an identification of a CATI family was done from Allied Hospital, Faisalabad (February to August 2017). This study was approved by the institutional review board of Institute of Biochemistry and Biotechnology (IBB). 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

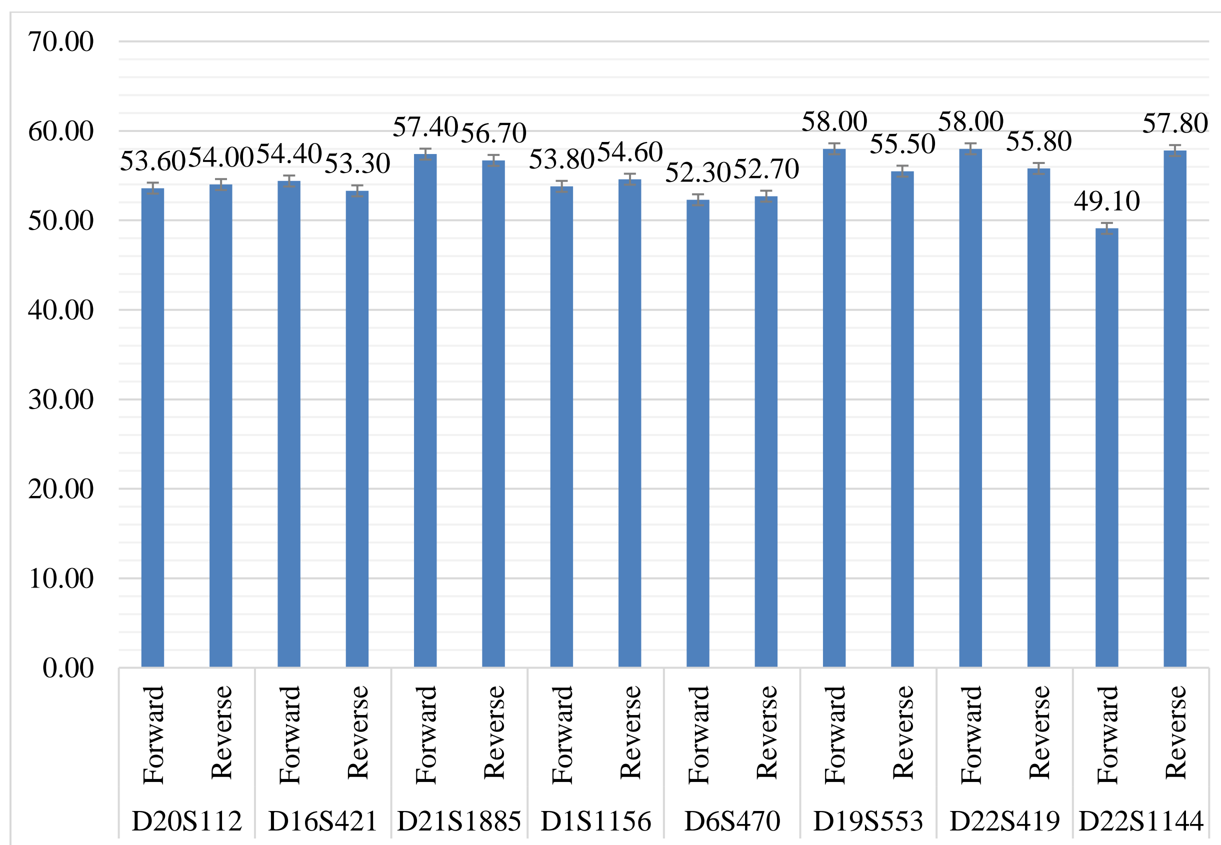
From each of the family members, blood sample withdrawn was 5ml. According to the standard method, extraction of genomic DNA was done [22] and we performed agarose gel electrophoresis. By starting the construction of the master mixture, PCR was performed. 2mi of template DNA (80ng), 2-5ml of 10x PCR 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, there is a list of the 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 as per the outcomes.

