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A novel splice acceptor site mutation (IVS11 G > A) of *PEPD* gene causing prolidase deficiency associated with hyperimmunoglobulinemia E

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ABSTRACT

Prolidase deficiency is a rare, autosomal-recessive, inborn error of collagen metabolism. We report a 20-year old girl with prolidase deficiency, who presented with spontaneous, refractory, lower extremity ulceration, elevated immunoglobulin E levels (IgE) and normal intellect. Hyperimmunoglobulin E syndrome was considered as a possible diagnosis but Th1 and Th17 T cell subsets were normal. Thin layer chromatography for examination of amino acids in random urine sample revealed proline containing peptides. Prolidase enzyme activity was undetectable in dried blood spots as well as in plasma using glycylproline as substrate. Sequence analysis of *PEPD* gene revealed a single nucleotide substitution (G > A) at the splice acceptor consensus sequence of AG resulting in AA at 3′ of intron 11 in both the alleles leading to skipping of entire exon 12, frame shift and premature stop codon at 278.Comparative expression profile of selected inflammatory genes was carried out in the index patient with prolidase deficiency and high immunoglobulin E levels, another patient with hyperimmunoglobulin E syndrome and a normal control. Increased expression of *IL-23 and TNF-α* in prolidase deficient patient while *IL-6 and TNF-a* in hyperimmunoglobulinemia E patient was noted. This indicates a pro-inflammatory state in hyperimmunoglobulin E syndrome compared to the stable regulatory immunological state in prolidase deficiency. To the best of our knowledge, 819-1G > A is a novel mutation of *PEPD* gene in patients with prolidase deficiency and elevated immunoglobulin E levels.

Introduction

Human prolidase is a widely distributed dimeric metalloenzyme encoded by *PEPD* gene located on chromosome 19 and is composed of 493 amino acids (Lupi et al., 2008). It cleaves iminopeptides with C-terminal proline or hydroxyproline, which result from degradation of collagen. Mutations in *PEPD* gene cause reduction or loss of prolidase enzyme activity. Prolidase deficiency (PD) is an extremely rare autosomal-recessive disorder with less than 100 cases described in literature (Ferreira and Wang, 2015). The correlation between genotype and phenotype is not clear in literature; however the dominant clinical manifestations are recurrent and refractory chronic leg ulcers.

We describe here a girl with PD and characteristic facial dysmorphism, chronic leg ulcerations, venous insufficiency and hyperimmunoglobulin E (IgE) due to a novel homozygous c.819-1G > A mutation. We also report increased expression of *IL-23 and TNF-α* in PD patient while increased expression of *IL-6* and *TNF-a* in a patient with hyper IgE syndrome. This may give newer insights into the pathogenesis as well as varied phenotypic spectrum of this rare disorder.

Case report

A 20-year old girl, born to consanguineous Muslim parents, presented with spontaneous, persistent, treatment-refractory lower extremity ulceration for 4 years. There was no history suggestive of distal neuropathy, trauma, diabetes, photosensitivity or malar rash. Birth and development milestones were normal. Family history was non-contributory. Examination revealed normal anthropometry and vital parameters. She had large, dirty-looking ulcers over the posterior part of bilateral ankle joints with diffuse dyspigmentation,

Abbreviations: PEPD, *Paroxysmal extreme pain disorder*; PD, Prolidase deficiency; IgE, Immunoglobulin E; Th1, T helper cell 1; IL, Interleukins; TNF-α, Tumour necrosis factor alpha; STAT, Signal Transducer and Activator of Transcription; G, Guanine; A, Adenine.

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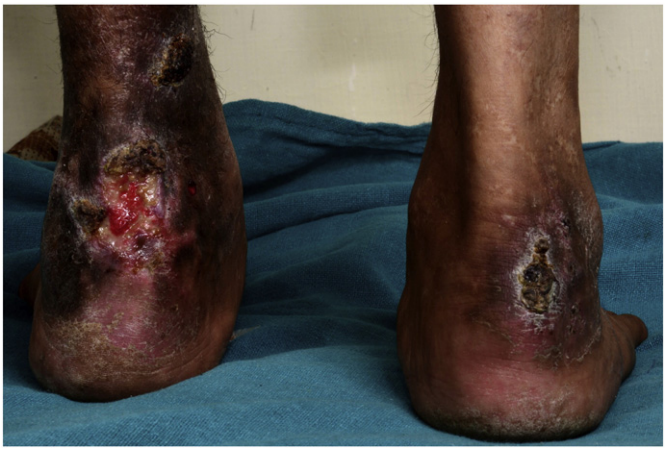

Fig. 1. Non-healing ulcers on the posterior aspect of both ankles.

granulation tissue, erythema and scaly skin with surrounding induration (Fig. 1**).** She also had left corneal keratitis, firm nontender splenomegaly, high plantar arches and antalgic gait. Facial dysmorphism with small almond-shaped eyes, hypertelorism, epicanthic folds and bilateral clinodactyly was noted. Rest of the systemic examination was not contributory.

