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Research Article

Linkage Analysis of Non-Syndromic Congenital Cataract in a Pakistani Family

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ABSTRACT

Purpose: Finding a cataract locus which affects a family of province Punjab in Pakistan through homozygosity mapping and linkage analysis. A CAT1 family was identified from Ganga Ram hospital having Autosomal Recessive Non-Syndromic Congenital Cataract (ARCC) having 4 affected members and a common practice of consanguineous marriages in the family. DNA was extracted using standard phenol chloroform method. Genotyping was done with 28 sets of Microsatellite markers to find linkage with 12 reported loci of ARCC. The DNA obtained was run on SSR markers. Native Polyacrylamide gel electrophoresis was done to find the banding pattern.The results showed that the kindred were not linked with all known reported loci of ARCC.After performing this study, it is concluded that there might be an indication of a novel geneinvolvement in the cause of autosomal recessive congenital cataract. It is therefore needed to carry out genome wide association scan using 392 sets of microsatellite markers or Whole Exome Sequencing to find the causative gene.

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

INTRODUCTION

A Cataract is an opacification of lens and is one of the most common causes of childhood blindness throughout the world. Congenital cataract is a Mendelian disorder with an estimated frequency of about 1 per 4000 live births [1]. Congenital cataract can be either Syndromic or Non-sydromic with an estimated occurrence of about 1-15 per 10,000 live births [2-4]. Congenital Cataract has a great variation in its morphology and severity, which affects mainly the nuclear, cortical, polar, sub-capsular part of the lens or in rare conditions the entire lens [5]. Till now, via linkage analysis about 30 genes have been reported and have been identified. Most of these genes are included from Crystallin family which include alpha crystallin (CRYAA and CRYAB), beta crystallin (CRYBB1, CRYBB2, CRYBB3, CRYBA1, CRYBA3, CRYBA4) and gamma crystallin (CRYGA, CRYGC, CRYGD, CRYGS) [6-7]. Rest of the mutations 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 homeodomain transcription factor 3. Till now, 28 autosomal dominant and 12 autosomal recessive loci have been mapped. From the 12 mapped autosomal recessive loci, 8 mutations have been reported [8-18]. Linkage Analysis is one of the conventional methods which not only allow us to map novel locations but also also do positional cloning of previously known loci [19-20]. This becomes useful in societies where consanguineous marriages are a common practise, where according to a survey, 60 % of the marriages are within families [21].

MATERIALS AND METHODS

Family enrolment and clinical evaluation

Family was enrolled from Gangaraam hospital Lahore. Institutional Review Board of Institute of Biochemistry and Biotechnology (IBB), University of Punjab approved this study. A written and signed consent form was obtained from 9 participating subjects with the study being performed in accordance with the tenets of the Declaration of Helsinki. A detailed medical history was obtained from all the family members.

Linkage Analysis

5mL blood sample were withdrawn from each of the family members. Genomic DNA was extracted according to the standard procedure [22] and agarose gel electrophoresis was performed as shown in Figure 1.

PCR was performed by starting the preparation of master mixture. The reaction mixture contains 2µl of template DNA (80ng), 2.5µl of 10X PCR Buffer, 1.0µl of dNTPs (100 mM), 2.5 µl $MgCl_2$ (1.5mM), 1.25 µl of both forward and reverse primer (10picomoles) each, 13.9 µl of water and 0.5 µl of *Taq*(0.5U) DNA polymerase (*Taq* Gold; ABI). Amplification was done in a Bio-Rad thermal cycler. Sequence of some widely used primers, along with their Tm is listed in Table 1. PCR conditions were set with an initial inactivation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45sec, annealing at 58°C for 45 seconds and extension at 72°C for 45 seconds with a final extension at 72°C for 10minutes. The PCR product was run on 8% non-denaturing polyacrylamide gels. The banding pattern was observed on gel documentation system. Haplotypes were constructed at last as shown in Figure 2 and Figure 3.

RESULTS

The result showed after screening 12 reported loci of ARCC with the CAAT family. In this study no linkage was observed and all reported loci were excluded as shown in the banding pattern in figures 4-6. It is therefore needed to carry out further work using genome wide scan or Exome sequencing which will result in identifying the position of the desired locus and the possible causative gene present within that area

DISCUSSION

Further study is therefore needed for the screening of the genome of this family which can be done by using392 highly polymorphic microsatellite markers or by Whole Exome Sequencing. CRYBB3 is the gene which showed partial linkage screened using closely spaced markers. However no complete linkage was established with CRYBB3 gene while doing fine mapping of this gene. With our current knowledge, in a zonular form of cataract only one mutation has been Reported in CRYBB3 in a family with autosomal recessive mode of inheritance with a change of G to C nucleotide at position number 493 in exon 6 [23]. There have been numerous polymorphisms detected in genes encoding B Crystallin most of them with the autosomal dominant mode of inheritance. Previous work have found that three different families were linked to 22q11 region in the position of CRYBB2 gene and have the same mutation despite having different ethnic background [24-26].