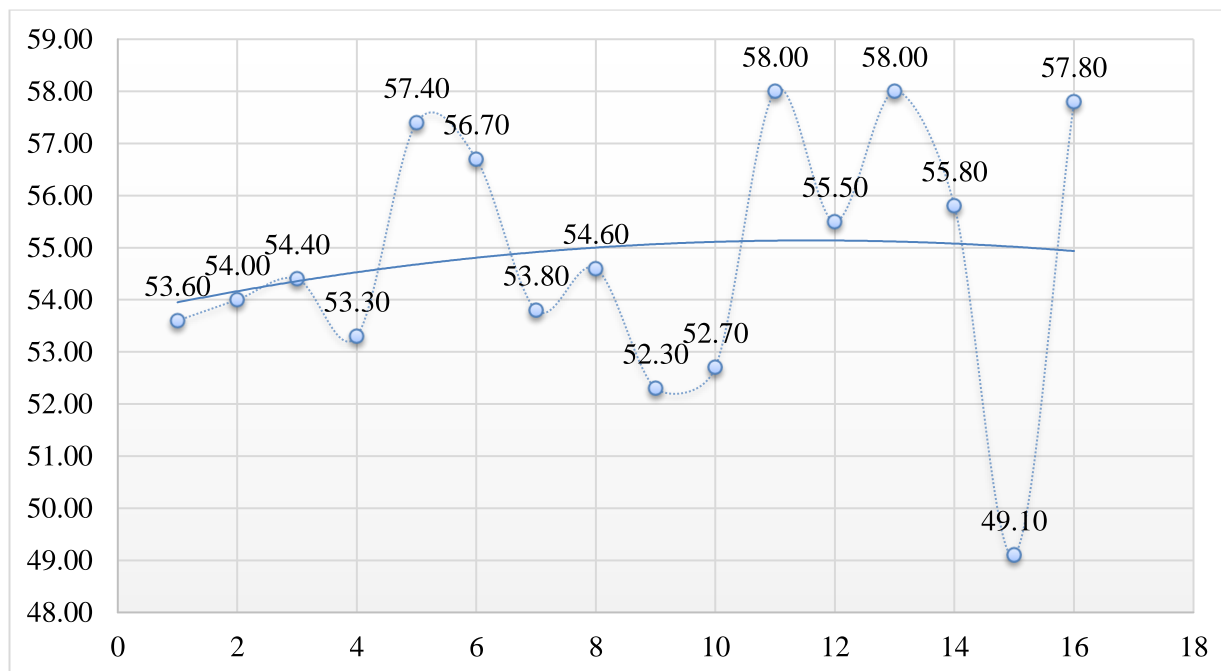
RESULTS:

After screening, 12 reported a locus 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
D20S112	Forward	ATGGGTGTGCCAAATCTC	53.60
	Reverse	TTCTTGTAAGTCAGACAGCATCA	54.00
D16S421	Forward	ACATGAACCGATTGGACTGA	54.40
	Reverse	CCGTTCCCTATATTTCCTGG	53.30
D21S1885	Forward	AGCATGGCACTGGCATC	57.40
	Reverse	AGGACAAGTTTGGCCCC	56.70
D1S1156	Forward	GCAACAGAAGGAGACTCTG	53.80
	Reverse	TGAAGCCTCGGTCATAGAG	54.60
D6S470	Forward	AAGCGATCTCACCATATACAC	52.30
	Reverse	ACACTGCAAAACGATTACCA	52.70
D19S553	Forward	CATGCCTCTAGTCCCAGCT	58.00
	Reverse	GACAAATGCCAGAAAGCCTG	55.50
D22S419	Forward	GGCTCAGGGACTCTGGA	58.00
	Reverse	GGCCAATCGGTAGGTCA	55.80
D22S1144	Forward	GCTGAAATTGCCAAAGTTTA	49.10
	Reverse	GAGCCTCTGGTCCTCTGT	57.80





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 the autosomal recessive mode. Of inheritance with the change at position number 493 in exon 6 with a change 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 the position of the CRYBB2 gene, three different families were linked to the 22qll region. In spite of having the different ethnic background, these genes have some mutation [24 – 26].

CONCLUSION:

At the end of this particular research, we conclude that there is a new indication of involvement of the gene in the incidence of autosomal recessive congenital cataract. Therefore, in order to find out causative gene the genome-wide related scan with the help of 392 microsatellite sets markers or a sequencing of the whole exome in order to locate the causative gene.

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