Laboratory investigations showed haemoglobin 10.6 g/dL, platelet count 141 × 10^9/L and total leukocyte count 4.2 × 10^9/L (80% neutrophils, 14% lymphocyte, 4% monocytes and 2% eosinophils). Serum electrolytes, coagulation profile, renal and liver function tests were normal. Serum immunoglobulin profile showed significantly elevated IgE levels [3300 IU/ml (normal < 100 IU/ml)]. Complement levels (C3) were mildly elevated 236 mg/dl (normal 50–150 mg/dl). Dihydrorhodamine assay and nitrobluetetrazolium dye reduction tests were normal. Human immunodeficiency virus serology by ELISA and anti-nuclear antibodies by indirect immunofluorescence were negative. Proportion of IL-17 producing CD4+ T cells was 1.2% (normal >1%), IFN-γ producing CD4+ T cells (Th1 cells) were 16.6% (normal 5–20%). Flow cytometric detection of pSTAT3 on peripheral blood mononuclear cells (PBMCs) revealed a mean fluorescence intensity of 74 in unstimulated PBMCs which increased to 329 upon stimulation with interleukin 6 (IL-6). Also, the percentage of pSTAT3 positive PBMCs increased from 0.2% to 46.7% on stimulation with IL-6. Based on the above findings, *STAT 3* deficient hyper IgE syndrome was ruled out. Thin layer chromatography in the urine sample revealed large ninhydrin stained yellow spots suggesting proline containing peptides. Prolidase levels in dried blood spots and in plasma were undetectable. Tandem mass spectrometry and gas chromatography/mass spectrometry for fatty acid oxidation disorders were normal. Doppler ultrasound of lower limb vessels showed incompetent perforating veins in mid and upper calf. Biochemical investigations were normal in the siblings and parents of the patient.

Sequencing of *PEPD* gene was performed using genomic DNA of patient and her parents. Fifteen exons of the gene were amplified by polymerase chain reaction (PCR) using primers sequences as described by Pandit et al. (Pandit et al., 2013). Sequencing performed using the automated DNA sequence ABI prism 310 revealed homozygous c.819-1G > A mutation (Fig. 2A). In silico analysis using MaxEnt, Sroogle, F splice, NN splice and ASEEDA predicted Exon 12 skipping that resulted in premature stop codon at 278. Chromatographs of parents revealed the presence of heterozygous mutation in their *PEPD* gene. Complementary DNA prepared from the patient and her parent's blood samples confirmed these findings (Fig. 2B).

Furthermore, we studied the expression of some specific genes that are commonly involved in the immune responses related to infections and cancers such as*IL-6*, *IL-23*, *TNF-a*, *STAT3*, *IFN-γ* and *ROR-g* in the index patient. Further, these results were compared with another patient diagnosed to have hyper IgE syndrome and a normal control. Total RNAs were extracted from the whole blood of all the 3 individuals using RNA-4PCR kit (Ambion, Life Technologies, Carlsbad, CA, USA). Complementary DNA was synthesized using High Capacity cDNA synthesis kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and SYBR Green based Real-Time PCR reactions were carried out in StepOne plus system (Life Technologies, Carlsbad, CA, USA). The expression levels were analysed on the basis of Ct values obtained in triplicates of the genes in each individual sample. The fold change in

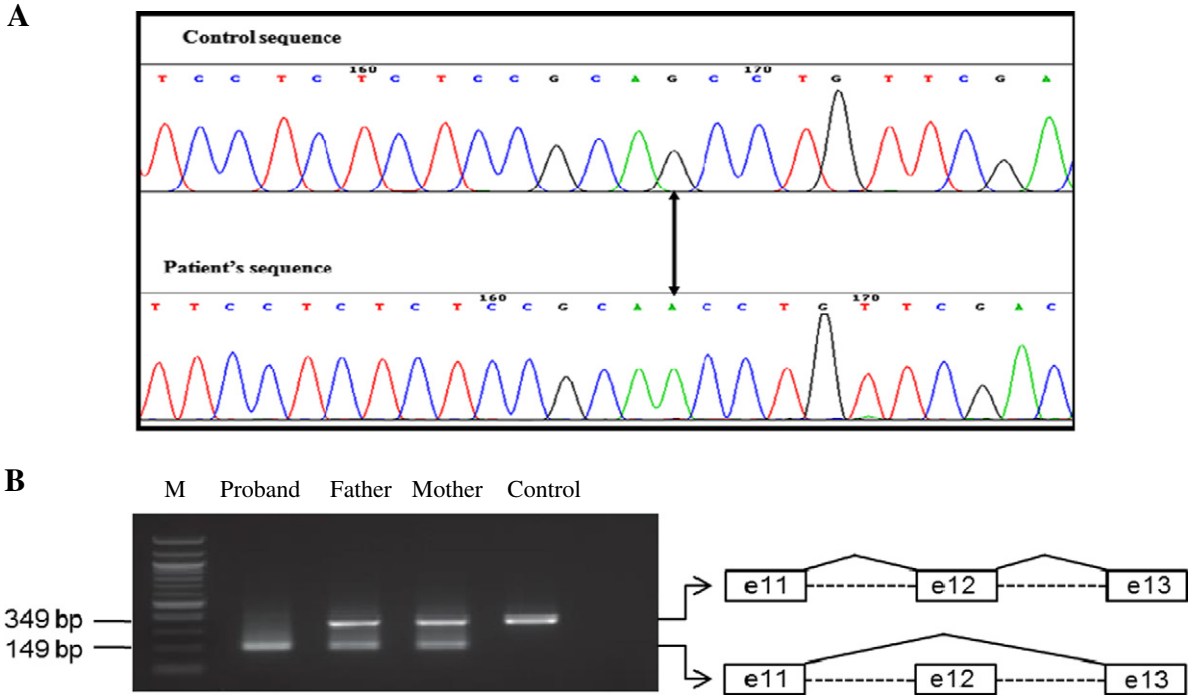

Fig. 2. A) Partial chromatogram sequence (forward) of the control (above) and patient (below). Arrow indicates the position of single nucleotide substitution at the splice donor site from AG to AA (G > A) at 3′ of intron 11 in two alleles B) Agarose gel electrophoresis showing large deletion because of exon 12 skipping.