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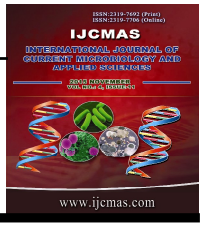

Original Research Article

Homozygosity Mapping of Consanguineous Families with Leber's Congenital Amaurosis

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A B S T R A C T

The human eye is a complex organ and is extremely important for vision related functions. Leber congenital amaurosis (LCA) is clinically and genetically heterogeneous disease with autosomal recessive pattern of inheritance and is characterized by severe vision loss present at birth or early childhood. Up to now 19 genes have been identified in pathogenic course of LCA, but mutations of few genes are more frequent than others. Interestingly, some of the known LCA genes also cause retinitis pigments and cilia related disorders, which creates extreme clinical heterogeneity and poses problems for accurate diagnosis. Two Pakistani families (A and B) with clinical signs like visual impairment since childhood, abnormal movement of eye, flat electroretinogram and abnormal retinal appearance were included in this study. Additionally, affected individuals of both families present oculodigital sign, keratoconus and cataract, which are indicators of segregation of LCA phenotype in both families. Both families were subjected to candidate gene analysis to test the involvement of currently known LCA genes. One family (A) did not show homozygosity shared by affected individuals for the all known candidate genes. However, family B revealed homozygosity at *RPE65* genes for two markers, but when typed with additional markers the pattern did not last further. Further analysis showed linkage in a region having spermatogenesis associated protein *7 (SPATA7;* MIM 609868) gene. This region was homozygous in all affected individuals for six markers on chromosome 14 and region contained *SPATA7* gene. The exact function of *SPATA7* gene is unknown, but studies have shown its role in vesicular transport. The gene is sequenced for the exons which were extensively documented to have mutations in earlier literature, but DNA sequencing did not show any pathogenic variation in all 12 exons of *SPATA7* gene. The future identification of pathogenic variation in family A and B require genome wide analysis and next generation technologies respectively.

Introduction

The human eye is a complex organ and by in large most important part of the human body. Eye genesis normally starts at three week embryo and is developed from all the three germinal layers (ectoderm, endoderm and mesoderm). The first three years of life are important for eye development as tremendous growth occurs during these years. Normal vision is essential for the growth and development of the visual cortex in this phase of development. It is normally agreed upon that visual acuity and capacity develops at first early three years of life (Fredrick, 2004).

Photoreceptors

Special kind of cells is located in retina which is responsible for detecting light, called photoreceptors. There are two types of photoreceptors. Rods are more abundant in number than cones which are less in number. The rods are stretched long and tube like while cones are small, broad and pointed. Rods and cones are dispersed in unusual numbers throughout the human eye with rods more in number than cones. Cones are covering the central portion while rods are mainly located in the outer segments of the retina. Just in the central portion of the retina there is a small “cup” or “pit” like depression called fovea or macula, which is responsible for most of vision as has a 100% visual sharpness or acuity. Mostly cones are present in fovea and are devoid of rods. High resolution images are formed when light falls on the fovea portion of the eye (Provis *et al.,* 2013).

A marked difference in structure occurs not only between rods and cones, but also between their functions. Rods are more adapted for function in dim light and are responsible for scotopic vision whereas cones for phototopic vision. The cones are active in bright illumination, whereas the rods are active in dim illumination (Pearring *et al.,* 2013). A tiny portion of the retina is left behind which is called blind spot. Here retinal blood vessels and optic nerve separate from retina. Natural blind spot are completely void of rods and cones so there is no vision in that spot (Frank and Stephen, 2001).

Retinal diseases

Retina has numerous cell types and have a high metabolic rate among its tissues so new mutation in different proteins of retina leads to the disruption of different physiological as well functional characteristics and ultimately leads to its deterioration. Hereditary diseases are arranged or classified according to observable differences. It includes dystrophies which has a devastating effect on central retina and then on peripheral retina. The former includes age related macular degeneration (ARMD), cone rod degeneration (CORD) which affect middle retina and leads to vision loss while the later affect outer boundary of retina which consists of retinitis pigmentosa, congenital stationary night blindness (Musarella, 2001). LCA is caused by both peripheral and central retinal abnormalities leading to colossal loss of vision. Most of the congenital eye disorders are bilateral and their mode of inheritance can be autosomal recessive or dominant. Sometimes it can be of mitochondrial or X linked in nature.

