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Autozygosity in a Turkish family with scoliosis, blindness, and arachnodactyly syndrome

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BACKGROUND AND OBJECTIVES: Blindness-scoliosis-arachnodactyly syndrome has been described in a family with parental consanguinity. We present the strategy employed to determine the gene locus responsible for the syndrome.

DESIGN AND SETTING: A retrospective study of blindness-scoliosis-arachnodactyly syndrome patients at the Department of Medical Genetics, Erciyes University, between 2009-2010.

PATIENTS AND METHODS: Whole genome single nucleotide polymorphisms (SNPs) were scanned using a 250K Affymetrix array. We visually evaluated runs of homozygosity shared by two affected brothers that segregated in the entire pedigree with different combinations due to the unclear affected status of some siblings. Two and multiple-point LOD (logarithm [base 10] of odds) score analyses were performed by easyLINKAGEplus v5.08.

RESULTS: Five homozygous blocks over 2 Mb shared by two affected brothers segregated with phenotype in two affected and three unaffected siblings and in the mother whose phenotypes were unequivocal. The longest homozygous block in this analysis was on chromosome 14 between 67817621bp (rs7148416) and 82508151bp (rs17117757). When another sister with positive eye findings was added to the analysis, this region was narrowed to between 67817621bp (rs7148416) and 75657598bp (rs11626830), with a maximum LOD score of 2.3956 by two-point analysis. Three candidate genes were detected in this region.

CONCLUSION: This study contributes to the existing literature on the region 67817621 bp 82508151 bp (rs17117757) on chromosome 14 and the three candidate genes, which could be responsible for the syndrome.

The identification of genes for syndromic disorders not only provides insights into the cause and molecular pathology of disease, but also advances our understanding of their normal function. Although technology has advanced to the point where large-scale genome-wide association studies for complex diseases are now feasible, family-based disease gene discovery still plays an important role, particularly in identifying the genetic causes of Mendelian disease.[1] Even in the case of rare syndromes, patients and families benefit from a clear molecular diagnosis which may assist with prognosis and clinical management as well as risk prediction, enabling prenatal and preimplantation genetic diagnosis.[2] In addition, novel disease gene discovery can lead to insights into the function of those genes and a better understanding of the underlying biology.[3,4] Many

syndromes have been described where the causative gene mutation has yet to be identified.

Scoliosis, blindness, and arachnodactyly syndrome has been described by Dundar et al (2008)[5] in a family with parental consanguinity. This family has been followed since 1996. Biochemical analyses, cytogenetic and aminoaciduria analyses, echocardiography, psychomotor development and intellectual levels were normal. All affected individuals had variable degrees of scoliosis, blindness and arachnodactyly (**Figure 1**). They had sufficient vision for childhood activities and started to lose vision following lens luxation due to unknown causes between 8-12 years of age. Ocular progress included total retinal detachment, phthisis, opaque small cornea, and finally blindness due to retinal detachment. Dundar et al (2008)[5] reported the differences in scoliosis, blind-
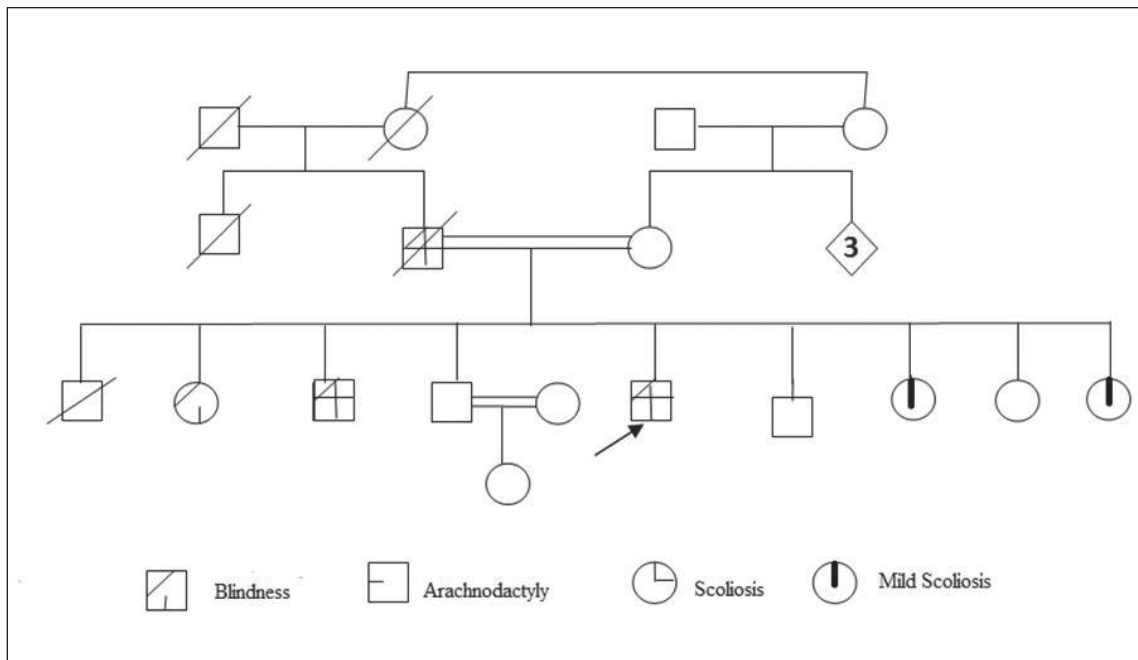
Figure 1. Family's pedigree.

