Two families of nail-patella syndrome with splice site mutations in the *LMX1B* gene

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<Introduction>

Nail-patella syndrome (NPS; OMIM #161200) is characterized by nail dysplasia and skeletal anomalies such as aplastic or hypoplastic patella, elbow abnormalities and iliac horn, which is frequently associated with nephropathy.¹ NPS is caused by heterozygous mutations in the *LMX1B* gene, encoding a member of the LIM homeodomain protein family.² Gene analysis in NPS has so far disclosed that haploinsufficiency of *LMX1B* leads to the clinical manifestation of NPS.³ In this study, we evaluated two families with NPS.

<Family 1>

Family 1 was referred to us with nail deformities from birth. The affected family members were mother (I-2), 9-year-old boy (II-1), 7-year-old twin sisters (II-2 and II-3) and a 7-month-old younger brother (II-4). Their thumbnails exhibited dysplasia. Some of them showed triangle lunules on their index, middle and ring fingers. The creases of the skin overlying the distal interphalangeal (DIP) joints of their fingers were lost.

Figure 1. Pedigree and clinical features of Family 1.

(a) Pedigree of Family 1. Affected individuals are indicated by black fills. (b-f) Clinical manifestations. The creases of the skin overlying the DIP joints are lost (arrow) (b). Thumbnails dysplasia of the children (c-f).

<Family 2>

The proband was a 6-month-old boy. There was no family history of nail deformity. Clinically he showed thumbnail dysplasia. Triangle lunules and spoon nail were observed on his index, middle, ring and little fingers. The creases of the skin overlying DIP joints of their fingers were lost.

Figure 2. Pedigree and clinical features of Family 2.

(a) Pedigree of Family 2. The proband is indicated by an arrow. (b,c) Clinical manifestations of thumbnails (b) and the other finger nails (c).

<Gene analysis>

Direct sequencing analysis of exons and intron-exon boundaries of *LMX1B* revealed Family 1 and Family 2 to be heterozygous for the mutations c.140-1G>C and c.326+1G>C, respectively (RefSeq: NM_001174146.1).

Figure 3. Gene analysis.

Affected family members of Family 1 and Family 2 are heterozygous for the splice site mutations c.140-1G>C and c.326+1G>C, respectively.

<Reverse transcription-PCR>

Since c.140-1G>C and c.326+1G>C were located at the exon-intron boundaries, we performed splice variant analysis using RT-PCR of cDNA extracted from the dorsal thumb skin of the affected mother (I-2) of Family 1 and the proband of Family 2. The cDNA were amplified using primers designated in exon 1 and exons 5-6, which showed two transcripts, 827 bp (transcript 1) and 640 bp (transcript 2), in both Family 1 and Family 2 cDNA. Cloning and direct sequencing of the amplified fragments revealed that transcript 1 was wild-type. In transcript 2, exon 2 was skipped and exon 1 was directly connected to exon 3, resulting in a frameshift and a subsequent premature termination codon (p.Asp49Serfs*17).

Figure 5. Results of RT-PCR.

(a) RT-PCR shows two splicing variants. N.C. indicates normal control. (b) The sequences of the RT-PCR products are subcloned using TA cloning vector, showing a splice variant.

<Conclusion>

In this study, we evaluated two families with NPS. Direct sequencing analysis revealed Family 1 and Family 2 were heterozygous for the splice site mutations, c.140-1G>C and c.326+1G>C, in *LMX1B*, respectively. To identify the mechanism of pathogenesis, we performed RT-PCR of cDNAs extracted from the dorsal skins of their thumbs, which showed normal and shortened PCR products in both samples. Intriguingly, sequencing of the shortened PCR products revealed the same splice variant with deletion of exon 2, leading to a frameshift (p.Asp49Serfs*17). Our data confirm that NPS is caused by haploinsufficiency of *LMX1B*.

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<References>

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