A famous figure of international repute Theodar Leber discovered a disease from German School of ophthalmology which was characterized by extreme vision loss since birth and was found in the offspring of healthy individuals. In the coming years he and others renowned eye specialists noted

occurrence of this disease in consanguineous families. Symptoms of the disease were decreased or nearly extinguished response to light reflex, rolling eye movement, but fundus was of normal architecture at birth showing signs of retinitis pigmentosa. Leber congenital amaurosis has been wrongly diagnosed for cortical blindness because of the normal appearance of fundus at the beginning. It was noticed six years later in a report related to infant blindness in which the disease was defined as "Congenital blindness with extinguished ERG" with a fine appearance of fundus, but often followed by ocular malfunctions such as keratoconus.

Mapping of LCA genes: the dawn of linkage era

Shomi Bhattacharya was the first who mapped a gene responsible for X linked Retinitis Pigmentosa on the short arm of the X chromosome (Bhattacharya *et al.,* 1984). Later, Peter Humprhies *et al.* (1990) mapped a gene responsible for autosomal dominant RP on a chromosome 3. A ray of hope came with the new "Homozygosity mapping" technique which was applied for extended as well as closely related families afflicting with LCA. Many families of North Africans roots were taken and were scanned for homozygosity mapping via new markers called "microsatellite markers" spanning a 10 cM region. Subsequently, this methodology was used to identify the LCA locus, LCA1 (Camuzat, 1995, 1996).

LCA is a collection of congenital retinal dystrophies manifested by severe vision loss nystagmus, roving eyes and other retinopathies. The vulnerability of getting LCA globally is three out of every 100, 000 approximately (Perrault *et al.,* 1996). LCA has a significant impact and is responsible for 20% of all the children attending blind schools across the globe (Marlhens *et al.,* 1997). Advancements in the realms of human genetics have brought this disease to a new horizon, with screening mutations in approximately 70% of all the LCA individuals (Perrault *et al.,* 1999).

Genes and loci

Molecular genetics of LCA the has been thoroughly explained for the last decade thus creating new insights from the research into Leber congenital amaurosis. So far 19 genes have been implicated in the pathogenic cause of LCA and these nineteen genes are concerned with different diverse functions of the retina. The LCA genes are *GUCY2D* (LCA1), *RPE65* (LCA2), *SPATA7* (LCA3), *AIPL1* (LCA4), *LCA5* (LCA5), *RPGRIP1* (LCA6), *CRX* (LCA7), *CRB1* (LCA8), *NMNAT1* (LCA9), *CEP290* (LCA10), *IMPDH1* (LCA11), *RD3* (LCA12), *RDH12* (LCA13), *LRAT* (LCA14), *TULP1* (LCA15), *KCNJ13* (LCA16), *IQCB1* and *MERTK* (Stockton *et al.,* 1998; Dharmaraj *et al.,* 2000; Keen *et al.,* 2003; Perrault *et al.,* 2003).

LCA, despite being relatively clinically and genetically heterogeneous disease, has been well studied over the last years. Understanding the genetics has also improved lately with mutation in 19 genes now identified for this disease. Analysis of the phenotype and establishing a relationship with the genotype remains a challenge. The study is based on families with LCA showing autosomal recessive inheritance. Candidate genes which are known to be involved in disease are first checked. The purpose of the study was to investigate the molecular basis of such disorders whose clinical examination often leads to an imprecise and poor diagnosis. Our study is, therefore, important to explore the novel genes and mutations involved in

different ocular anomalies as well as growing awareness among the masses about these complex disorders.

Materials and Methods

The study was approved by the Institutional Review Board of Quaid-i-Azam University, Islamabad, Pakistan.

Ascertainment of families with inherited LCA

The families were visited at their places of residence to generate pedigrees, to collect blood and other relevant information. All participants gave their consent to take part in the study.

Pedigree construction and analysis

For genetic implication, an extensive pedigree was constructed for each family by the standard methods. Family pedigrees were constructed from available information for each family using the methods described by Bennett *et al.* (1995). In the pedigrees (Fig. 1 and 2) males are symbolized by squares and females by circles. Filled circles and squares represent affected individuals, while unaffected individuals are represented with unfilled symbols. Each generation was indicated by a Roman numeral. The individuals within a generation were designated by Arabic numerals. A number enclosed within a symbol indicates the number of siblings males or females, as the case may be. Double lines in the pedigrees represent consanguineous marriages. The mode of inheritance of LCA was inferred by observing segregation of disease within the family.