ness, and arachnodactyly syndrome from Marfan and other connective tissue syndromes through both mutation analysis on *FBN1*, *TGFBR1*, and *TGFBR2* genes and clinical signs.[5]

Persons in consanguineous families with rare recessive diseases such as scoliosis, blindness, and arachnodactyly syndrome are likely to be autozygous for markers linked to the disease locus. Autozygosity mapping is a frequently used method for discovering autosomal recessive disease loci. The method is based on the fact that the affected individuals for an autosomal recessive trait who have consanguineous parents have most likely received the disease locus in the homozygous state identical by descent, from a common ancestor.[6,7] Searching for common homozygous regions shared by such individuals by using autozygosity mapping is a highly effective method for mapping loci, an initial step for the identification of genes responsible for autosomal recessive disorders.[8] In the present study we aimed to determine the gene locus of this syndrome by autozygosity mapping.

PATIENTS AND METHODS

Participants

Case #1 (Proband)
The proband, who was 29 years old at the time of study, was referred to our clinic with severe scoliosis at the

age of 16. Although he had undergone surgery, at the follow-up examination he had more significant scoliosis. At the age of 16, the proband's height was 153 cm (<p25), upper body 76 cm, upper to lower body ratio 0.987 (+1SD), and armspan 156 cm. Arachnodactyly was present in both hands and feet (>95) and the fifth toes were situated bilaterally in the back position. The proband showed neither wrist and thumb signs nor joint laxity. He also showed no skin, face, tooth, and palate malformations. No skeletal findings indicating Marfan syndrome were detected by whole body radiography. The proband had suffered from strabismus and progressive visual loss since the age of 8. Ophthalmic examination revealed bilateral lens luxation and hypertrophy in retinal pigment epithelium. A visual acuity of 0.2 was achieved in both eyes with +6.0 glasses. These findings were consistent with severe myopia, which turned to hypermetropia after lens luxation. Corneal diameters were 10 mm horizontally and could be considered microcornea. Over 2 years, retinal detachment was observed in the right eye.

Case #2
The proband's 20-year-old brother suffered from kyphoscoliosis and bilateral arachnodactyly was present in his both fingers and toes. He was completely blind and ophthalmic examination found bilateral phthisis with opaque small lenses. He was 130-cm tall without joint laxity. Skin and facial malformations were not observed.

Case #3

The proband's sister (proband's sister 1), who was 23 at the time of her first examination, presented with eye findings only with no arachnodactyly or scoliosis. Ophthalmic examination revealed bilateral phthisis with totally opaque small lenses and total blindness. The sister was 150-cm tall and had no skin or facial malformations, and no joint laxity.

Other family members

When the proband was first admitted to the clinic his mother and father were 55- and 60-years old, respectively. The parents were first cousins without remarkable family histories. Although two siblings of the proband shared similar clinical features, the five unaffected siblings did not present any findings. The deceased father shared similar clinical features as well.[5]

Procedures

Peripheral blood samples were obtained for genetic analyses with informed consent from the mother and from the patients and unaffected siblings according to our study protocol approved by the ethical committee. Genomic DNA was extracted by the standard phenol-chloroform method. A genome-wide search for homozygosity was performed on the family using a 250K Affymetrix® microarray(Affymetrix UK Ltd, High Wycombe, UK, *http://www.affymetrix.com/*) of 250 000 autosomal single nucleotide polymorphisms (SNPs) according to the instructions of the manufacturer.

Data analysis

We visually evaluated runs of homozygosity (ROH) shared by two affected brothers and segregating in the entire pedigree with different combinations due to the unclear affected status of some siblings. Two and multiple-point analyses were computed using easy LINKAGE plus v5.08 software postulating a gene frequency of 0.005 and marker allele frequencies as observed in a series of control subjects, with a minimal minor allele frequency of 0.10. All known genes located in the region determined by the calculated LOD (logarithm [base 10] of odds) scores were detected by electronically scanning the online databases (NCBI-Map Viewer, Ensembl, OMIM, KEGG). DNA sequence analysis was performed for the gene that was decided to most likely cause the syndrome among the candidate genes. Primers were designed to amplify the coding exons (including splice sites) of each gene, and PCR products were directly sequenced on the CEQ™ 8000 Genetic Analysis System (BeckmanCoulter, Fullerton,

CA, USA) automatic capillary DNA sequencer according to standard protocols. Each exon was sequenced in affected individuals, and sequences were compared to the reference sequence.

