Novel compound heterozygous mutations in PLEC1 causing epidermolysis bullosa simplex with muscular dystrophy

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INTRODUCTION & CLINICAL FINDINGS

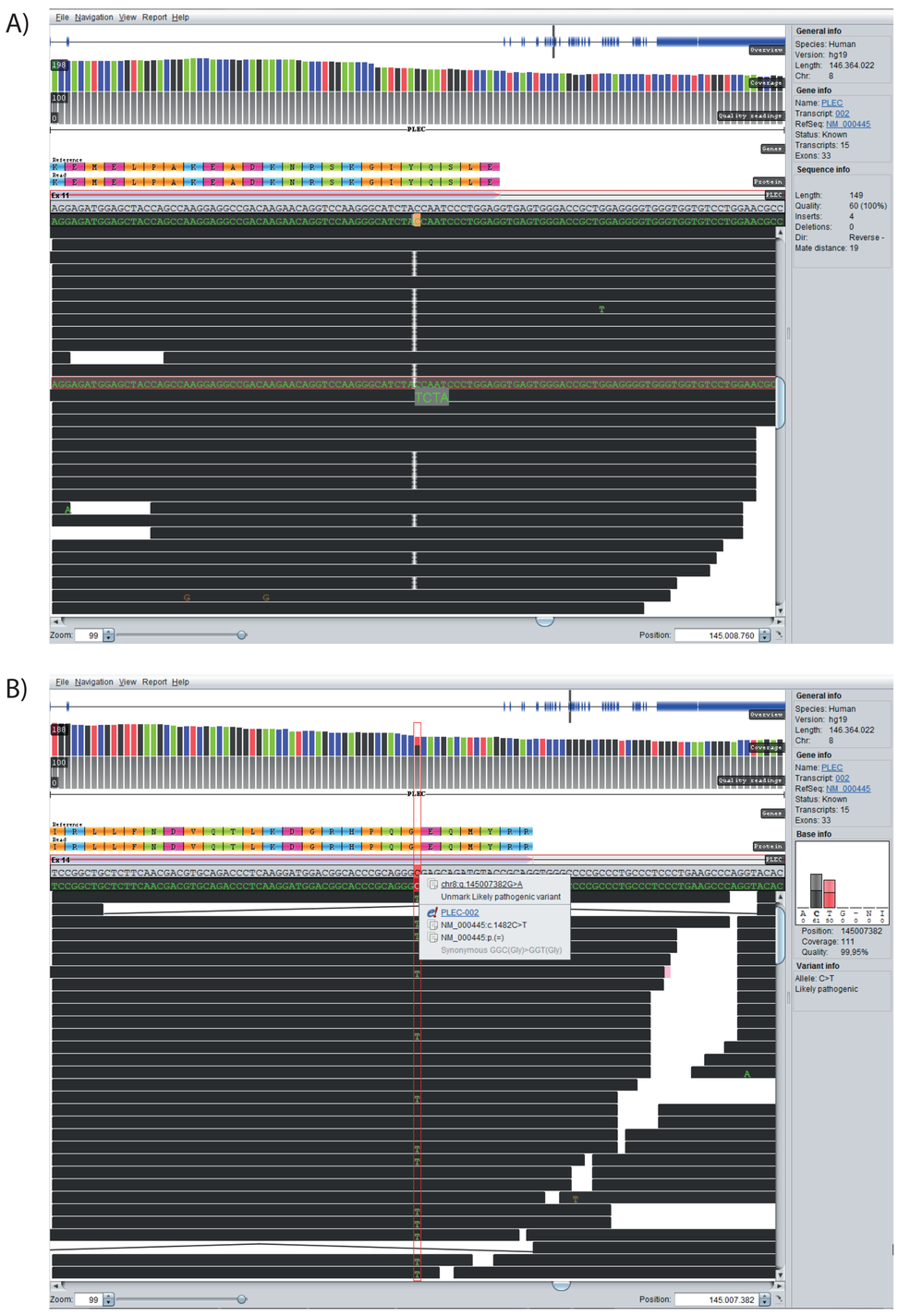
Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) is an autosomal recessive disorder caused by mutations in the plectin 1 gene (PLEC1). EBS-MD is characterized by early childhood onset of progressive muscular dystrophy and blistering skin changes (OMIM #226670).

Here, we report on a 28-year-old man who has been suffering from chronic skin disease with blistering and yellow nails since his birth. In addition, he developed a reduction of his muscular strength over at least the past five years. The patient showed generalized muscular atrophy, particularly affecting proximal muscles, and a moderate proximal but also distal tetraparesis. Finger extension was severely impaired on both sides while finger flexion showed normal strength. No deep tendon reflexes could be elicited.

Neurological examination further revealed mild bilateral ptosis, moderate bilateral ophthalmoparesis into all directions and a moderate generalized facial weakness. A biopsy of the left deltoid muscle showed signs of muscular dystrophy and mitochondrial disease.