Collection of blood

Venous blood samples from both normal and affected individuals, including their parents were collected by 10 ml syringes (BD 0.8 mm x 38 mm 21 G x 1 ½ TW, Franklin Lakes, USA) and from children below 2 years of age by butterflies, in potassium EDTA vacutainer sets.

Genomic DNA extraction

Genomic DNA was extracted from whole blood using standard phenol-chloroform procedure (Sambrook *et al.,* 1989).

Genomic DNA extraction by commercially available kit

DNA extraction was also carried out using Genomics Isolation Kit (Sigma Chemical Co. St. Louis, USA). 150 µl blood was taken in a 1.5 ml microcentrifuge tube along with 250 µl of lysis solution A, mixed by inversion, incubated at 65^{o} C for 6 minutes. Clear aqueous phase was transferred to a new 1.5 ml microcentrifuge tube after adding 100 µl of precipitation solution B and centrifuge at 14,000 RPM for 5-10 minutes. DNA was then precipitated by adding 500 µl of 100 % ethanol. Ethanol was removed after centrifugation at maximum speed for two minutes, and then washed with chilled 70 % ethanol. After evaporation of residual ethanol DNA was dissolved in appropriate amount of Tris-EDTA (TE) buffer for incubation at 65^{o}C for 5 minutes.

Agarose gel electrophoresis

Extracted DNA was analyzed on 1% agarose gel prepared by melting 0.5 gm of agarose in 50 ml 1 X Tris-Borate-EDTA (TBE) in a microwave oven for 1-2 minutes. Ethidium bromide was added to the gel to stain the DNA. The DNA was mixed with loading dye (bromophenol blue) and loaded into the wells on the agarose gel. The electrophoresis

was performed at 120 volts for 25-30 minutes. Then the DNA was visualized under UV transilluminator and results were recorded by using a gel documentation system.

Genotyping and linkage analysis

To reveal the genetic defect in the families, presented here, an initial search for linkage was carried out by using polymorphic microsatellite markers corresponding to candidate genes involved in LCA and related phenotype. The families A and B were tested for linkage by using a minimum of 5 microsatellite markers for each of the candidate region of known loci, associated with various forms of LCA. Table 2 summarizes microsatellite markers located in the region of known LCA loci, which were used as a first pass analysis for the genetic linkage in the families.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) amplification of microsatellite markers was carried out in 0.2 ml tubes (Axygen, California, USA) according to a standard procedure in a total volume of 25 µl containing 1 µL DNA dilution, 0.3 µl of each forward and reverse microsatellite marker (20 ng/ µl), 2.5 µl 10X PCR buffer (200 mM $(NH_4)_2SO_4$, 750 mM of Tris-HCl pH 8.8, 0.1 % Tween 20), 1.5 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTPs and 0.2 µl of 0.5 unit Taq DNA Polymerase (MBI-Fermantas, Burlington Canada) in 18.7 µL PCR water. The reaction products were centrifuged for 30 seconds at 8,000 RPM for thorough mixing. Reactions were performed by means of T3 thermocyclers (Biometra, Gottingen, Germany).

PCR was carried out with the following thermal cycling conditions: an initial denaturation of template DNA at 95^{o}C for 4 minutes, followed by 40 cycles of amplification, each consisting of 3 steps: denaturation of DNA into single strand at 95°C for one minute, annealing, or hybridization of microsatellite markers to their complementary sequences on either sides of target sequence at 54–59°C for one minute, and 72°C for one minute for extension of complementary DNA strands from each primer. This was finally followed by a final extension at 72°C for ten minutes.

Polyacrylamide gel electrophoresis

The amplified PCR products were resolved on 8% non-denaturing polyacrylamide gel. Gel solution was made in a 250 ml conical flask, and was poured into the space between two glass plates separated at a distance of 1.5 mm. After placing the comb, it was allowed to polymerize for 45 minutes at room temperature. Samples were mixed with 6 µl loading dye and loaded into the wells. Electrophoresis was performed in a vertical gel tank of Whatman Biometra (Biometra, Gottingen, Germany) at 100 volts (30 mA) electric current for 120-150 minutes. The gel was stained with ethidium bromide solution (0.5 µg/ml final concentration) and visualized on a UV transilluminator (Biometra, Gottingen, Germany) and photography was done with the help of a Digital camera DC 290 (Kodak, New York, USA).