RESULTS

The homozygosity mapping performed in the family using 250K Affymetrix array identified large homozygous segments. Five homozygous segments over 2 Mb segregated over two affected and three unaffected siblings were detected. Three siblings were not included in this first analysis. The largest one among these five homozygous blocks was on the 5th chromosome between 29962835 bp (rs2914130) and 16760837 bp (rs17501392). When case#3 and one of the sisters (proband's sister #2) were included as affected individuals, the block was shortened while the addition of case #3 only resulted in disappearance of the homozygous block. The second largest segment was on the 14th chromosome between 67817621 bp (rs7148416) and 82508151bp (rs17117757). When case #3 was considered as affected and 2 siblings (proband's sisters #2 and #3) unaffected, the block was shortened between 67817621 bp (rs7148416) ile 75657598bp (rs11626830). The largest segment on the 5th chromosome did not segregate with the phenotype; thus further analyses focused on the region in the 14th chromosome, for which a maximum LOD score of 2.3956 was calculated with two-point analysis. LOD score for the same region was calculated as 2.1335 in multipoint LOD score analysis. As there were no previously reported genes related to the syndrome in the region, three candidate genes that could be related to the mechanism of the syndrome were determined by scanning the electronic databases. PCR forward –reverse primers of 2-17 exons for *LTBP2* gene sequencing were designed with PRIDE 2.0 (**Table 1**). Exon 1 was not included in the study as suitable primers could not be designed due to its length. Sixteen exons between exons 2 and 17 of the *LTBP2* gene were sequenced and compared to the databases using Chromas 2.32 and NCBI-BLAST programs. A G/C mutation was found in the first intron, 85 bp upstream of exon 2, but it was disregarded as it would not be translated into protein. No other mismatches were found in the remaining exon sequences. As a result, no mutations were detected between exons 2 and 17 of *LTBP2* gene.

DISCUSSION

In this study, aimed at finding the responsible gene(s) for autosomal recessive scoliosis, blindness, and arachnodactyly syndrome, homozygosity mapping was per-

Table 1. *LTBP2* gene PCR primers (exon 2-17).

Exon 2	F Primer 5'-TAGATTTGCAAGTAGAATAG-3' R Primer 5'-GTTGCCTCCACTTTAAATA-3'
Exon 3	F Primer 5'-CTCATATCTCTGGCCATGC-3' R Primer 5'-TTCTGACAGGAAGGTGTG-3'
Exon 4	F Primer 5'-AGGAGTGGCACAGACACA-3' R Primer 5'-ACAAGGTGACAACTCAGC-3'
Exon 5	F Primer 5'-GGAACCATCTGAGCAGGA-3' R Primer 5'-CCTTGAGATGAATGCAGC-3'
Exon 6	F Primer 5'-CACTCTTCTTCACTCTACAT-3' R Primer 5'-GATCATAAAGGAAGGACTAC-3
Exon 7	F Primer 5'-ACACATAGCCAGTGGCCT-3' R Primer 5'-ACAACTGCCATCCTGGAA-3'
Exon 8	F Primer 5'-GAGTGAGCTCTGTGTCCTA-3' R Primer 5'-TACCAGCCTGTTCTACCT-3'
Exon 9	F Primer 5'-GAGAGGAGTCTGGTGAGAAC-3' R Primer 5'-AGTCCCTGGATTTCCTTAGT-3'
Exon 10	F Primer 5'-GAATCCCACTGAATACATAG-3' R Primer 5'-GAAGTGTCCCATCTTGTCTC-3'
Exon 11	F Primer 5'-CTAAAGTTCTTGTGAGCATT-3' R Primer 5'-CTACTTCAGTCTTCCAGATT-3'
Exon 12	F Primer 5'-GGAAGACTGAAGTAGCCT-3' R Primer 5'-ACTTCTCTGAGGTGCTGG-3'
Exon 13-14	F Primer 5'-GAGCTCACGCTGTTACCTT-3' R Primer 5'-GAACCAGCTCACACATAG-3'
Exon 15	F Primer 5'-CTCTCAGATTCTCTAAGTGG-3' R Primer 5'-GGGACTTTACCTAGTTGGA-3
Exon 16	F Primer 5'-AGTTCTGGTCTCTCCTCCT-3' R Primer 5'-GACTCTCAACTCGGCCT-3'
Exon 17	F Primer 5'-CTTCCAGCCTTAGAGATT-3' R Primer 5'-AGTTCTGGTCTCTCCTCCT-3'