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APPENDICE

Table1: Sequence of some commonly used primers along with their melting temperature

Oligo name	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

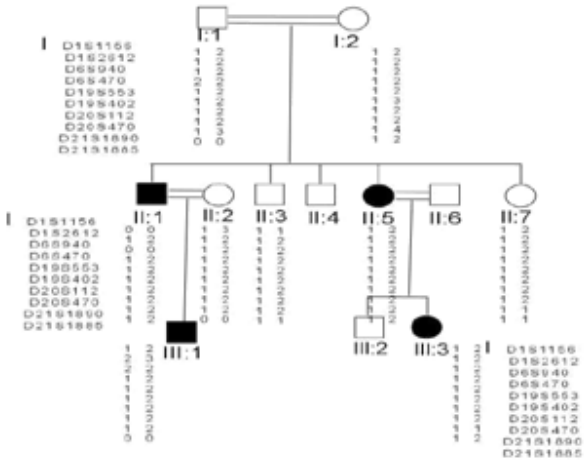

Figure 1: Pedigree and Haplotype of the family segregating for autosomal recessive Congenita Cataract. Circles represent females, squares represent males. Filled circle and squares represent affected individuals

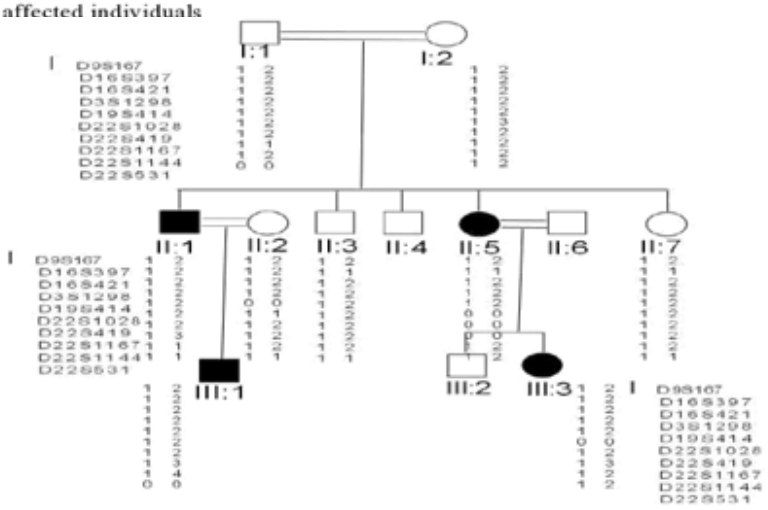

Figure 2: Another haplotype using different sets of microsatellite markers

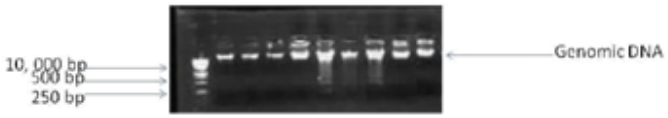

Figure 3: Agarose gel electrophoresis of DNA sample of nine persons. Sample 1, 2, 3, 4, 6 represent normal individuals while 5, 7, 8, 9 represent affected individuals. (M) represents Marker which is a 1kb DNA ladder

Screening of GJA8

M N N N N A N A A A

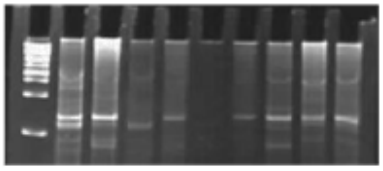

Figure 4: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for primer D1S1156 (155.89cM) showing heterozygosity among all affected (A) and normal (N) individuals. (M) represents marker

Screening of GCNT2

M N N N N A N A A A

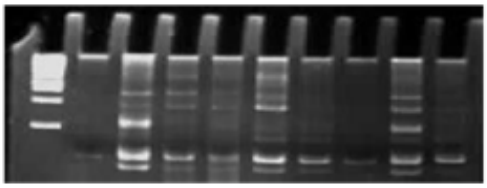

Figure 5: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for primer D6S470 (18.22cM) showing heterozygosity among all affected (A) and normal (N) individuals except sample 1 and 7 which shows homozygosity

Screening of LIM2

M N N N N A N A A A

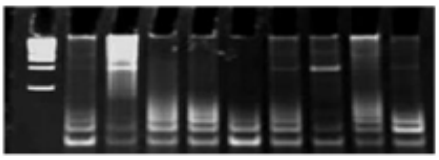

Figure 6: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for primer D19S553 (81.51cM) showing same banding pattern among all affected (A) and normal (N) individuals