***SPATA7* gene sequencing**

Ensemble genome browser (http://useast.ensembl.org/index.html) was used to download the sequence of SPATA7 gene. Primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/) tool was used to design the primers. From UCSC genome browser, BLAST search tool. (http://genome.ucsc.edu/cgi-

bin/hgBlat?hgsid=358608373&command=start) was used to check the specificity of the primers. Forward and Reverse primers for each exon with single hit were selected and purchased from Gene Link (USA).

Sequence analysis

Chromatograms from normal and affected individuals were compared with the corresponding control gene sequences from Ensemble Genome Browser database (http://www.ensembl.org/index.html) to identify any nucleotide base pair change. Sequence variants were identified via BIOEDIT sequence alignment editor version 6.0.7.

Results and Discussion

Family A

Patients of family A were suffering from LCA. The pedigree is shown in figure 1. The pedigree shows four generations. There were six affected male members of the family in fourth generation labeled (IV-7, IV-6, IV-5, IV-12, IV-13, and IV-4) and normal (III-1, III-10, IV-3, and III-4). The pedigree analysis indicated that the disease is transmitted in autosomal recessive pattern. For linkage studies blood was taken from ten members of the family including six affected (IV-7, IV-6, IV-5, IV-12, IV-13, IV-4) and four normal (III-1, III-10, IV-3, III-4) individuals.

Family B

Patients of family B were suffering from LCA. The pedigree is shown in figure 2. The pedigree shows four generations. There were two affected male members of the family in fourth generation labeled IV-6 and IV-7. The pedigree analysis indicated that the disease is transmitted in autosomal recessive pattern. For linkage studies blood was taken from five members of the family including two affected (IV-6, IV-7) and three normal (III-1, III-2, IV-4) individuals.

Linkage analysis

The families were genotyped by using microsatellite markers for the candidate genes involved in LCA.

Family A: The linkage analysis of the Family A did not give any region of homozygosity for the affected individuals. All candidate genes were analyzed. Thus, Family A was excluded, so advance research methodologies like SNP microarray, exome sequencing have to be carried out to know the cause of disease.

Family B

In Family B, DNA of the entire available five members was genotyped for the microsatellite markers in order to find out the causative agent of the disease. The markers are listed in table 2. Genotyping was carried out to map homozygous region shared by affected individuals. Two microsatellite markers D11S1180 and D1S2761 at a genetic distance of 104.28 cM and 105.16 cM respectively, for gene RPE65 show homozygous pattern. This region was further tested by different markers D1S3467, D1S1162 to find out further pattern of homozygosity but it didn't continue so the linkage for the gene *RPE65* was not established.

Another candidate gene *SPATA7* showed homozygous pattern. Markers D14S1044 and D14S1063 at 83.36 and 83.69 cM were homozygous for all affected individuals whereas all normal were heterozygous. To further evaluate this linkage marker D14S67, D14S1066, D14S256 and

D14S617 were tested further which also showed homozygosity. Markers D14S61 and D6S1031 at 72.82 cM and 93.14 cM were heterozygous for normal as well as affected members which create significant critical boundaries of homozygosity at the start and end of gene identified in family B.

Sequencing of *SPATA7* gene exons

Linkage in family B was established on chromosome 14q31.3 bearing *SPATA7* gene. In order to find any variant in *SPATA7* gene 12 exons including exon intron boundaries were sequenced by Beckman sequencer. Sequencing data were analyzed by using clustal W multiple alignment tools. However, no such disease causing mutation was found in the aforementioned twelve exons. Leber congenital amaurosis (LCA) (OMIM: 204000), is severe and diverse kind of retinopathy frequently associated with loss of vision, at or after birth, wandering eyes, Franceschetti oculodigital sign and degeneration of retinal pigment (Hollander *et al.,* 2008). Visual sharpness or keenness is up to 20/400 and fundus appearance is very paradoxical, varying from normality to degeneration of retinal pigment as in case of retinitis pigmentosa (Cremers *et al.,* 2002). Electroretinogram is completely diminished (Hollander *et al.,* 2008).

In the study presented here, two Pakistani families (A, B) demonstrating autosomal recessive LCA was collected. To hunt down gene underlying LCA in these families linkage analysis was performed by typing various microsatellite markers linked to the known gene loci involved in causing LCA. Linkage analysis of family A failed to reveal any linkage to the candidate genes loci. The result signifies the involvement of some novel genes in the pathogenesis of LCA in family A. Therefore, we suggest that advance analysis like SNP Microarray, Exome sequencing, next generation sequencing should be carried out in this family to find any causative gene for the LCA.