ed to Marfanoid features. In this search, the TGF-α pathway related to the Marfan syndrome and Notch pathway causing scoliosis were used. The genes in the region with known pathway information were investigated for their product activities. The three candidate genes were the 36-exon *LTBP2* gene in the TGF-α pathway,[9,10] The *PSEN1* gene responsible for spinal development in Notch pathway,[11,12] and the Numb gene, also in the Notch pathway, is an important pathway in scoliosis.[13,14] Numb was chosen as a second target gene only to study its relevance in scoliosis. *PSEN1* was not studied because it is known to be an important gene in Alzheimer disease. *LTBP2* gene mutations, especially in connective tissue, result in Marfanoid features such as eye lens dislocations (microspherophakia, ectopia lentis), myopia, megalocornea, glaucoma, excessive height and arm length.[15-17] Therefore, *LTBP2* was sequenced as the 1st candidate Human *LTBP2* has 36 exons and is localized in 14q24.3 encoding for latent TGF-α Binding gene.

The proteins encoded by this gene are multi-domain extracellular matrix proteins. The components of extracellular matrix and their varying expression models play important role in tissue formation. Latent TGF-α proteins are the largest members of the LTBP family with their multi-domain structures showing strong resemblance to fibrillins. Considering the important role of fibrillin mutations in Marfan syndrome,[18-22] the first 16 exons of *LTBP2* gene that are highly dense with EGF like, EGF-like Ca binding,[8] and 4 sistein domains also found in fibrillins were sequenced.

Beals syndrome (congenital contractural arachnodactyly) with arachnodactyly, ectopia lentis, and kyphoscoliosis is another syndrome presenting with scoliosis, arachnodactyly, and blindness besides scoliosis, arachnodactyly, and blindness and Marfan syndromes. Presenting with lighter findings than Marfan syndrome, Beals syndrome is the result of mutations in the *FBN2* gene on the fifth chromosome.[17,23-25] In the literature, cases with mutations in the *ROBO3* gene localized on the 11th chromosome present with progressive scoliosis and horizontal gaze palsy.[26,27] Cases with adolescent idiopathic scoliosis only were connected to chromosomes 1, 6, 7, 8, 12, and 14.[28] In a case study with scoliosis and deafness, mutations in *PPP1R9B* and *COL1A1* genes were detected,[29] and a family with adolescent idiopathic scoliosis and pectus excavatum, a link was detected in 18q.[30] Other than Marfan and Beals syndromes, no other syndrome or responsible chromosome region has been reported that represents the scoliosis, arachnodactyly, and blindness findings together. With this study, we propose that the gene re-

formed to detect gene loci. Genome wide SNPs were scanned for using 250K Affymetrix array. Due to the equivocal conditions of some siblings, segregation was evaluated with all possible combinations in the pedigree using the two affected brothers sharing the homozygosity. Two- and multi-point LOD score analyses were performed by easy LINKAGE plus v5.08. Two-point analysis resulted in a maximum LOD score of 2.3956 for the region between 7817621bp (rs7148416) and 75657598bp (rs11626830) on 14th chromosome. Using the electronic databases, 87 genes were located in this region and further literature investigation helped us to choose 3 candidate genes. DNA sequence of 16 exons between 2 and 17 of the most probable candidate gene, *LTBP2*, was analyzed and no mutation was detected.

Since scoliosis, arachnodactyly, blindness syndrome carries Marfanoid features, three candidate genes were chosen around the pathways known to be relat-

gion between 14q3.3-q24.3 is linked with the scoliosis, arachnodactyly, and blindness syndrome.

As a result of the gene detection strategy postulated and followed in this study, three candidate genes were determined that could be responsible for the scoliosis, arachnodactyly, and blindness syndrome in the region between 7817621bp (rs7148416) and 75657598bp (rs11626830). No base change was detected in the protein coding regions by DNA sequence analysis of the 16 exons of the *LTBP2* gene. Electronic databases need to be closely followed for newly found genes in the region and their possibilities for being the candidate gene

should be evaluated. Another method, STR marker, could be applied to the cases presented here to find the responsible gene as an additional secondary strategy. Thirdly, exome sequencing of the patients' genome is being planned to identify the responsible gene(s) for the syndrome. A new gene could be added to the literature after these processes. Our study contributes to the disclosure of the chromosome region [67817621bp (rs7148416) - 82508151bp (rs17117757) on chromosome 14] and the three candidate genes (*LTBP2*, Numb, *PSEN1*), which could be responsible for the syndrome.

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