

Lipoid Proteinosis: Identification of Two Novel Mutations in the Human *ECM-1* Gene and Lack of Genotype-Phenotype Correlation

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Sir,

Lipoid proteinosis (LP; OMIM #247100), first described by Urbach & Wiethe in 1929 (1), is a rare autosomal recessive disorder. It is characterized by a hoarse voice and skin and mucosal changes. Beaded papules along the eyelid margins are characteristic, as are infiltration of the oral mucosa. Associated findings include epilepsy, mild mental retardation, respiratory tract obstruction, abnormal dentition and ocular abnormalities. Histological findings include an extensive deposition of amorphous eosinophilic material around capillaries, sweat coils and in the thickened papillary dermis, which stains with periodic acid-Schiff (PAS). Molecular genetic studies of LP consanguineous families have revealed mutations in the *ECM1* gene located on 1q21.2 (2, 3). There are three known splice variants: ECM1a, ECM1b and ECM1c, encoding proteins of 540, 415 and 559 amino acids. To date, 41 distinct germline missense, nonsense, splice site, small and large deletions and insertions, have been reported (2–12). Approximately 50% of the mutations cluster to exon 6 and 7 of the gene. We report here two novel mutations and one recurrent mutation in two unrelated patients of German and Arab-Israeli ancestry.

CASE REPORTS AND METHODS

Case 1. A 45-year-old Arab man from Israel, the eldest son of first cousins, presented with longstanding disfiguring nodules and tumours of the skin. Two of his nine siblings had similar disease characteristics, but to a lesser degree, and two others were congenitally deaf. The patient had worked in manual jobs for many years. He noticed that his lesions improved slightly at times of unemployment. His voice had been hoarse since early childhood, and he had experienced numerous episodes of shortness of breath, usually triggered by an upper respiratory tract infection. There was no history of seizures and his intelligence was normal. He smoked 20 cigarettes daily and was not taking any medications. On examination, large verrucous nodules and plaques were present on both elbows and knees. Smaller lesions were present on the knuckles, around the wrists, and in the armpits. The eyelids were oedematous and red, with warty projections in their margins and eyelashes were sparse. The lips were swollen and the frenulum of the tongue was thickened with reduced movement, but dentition was normal. No other abnormalities were observed.

Case 2. A 37-year-old man from Germany, with no family history or consanguinity, had experienced hoarseness since childhood. Physical examination revealed an otherwise healthy man with ma-

croglossia, yellowish deposits on the tongue and soft palate, beaded papules on the thickened margins of the eyelids, yellowish papules in the axillae and nodules of variable sizes on both elbows. There were no other disease features and his intelligence was normal.

Methods. A skin biopsy was performed and the diagnosis of LP was verified histologically by haematoxylin and eosin (H&E) and PAS stains. Following informed consent, genomic DNA was extracted from peripheral blood lymphocytes. All 10 exons and exon-intron boundaries of the *ECM1* gene were amplified via PCR, as previously described (3, 4). PCR products were checked on 1.5% agarose gel, purified and sequenced, using Big Dye Terminator kit (PE Applied Biosystems, Foster City, CA, USA) and the ABI PRISM DNA Analyser 3700 (Perkin-Elmer).

To verify the c.1393delA mutation we used a mismatched PCR strategy, with the following primers: 5'-CAGTAGAAGC-TGATGAAAGG-3' (forward) and 5'-CCACACAGATCATT-GATGAAGGTGAA-3' (reverse) (underlined letter denotes a nucleotide mismatch). The reverse primer introduced an *EcoRI* restriction site (GAATTC) in the mutated allele.