Linkage in family B was established to LCA3 locus on chromosome 14q31.3. LCA3 locus harbors *SPATA7* (spermatogenesis associated protein 7) gene. The human *SPATA7* gene *(*MIM 609868) contains 12 exons encompassing about 52.8 kb in size, which translates into a protein product of about 599 amino acids. *SPATA7* remained conserved evolutionary from sea urchin to human, but lacks in lower eukaryotes. *SPATA7* expression has been reported in many retinal layers, incorporating in ganglion cell and inner portions of the photoreceptor pigments. Various stages of expression suggest that *SPATA7* is necessary for the normal regulatory role of retina instead of development. Two isoforms of *SPATA7* have been reported till date in the retina, cerebellum, and testis. Recent studies reveal that the expression of isoform 1 is higher in neuronal tissues as compared to isoform *2* which was mainly manifested in testis (Perrault *et al.,* 2010*).* It is interesting to note that mutations in *SPATA7* cause LCA and retinitis pigmentosa (RP), two overlapping but distinct human retinal diseases.

An interesting side of retinopathies is both clinical heterogeneity and their fundamental molecular mechanisms which are due to intricate genetic inheritance. For instance, digenic triallelic inheritance has been reported in some families, separating the Bardet-Biedl phenotype, in which mutation in a second gene is necessary for an individual who has two mutations in the first gene to exhibit a clinical phenotype or to modify the severity of the primary phenotype (Katsanis *et al.,* 2002).

Table.1 Genes implicated in LCA

Study (year)	Gene	Function	Identification method (RetNet)	Mutation frequency (RetNet)
Perrault *et al.* (1996)	*AIPL1*	Phototransduction	Linkage analysis	Accounts for 5–10% of recessive LCA
den Hollander *et al.* (2006)	*CEP290*	Transport across photoreceptor	Homozygosity and linkage mapping	20% of LCA
Lotery *et al.* (2001), den Hollander *et al.* (2001)	*CRB1*	Photoreceptor development	Linkage mapping	9–13% of LCA
Freund *et al.* (1998)	*CRX (AD)*	Photoreceptor development	Mutation analysis and causes	1–3% of LCA
Sohocki *et al.* (2000)	*GUCY2D*	Phototransduction	Linkage analysis	10–20% of recessive LCA
Bowne *et al.* (2002) and (2006)	*IMPDH1 (AD LCA)*	Unknown	Mutation analysis and linkage mapping	NA
den Hollander et al. (2007)	*LCA5*	Transport across photoreceptor	Identity-by-descent mapping and linkage mapping	NA
Thompson *et al.* (2001)	*LRAT*	Retinoid cycle	Mutation analysis	NA
Gal *et al.* (2000)	*MERTK*	Failure to phagocytose outer segment	Mutation analysis	NA
Friedman *et al.* (2006)	*RD3*	Unknown	Mutation analysis	NA
Janecke *et al.* (2004)	*RDH12*	Retinoid cycle	Linkage analysis	4% of recessive *LCA*
Aguirre et al.(1998), Gu *et al.* (1997), Marlhens *et al.* (1997), Morimura *et al.* (1998)	*RPE65*	Retinoid cycle	Mutation analysis	6–16% of *LCA*
Dryja *et al.* (2001), Gerber *et al.* (2001)	*RPGRIP1*	Transport across photoreceptor	Mutation analysis	4–6% of *LCA*
Wang *et al.* (2009)	*SPATA7*	Unknown	Homozygosity mapping	NA
Hagstrom *et al.* (1998)	TULP1	Transport across photoreceptor	Mutation analysis and linkage mapping	NA

