SHORT REPORT

Genetic mapping of autosomal recessive microspherophakia to chromosome 14q24.3 in a consanguineous Pakistani family and screening of exon 36 of *LTBP2* gene

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Abstract

Latent transforming growth factor beta binding protein 2 *(LTBP2)* plays a critical role in the development of connective tissue structure and function. Mutations in gene encoding *LTBP2* are known to cause syndromic and a non-syndromic microspherophakia. Here, we present a 'first' report of genetic linkage of microspherophakia (MSP) to *LTBP2* locus in a large consanguineous Pakistani family with four affected individuals in three loops. Using polymorphic microsatellite markers, haplotypes and linkage analysis, the diseased phenotype in MSP001 family was mapped to the *LTBP2* gene. A maximum two point Logarithm of the odds (LOD) score of 4.16 was obtained with marker D14S284 at θ=0. Mutational analysis of exon 36 of *LTBP2* using Sanger's sequencing did not reveal any previously reported mutations. Further analysis of the remaining exons are required to identify the causative variant.

Keywords: Microspherophakia, Autosomal recessive, LTBP2, Linkage analysis.

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Introduction

Microspherophakia (MSP) is characterised by bilateral small and spherical lens. It is usually responsible for high incidence of myopia, ectopia-lentis and secondary glaucoma.[1-3] MSP can be present in association with other anomalies or as an isolated condition. Isolated forms of MSP may predispose to ectopia-lentis or secondary glaucoma, usually segregated as an autosomal recessive trait.[2,4] To date, mutations in *LTBP2* gene are reported to cause microspherophakia,[4] microspherophakia with ectopia lentis[5] and secondary glaucoma.[6]

LTBP2 gene *(LTBP2* [MIM *602091]) has 36 exons encoding 1821 amino acid proteins with multi-domain structures. It has a structural and functional homology with matrix protein fibrillin-1 and fibrillin-2. During eye development it plays an important role in the formation of micro-fibril bundles in ciliary zonules.[7] *LTBP2* expression pattern in the anterior segment of the eyes and reports regarding identification of pathogenic mutations of *LTBP2* gene in the primary congenital glaucoma (PCG),[7] Weill-Marchesani syndrome (WMS) and Marfan syndrome[5] patients demonstrate *LTBP2* involvement in different eye disorders.[4,5,6,7,8]

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Case Report

Members of an inbred family inheriting an autosomal recessive form of microspherophakia were enrolled in December 2016 from the Punjab province of Pakistan (Figure. 1). A 21-year-old propositus (ID: V-2) visited Layton Rahmatulla Benevolent Trust (LRBT) hospital, Lahore, Pakistan. She presented with complaints of severe pain in the left eye after the use of an eye drop for the dilatation of her pupil following an eye examination. The ophthalmologic examination showed that the lens in her left eye had been dislocated and was now present in the anterior chamber (Figure 2C). It was also noted that both of her eyes had small spherical lenses (Figure. 2C and D). She underwent surgical removal of her lens for refractive purposes with implantation of an artificial intraocular lens. Detailed interview of the patient revealed that three of her cousins had glaucoma from early childhood. Upon clinical examination, they (V-3, V-4, and V-6) were found to have buphthalmos and enlarged corneas with corneal opacities (Figure 2B and Table 1). Their medical records indicated intra ocular pressures above normal ranges and history of multiple glaucoma surgeries/medications. None of these patients had any systemic involvement. Ophthalmological history of family concluded that one affected individual has microspherophakia without glaucoma whereas, three others may have micro-spherophakia and thereafter developed secondary glaucoma.

The study was approved by the Institutional Review Board

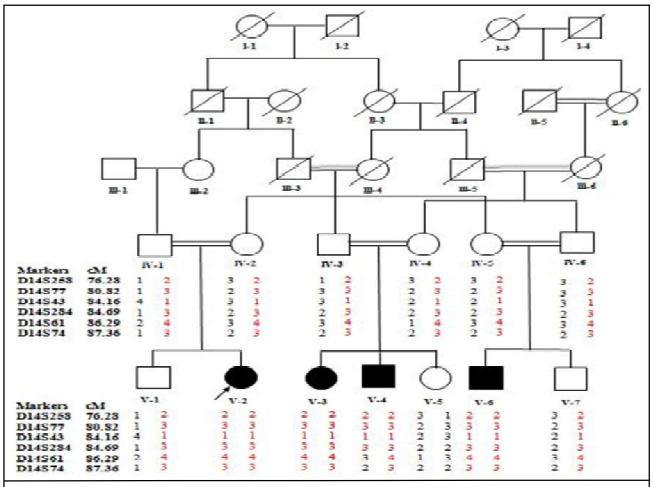

Figure-1: Pedigree drawing of family MSP001 along with haplotypes for markers of *LTBP2* harboring locus on chromosome 14q24.3.The disease haplotype is shown in colour.