RESULTS

Case 1 carried a homozygous deletion of an adenosine at position 1393, at the 5' end of exon 10 (Fig. 1a). The deletion led to a substitution of the amino acid lysine by asparagine, followed by an early stop codon, which resulted in a 75 amino acid protein truncation (c.1393delA; p.Lys465AsnfsX2). The screening of 60 control cases from unrelated individuals of Arab ancestry failed to disclose the presence of the mutation. In sequencing the *ECM1* gene of case 2, two mutations were identified: a c.240delTC in exon 4 and a c.1019delA in exon 7 (Fig. 1b, c). The mutation c.1019delA has been described previously as a homozygous mutation in a Kuwaiti individual with LP (2). The mutations c.240delTC and c.1019delA lead to a frameshift and premature stop codons 18 and 36 amino acids downstream to the mutations, respectively.

DISCUSSION

The ECM1 protein contains six cysteine doublets, with a CC-(X7–10)-C pattern (13). The cysteine arrangement leads to the formation of double-loop structures, which are involved in protein/protein interactions and therefore are essential for the function of the protein. The ECM1 protein interacts with fibulins 1C and 1D variants, extracellular matrix components of a wide range of connective tissues and various basement membranes, through its

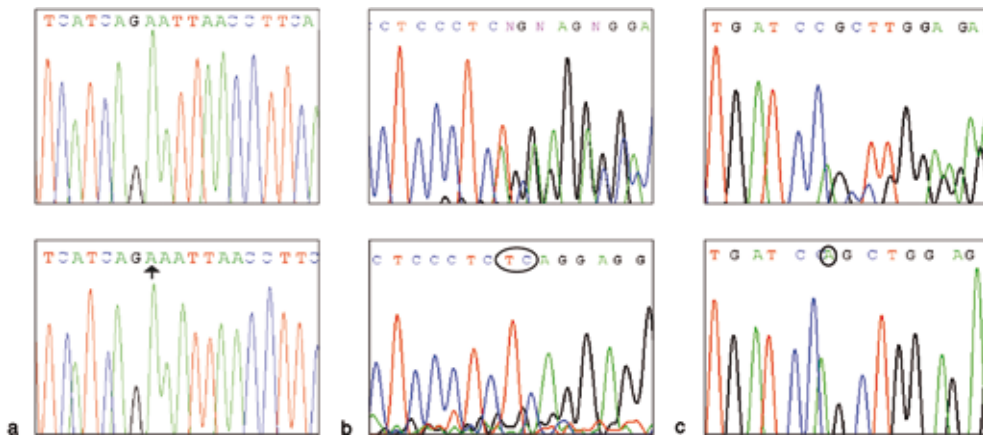


Fig. 1. (a) Homozygous 1393delA in exon 10 of the *ECM-1* gene. Upper panel: case 1; lower panel: unrelated control. Arrow represents site of adenosine deletion. (b) Heterozygous 240delTC mutation in exon 4. (c) Heterozygous 1019delA mutation in exon 7 of the *ECM-1* gene. (b+c) Upper panel: case 2; lower panel: unrelated control. The circled nucleotides are deleted in a heterozygous state in case 2.

central tandem repeat 2 region (14). It also interacts with perlecan, an intrinsic constituent of basement membranes, through its C terminus double-loop domain (15). The C terminal domain also contains two of the three N-glycosylation sites for protein kinase C. It can be assumed that a protein, truncated by c.1393delA, would be devoided of its C terminus dependent functions. The mutations c.1019delA and c.240delTC would create mRNAs that probably would be eliminated by the nonsense-mediated decay mechanism (16). However, since exceptions exist, the functional consequences of the premature termination codon mutations must be established by Northern blotting or quantitative reverse transcriptase PCR.

The mutation c.1393delA creates a stop codon in the last exon of the gene, so the mRNA transcript may be more stable and less prone to elimination by nonsense mediated mRNA decay (16). Some protein, albeit truncated of its highly functional C terminus, may be present. Of our two cases, however, case 1 carrying the c.1393delA mutation, did not show a milder phenotype in comparison with case 2, with more upstream mutations. In fact, in case 1, nodular infiltration localized to the knuckles, elbows, knees and armpits, was more severe at times of intense physical labour. Environmental factors, such as trauma and friction, as well as other genetic or epigenetic modifiers, may all contribute to the actual clinical severity seen in patients with LP.

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The authors declare no conflicts of interest.

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