Fig.1 Pedigree of family A

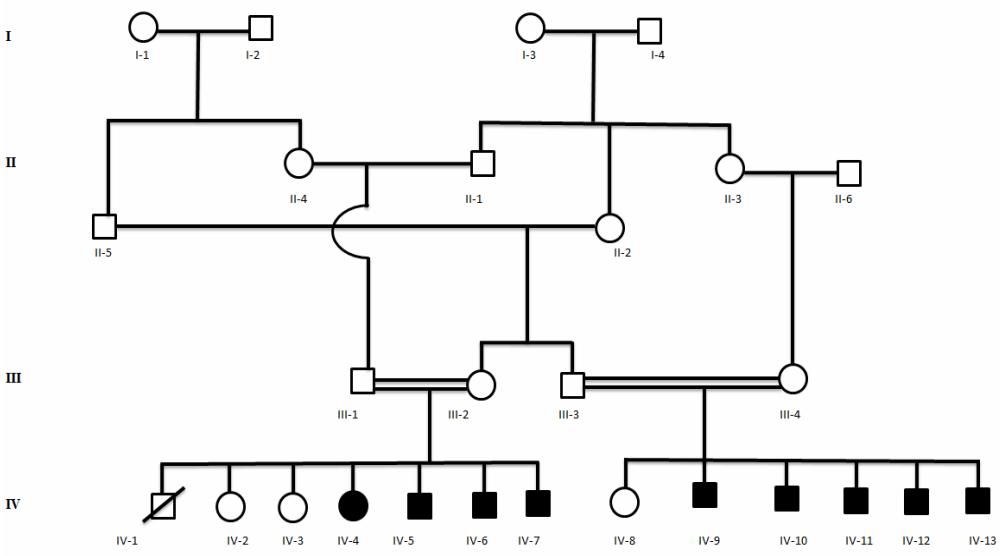

Fig.2 Pedigree of family B

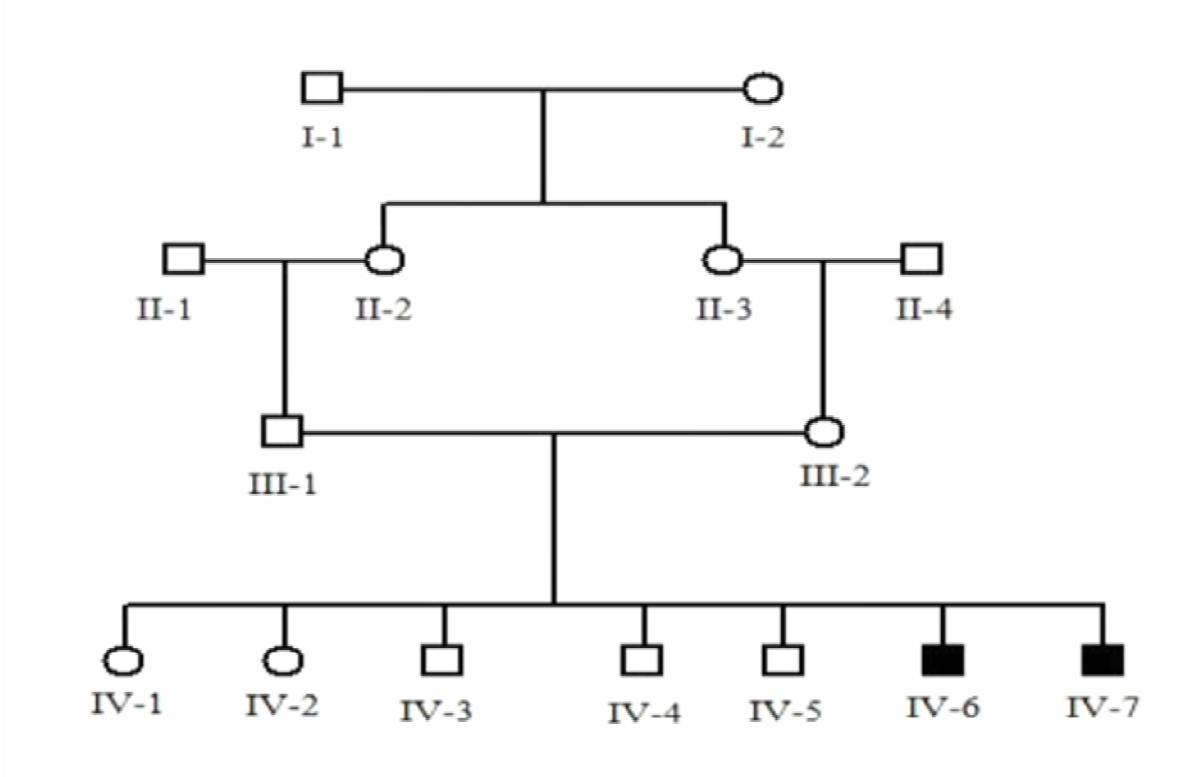

Fig.3 LOD score in the form of a graph attained for (SPATA7 gene) markers genotyped in Family B which yielded a LOD score of 1.32 by using easy LINKAGE