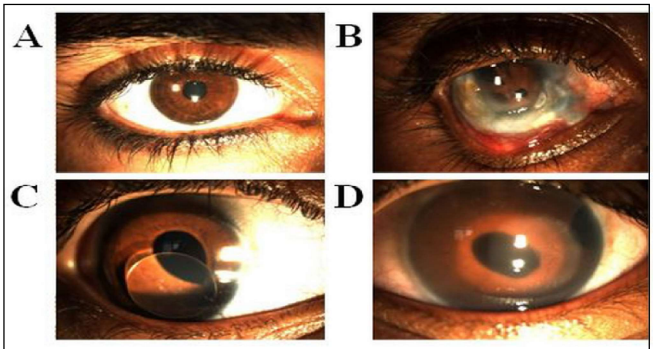

Figure-2: Slit lamp photographs of individuals of family MSP-001, (A) Photograph of normal eye of individual V-1, (B) Photograph of affected individual V-4 shows features associated with congenital glaucoma including buphthalmic eye and corneal opacity, (C and D) Clinical finding of affected individual V-2 shows micros-pherophakic dislocated lens (OD) but no features of congenital glaucoma.

of Quaid-i-Azam University (QAU), Islamabad and conducted at the Department of Animal Sciences during December 2016 to December 2017. Blood samples from four affected and nine unaffected family members were drawn after informed consent and poured in labelled ethylene diamine tetra acetic acid (EDTA) tubes. Genomic deoxyribonucleic acid (DNA) was extracted by a non-organic method.[9] Polymerase chain reaction (PCR) was performed in 20 µl reaction mixture containing 40 ng of genomic DNA, 10X PCR reaction buffer, 2.5 mM magnesium chloride (Solis BioDyne), 2.5 µl of 10 mM deoxynucleoside triphosphate (dNTP) mix, 0.5 µl of 10 pmol/µl of each forward and reverse primer and 0.3 µl of 0.5 U/µl DNA Polymerase (Solis BioDyne). Thermal cycling profile was set at T3 thermocycler (Biometra GmbH, Germany) at 95^{O}C for 5 min followed by 35 cycles of 95^{O}C for 45 sec, 55-58^{O}C for 45 sec, 72^{O}C for 90 sec and a final extension at 72^{O}C for 10 min followed by a final hold at 25^{O}C. Amplified products were electrophoresed on 8% non-denaturing polyacrylamide gels.

The family MSP001 was tested for linkage to GLC3D locus harbouring *LTBP2* gene using markers D14S258, D14S77, D14S43, D14S284, D14S61, and D14S74. These markers were highly polymorphic with an average heterozygosity above 70%. The affected individuals V-2, V-3 and V-6 showed homozygosity for all markers while a region of homozygosity was identified at D14S258, D14S77, D14S43 and D14S284 in individual V-4 with transmission of a recombinant allele with crossing over between D14S284 and D14S61 from his carrier father. Normal individuals except V-5 were carriers for the disease alleles (Figure. 1). Linkage and haplotype analysis showed that the pattern of inheritance of disease phenotype is autosomal recessive in nature. Logarithm of the odds (LOD) scores calculation was performed using the FASTLINK version of MLINK from the LINKAGE Program Package 10. Penetrance of phenotype was taken as 100% with a disease allele frequency of 0.001. The marker order and their relative positions were obtained from the Marshfield database10. Maximum LOD score of 4.16 with marker D14S284 was obtained at θ=0 which suggested linkage to LTBP2 gene (Table 2).

Table-1: Clinical Features associated with the affected individuals of family MSP001.

Marker ID	cM	Mb	Two-point LOD score values at recombination fraction (θ=)										
			0.00	0.01	0.03	0.05	0.07	0.08	0.09	0.10	0.20	Z max	θ max
D14S258	76.28	50.65	4.03	3.91	3.72	3.53	3.31	3.22	3.12	3.03	1.97	4.03	0.00
D14S77	80.82	53.63	2.33	2.29	2.20	2.11	2.00	1.94	1.89	1.83	1.19	2.33	0.00
D14S43	84.16	54.98	3.94	3.81	3.62	3.44	3.25	3.12	3.00	2.94	1.87	3.94	0.00
D14S284	84.69	55.75	4.16	4.06	3.84	3.66	3.47	3.37	3.28	3.19	2.16	4.16	0.00
D14S61	86.29	56.37	-4.34	2.12	2.44	2.44	2.44	2.37	2.31	2.31	1.62	2.44	0.03
D14S74	87.36	58.70	-4.34	2.03	2.34	2.37	2.34	2.31	2.25	2.22	1.56	2.37	0.05

F, Female; M, Male; OD, right eye; OS, left eye; PL, perception of light

Table-2: Two point LOD score of MSP001 family with five adjacent markers of chromosome 14q.