METHODS & RESULTS

Analysis of the large PLEC1 gene was performed by partial exome enrichment using the TruSight One panel consisting of 4813 disease-associated genes followed by next generation sequencing (NGS) on a MiSeq desktop sequencer (Illumina). Data analysis was done by the software GensearchNGS (PhenoSystems), pathogenicity and splice predictions were carried out using the algorithms embedded in Alamut (Interactive Biosoftware). More than 98 % of the coding region of PLEC1 was covered at least 20x. We identified two heterozygous mutations in PLEC1. The first one was a small duplication of 4 bp (c.1106_1109dup) in exon 11 leading to a frame-shift and a preliminary stop codon at amino acid position 417 (p.Gln371Leufs*47). The second variant was a synonymous mutation (c.1482C>T, p.Gly494Gly) which was predicted to create a novel 5'-splice site 19 bp upstream of the original 5'-splice site by high scores of five different splice prediction tools. The new splice site would result in a loss of the last 19 bp of exon 14 in the PLEC1 transcript and also in a frame-shift leading to a preliminary stop codon at position 503 (p.Glu495Cysfs*9). The effect of the synonymous potential splice mutation was checked by mRNA analysis in the patient. Both parents were sequenced for the PLEC1 mutations detected in their son in order to confirm the autosomal recessive inheritance of the disease: the small duplication was inherited from the mother and the synonymous mutation from the father. Both PLEC1 mutations have not been described before in any mutation or polymorphism database and could be classified as pathogenic because of their truncating effects.



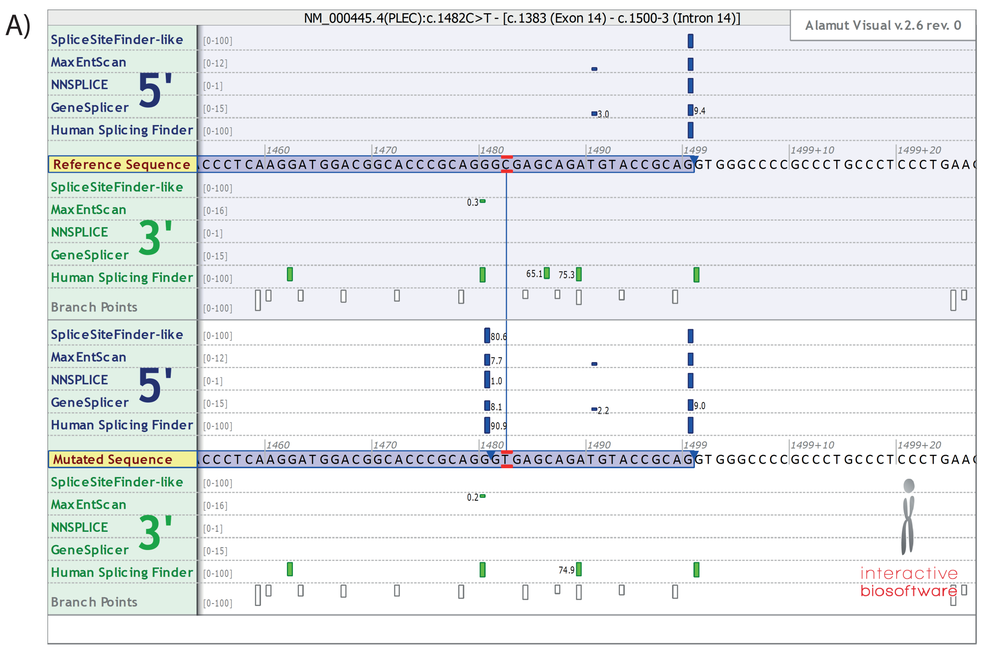


Figure 1:

NGS analysis of the index patient: A) PLEC1, exon 11, and B) PLEC1, exon 14. Black horizontal lines indicate reads which completely match to the reference sequence (ref: grey line with black letters); grey vertical lines in the black reads indicate inserted nucleotides which can be displayed by right mouse click (shown in one read of exon 11: insTCTA); the grey box displays the synonymous mutation detected in exon 14 of the PLEC1 gene; the nucleotide exchange is shown in the red column.

Figure 2:

A) Splice site prediction of the synonymous mutation detected in exon 14 of the PLEC1 gene. Five different splice prediction tools embedded in Alamut predicted a *de novo* 5'-splice site caused by the nucleotide exchange c.1482C>T.

B) PLEC1 mRNA analysis of exon 14 in the index patient (from blood and muscle) which confirmed the splice prediction for the synonymous mutation resulting in a loss of the last 19 bp of exon 14 and hence in a frame-shift and a preliminary stop codon.

CONCLUSION

In summary, we could identify the molecular cause of the EBS-MD disease in our patient by "clinical exome" sequencing.

The NGS approach applied for this case seems to be an efficient method for analysis of large genes for which Sanger sequencing is not established in routine diagnostics.

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