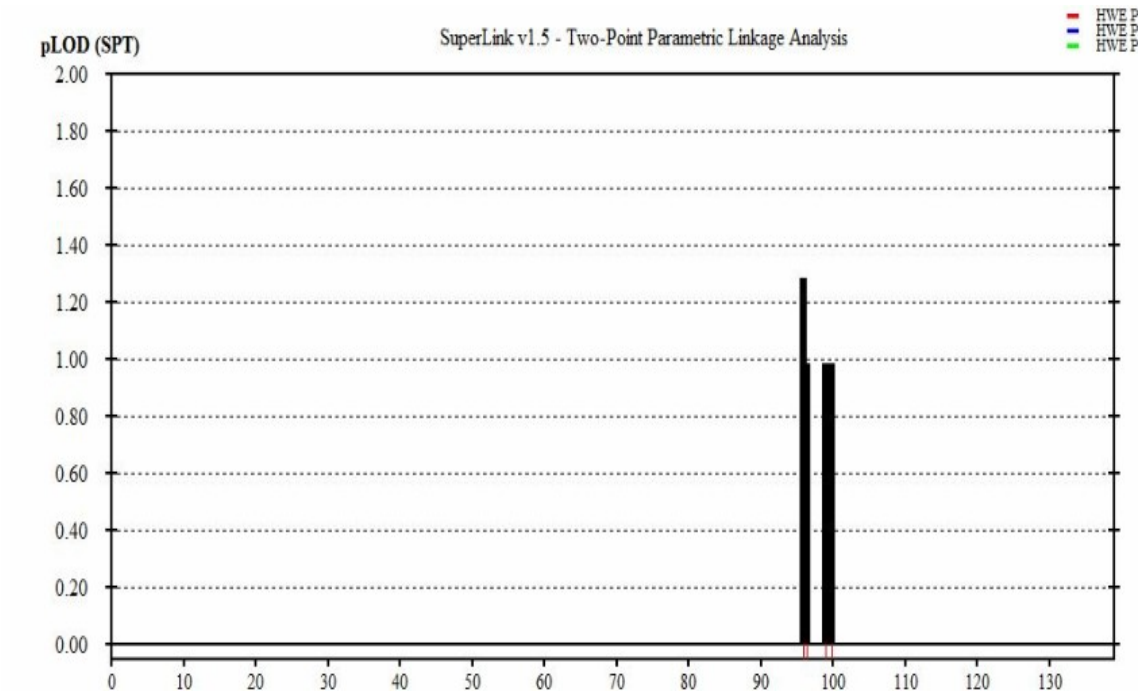

Table.2 List of microsatellite markers for LCA

S. No.	Gene	Markers	Distance (cM)
1	RPGRIP1	D14S122	4.91
		D14S742	9.27
2	AIPL1	D17S1149	19.95
		D17S1298	13.23
3	GUCY2D	D17S720	23.27
		D17S1879	32.73
4	NMNAT1	D1S1597	28.78
5	TULP1	D6S1611	57.04
		D6S1051	58.46
6	RDH12	D14S1038	57.19
		D14S1004	70.12
7	CRX	D19S543	71.49
		D19S902	75.28
8	CABP4	D11S4076	72.3
		D11S4136	80.2
9	SPATA7	D14S1063	83.69
		D14S1066	87.22
10	LCA5	D6S1282	86.81
		D6S1031	93.14
11	CEP290	D12S853	100.4
		D12S1598	101.32
12	RPE65	D1S3467	96.03
		D1S2761	105.16
13	MERTK	D2S293	119.81
		D2S1891	122.2
14	IMPDH1	D7S2543	124.42
		D7S530	132.96
15	LRAT	D4S43049	156.69
		D4S413	160.2
16	CRB1	D1S533	199.85
		D1S1660	202.08
17	RD3	D1S1667	221.7
		D1S2827	225.64
18	KCNJ13	D2S2344	242.78
		D2S2973	249.8