Individual ID	Gender	Age of onset	Age at time of study	Visual acuity (OD/OS)	Ocular problem	Medical Therapy
V2	F	~6 years	21 years	6/36/6/60	Myopia, Microspherophakia	Lens replaced
V3	F	Within 1st year	16 years	PL	Buphthalmous, cornea haze, megalocornea	Medical /Surgical treatment
V4	M	Within 1st year	14 years	PL	Buphthalmous, cornea haze	Medical /Surgical treatment
V6	M	Within 1st year	15years	PL	Buphthalmous, cornea haze	Medical /Surgical treatment

Primer pair for the exon 36 of *LTBP2* was adopted from Kumar et al., 2010.[4] DNA from three affected individuals (V-2, V-3 and V-4) and one unaffected individual (IV-3) of the family was amplified in a 20ul reaction volume containing 40 ng of genomic DNA, 2.5 µl of 10X PCR reaction buffer, 2.5 mM magnesium chloride (Solis BioDyne), 2.5 µl of 10 mM dNTP mix, 0.5 µl of 10 pmol/µl of each forward and reverse primer, and 0.3 µl of 0.5 U/µl Taq DNA Polymerase (Solis BioDyne). Thermal cycling profile was set as 95^{O}C for 5 min, followed by 35 cycles of 95^{O}C for 45 sec, 58^{O}C for 45 sec, 72^{O}C for 90 sec and a final extension at 72^{O}C for 10 min followed by a final hold at 25^{O}C. The amplified products were electrophoresed on 1.5% agarose gel. After ethanol purification of the amplified products, the sequencing reaction was done using Big Dye Terminator Ready reaction mix (Applied Biosystems) according to manufacturer instructions. Sequencing was performed on the ABI 3130 automatic sequencer, and results were analyzed using Sequencher software (version 5.4.6) (Gene Codes Co, Ann Arbor, MI). No mutation was identified in the exon 36 of *LTBP2* in analysed individuals of MSP001.

Discussion

Optical compromise in patients with Microspherophakia is attributed to a refractive error or secondary glaucoma which may occur due to pupillary block following anterior dislocation of the crystalline lens associated with weak zonules.[6] Clinical tests of the family MSP-001 exhibited typical symptoms of microspherophakia in a 21-year-old propositus (V-2 in Figure 1) and glaucoma secondary to microspherophakia in affected individuals V-3, V-4 and V-6. Individual 'V-2' had myopia with no corneal enlargement or buphthalmos similar to findings of Kumar et al., 2010.[4] However, secondary glaucoma in V-3, V-4 and V-6 could have resulted from the microspherophakic lens leading to the interruption of aqueous outflow. Alternatively, mutations in *LTBP2* gene can change the structural design of trabecular meshwork, inhibiting the aqueous outflow leading to an increased IOP and buphthalmos. *LTBP2* plays a significant role in the microfibrils development and ciliary zonules contains microfibrils mainly fibrillin-1. Zonule weakness and lens dislocation is apparent in patients having *LTBP2* mutations suggesting a primary defect in ciliary zonule abnormality.[6-8]

For recessively inherited diseases, the approach of screening for homozygosity by descent in affected individuals is used to test for co-segregation of a known loci with disease phenotype. In the present study, haplotype analysis and positive LOD scores (Maximum 4.16 with marker D14S284 in Table 2) suggests linkage of disease phenotype in MSP-001 family to *LTBP2* locus on chromosome 14q24.3. Previously, homozygous duplication mutation c.5446dupC, p.H1816PfsX28 in exon 36 of *LTBP2* was segregating with autosomal recessive microspherophakia with secondary glaucoma in two individuals and microspherophakia without secondary glaucoma in one individual of an Indian family.[4] Furthermore, a homozygous deletion (5376delC) in the same exon has been reported to cause PCG in a consanguineous Iranian family.[8] DNA sequence analysis of the entire coding region of exon 36 did not identify any pathogenic mutation in family MSP-001 supporting genetic heterogeneity.

Conclusion

Surgical management strategies for PCG and lens-associated glaucoma are very different. Therefore, phenotypic heterogeneity observed in MSP-001 family necessitates in-time screening and treatment of familial cases. Mutational analyses necessitates sequencing of other exons to look for the disease causing mutation in this family. Current study is a 'first report' of genetic linkages of MSP phenotype to *LTBP2* locus in a large consanguineous Pakistani family.

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Conflict of Interest: None.
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