Table.3 Primers of *SPATA7* Exons

Primer name	Primer sequence (5'-3')	Size	Temp
SPATA7-1-F	CTCGTGTAAAACGACGGCCAGTCGCAACTGTCCTCCTAGTACC	486	55
SPATA7-1-R	CTGCTCAGGAAACAGCTATGACACAAATTCAGGGCAAAGAAGC		
SPATA7-2-F	CTCGTGTAAAACGACGGCCAGTTTTAATGCTGTAACTCAGACTTCCT	369	55
SPATA7-2-R	CTGCTCAGGAAACAGCTATGACTGAAGTTCAAATATTCGTCAAATG		
SPATA7-3-F	CTCGTGTAAAACGACGGCCAGTAAGGTTTGAACCCAAATGGTC	475	55
SPATA7-3-R	CTGCTCAGGAAACAGCTATGACCAAAAATGGGTATGAATTTGCT		
SPATA7-4-F	CTCGTGTAAAACGACGGCCAGTCAAGGTCTGGAACATTTTGTGA	334	55
SPATA7-4-R	CTGCTCAGGAAACAGCTATGACTGTTTATGTGGCACAGGAATTT		
SPATA7-5-F	CTCGTGTAAAACGACGGCCAGTATCTAGAGGCACATGTGAAATAAA	398	55
SPATA7-5-R	CTGCTCAGGAAACAGCTATGACCAAAGTCAGATTGTACCACTAAAGAA		
SPATA7-6.1-F	CTCGTGTAAAACGACGGCCAGTTTTTGTAAACCCTTGAGGCTATC	458	55
SPATA7-6.1-R	CTGCTCAGGAAACAGCTATGACGGAGTGAATGGCAATTGTTTGT		
SPATA7-6.2-F	CTCGTGTAAAACGACGGCCAGTAGTCATCACAAATGGTCCTGAG	467	55
SPATA7-6.2-R	CTGCTCAGGAAACAGCTATGACTTCCAATCAAAAGGGCACTATC		
SPATA7-7-F	CTCGTGTAAAACGACGGCCAGTTCTGGCAGTAGGTTTTAGTTGTTTT	449	55
SPATA7-7-R	CTGCTCAGGAAACAGCTATGACTGTATGATAAGTGCCACCAACAG		
SPATA7-8-F	CTCGTGTAAAACGACGGCCAGTTGCTGTGTTATATTCTGCTTTCG	296	55
SPATA7-8-R	CTGCTCAGGAAACAGCTATGACTAGATTGGAGCATGCAATTAAA		
SPATA7-9-F	CTCGTGTAAAACGACGGCCAGTCATTAACCTTAGTCAAATTGTCATTG	650	55
SPATA7-9-R	CTGCTCAGGAAACAGCTATGACTGGTTTCTTTGATTCTTAATCCTTG		
SPATA7-10-F	CTCGTGTAAAACGACGGCCAGTCCCAGTGGATTGCATTTGA	500	55
SPATA7-10-R	CTGCTCAGGAAACAGCTATGACGGTGAACTTCCCCTAGAGTATGA		
SPATA7-11-F	CTCGTGTAAAACGACGGCCAGTTTTTCAACCTTTGTAGTTTCAGTG	300	55
SPATA7-11-R	CTGCTCAGGAAACAGCTATGACTTCCTTTCACTTCTCCCACCAC		
SPATA7-12.1-F	CTCGTGTAAAACGACGGCCAGTAATCCTGTGAGATTTTCAGCAC	450	55
SPATA7-12.1-R	CTGCTCAGGAAACAGCTATGACTCACAGAAGTTTCCCGATCTGT		

SPATA7 is a less common cause of LCA with screening mutations found in only 1.7% of the all LCA cases. Eight mutations

have been identified in *SPATA7* so for. Recently Hollander et al, (2008*)* reported additional three mutations in their study bringing the spectrum of *SPATA7* mutations to 11. Previous information about *SPATA7* mutations has very limited phenotypic information.

After establishing linkage in family B to *SPATA7* gene subsequently all 12 exons of *SPATA7* including exon intron boundaries were sequenced by Beckman sequencer. Sequencing data were analyzed by using clustal W multiple alignment tools. However, no such disease causing mutation was found in the aforementioned exons.

The aim of this study was to identify the disease causing genes and mutations involved in human congenital retinopathies. The objectives of the study included to investigate the disease pathogenesis at the molecular level and enhance genetic counseling for affected individuals. The study will help both the clinicians and researcher in better understanding of LCA which may lead to a targeted therapy or other possible managements. Exclusion mapping of family A from all known loci reported in LCA so far signifies the involvement of some novel gene causing LCA which may broaden the existing human genome database. Family B linked to *SPATA7,* however, did not reveal any pathogenic variant. It, is likely suggested that both families should be passed through latest and advance technologies like whole exome/genome sequencing or next generation sequencing to find any causative agent of LCA which will immediately lead

to improved diagnosis and helps in better understanding the molecular pathology of LCA, and hopefully more effective treatment including perhaps gene therapy.

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