

INVESTIGATIVE REPORT

Homozygous Missense Mutation in the *ECM1* Gene in Chinese Siblings with Lipoid Proteinosis

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Lipoid proteinosis is caused by loss-of-function mutations in the glycoprotein extracellular matrix protein 1 (*ECM1*). We report here mutation analysis of the *ECM1* gene in a Chinese family with lipoid proteinosis. A 10-year-old boy presented with a hoarse voice, acneiform scars and yellow skin nodules, as well as beaded eyelid papules and a thickened sublingual frenulum. Skin biopsy showed widespread deposition of hyaline material in the dermis and thickened basement membrane. His elder sister had the same clinical manifestations. The coding region of *ECM1* was amplified and sequenced and both affected siblings were shown to have a novel homozygous single nucleotide substitution, c.658T>G, in exon 6, which converts cysteine to glycine, designated p.C220G. Both parents were heterozygous for this mutation which was not detected in 100 control chromosomes. Missense mutations in the *ECM1* gene are an unusual finding in lipoid proteinosis, but this case adds to the spectrum of disease-associated mutations in this rare genodermatosis. **Key words: extracellular matrix protein 1; *ECM1*; lipoid proteinosis; LP; exon 6.**

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Lipoid proteinosis (LP), also known as Urbach-Wiethe disease, is a rare autosomal recessive disorder characterized by variable scarring and infiltration of the skin and mucosa (1). Classical clinical features include warty skin infiltration, papules on the eyelids and skin scarring as well as extracutaneous abnormalities, such as hoarseness of the voice, epilepsy and neuropsychiatric abnormalities (1). Histologically, there is widespread deposition of hyaline-like material and disruption or reduplication of basement membrane around blood vessels and at the dermal-epidermal junction. The aetiology of LP has recently been shown to result from loss-of-function mutations in the *ECM1* gene on 1q21 (2). The *ECM1* protein has important physiological and biological roles in epidermal differentiation, binding of dermal collagens and proteoglycans, and regulation of angiogenesis. More

than 20 pathogenic mutations have been reported so far, mostly nonsense, frameshift or splice site mutations with the majority occurring in exon 6 or 7 of the 10 exon *ECM1* gene. In this study, we describe the clinical and pathological features of a 10-year-old boy and his 18-year-old sister with LP from Shanxi province of China, in whom we identified a homozygous missense mutation in exon 6.

MATERIALS AND METHODS

Patient

The patient was a 10-year-old boy with LP who had papules on the margins of his eyelids (moniliform blepharosis) (Fig. 1A) as well as diffuse acneiform scars on his face, dorsae of the hands and on his buttocks, along with yellow waxy nodules on his lower legs. He had also suffered from a hoarse voice since infancy. The oral mucosa showed yellow-white infiltrates and was thickened (Fig. 1B). Movement of the tongue was limited because of a thick sublingual frenulum. Indirect laryngoscopy revealed infiltrated vocal cords. No obvious neuropsychiatric abnormalities were detected and there was no history of epilepsy. He had an 18-year-old sister who had similar, but somewhat milder, clinical features (Fig. 1C). Their parents were not affected and were not known to be related, although they originated from the same province.

Skin biopsy

Skin biopsy was taken from clinically affected skin. Skin sections stained with haematoxylin and eosin (H&E) showed widespread deposition of hyaline-like material around blood vessels and adnexal epithelia, and thickening of the basement membrane at the dermal-epidermal junction. On periodic acid-Schiff (PAS) staining and PAS-diastase, these sites were PAS-positive and diastase-resistant.

Mutation detection of *ECM1* gene

Genomic DNA sample of the 2 patients and their parents were extracted from peripheral blood by routine methods. The primers used for the amplification of the 10 exons and their flanking introns of the *ECM1* gene were as reported previously (4). The polymerase chain reaction (PCR) mixture contained 1×PCR buffer, 250 ng genomic DNA, 6.25 pmol/each primer, 1.5 mM MgCl₂, 0.2 mM/each dNTPs and 2.5 U Taq DNA polymerase in a total volume of 25 µl. A standard touch-down protocol was used for the amplification. For this, the annealing temperature began at 62°C, and was then lowered by 0.5°C per cycle to 52°C. PCR products were purified using a commercial kit and sequenced directly in an ABI 310 genetic analyser (Applied Biosystems, Foster City, CA, USA).

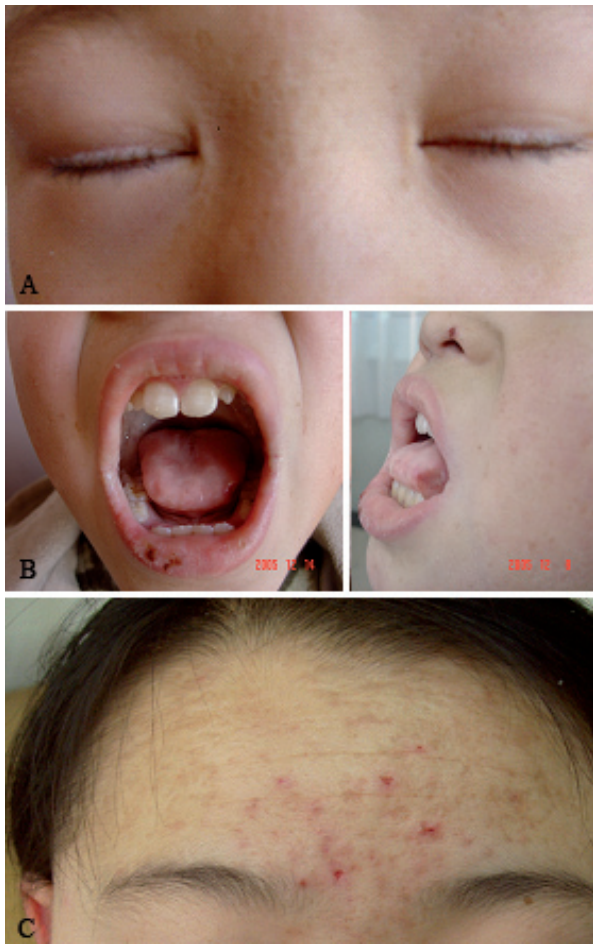


Fig. 1. (A) The male patient, fresh-coloured papules on the edges of his eyelids (moniliform blepharosis). (B) The male patient, oral mucosa with yellow-white infiltrates and tongue with dental impressions. (C) The female patient, diffuse acneiform scars on her face.

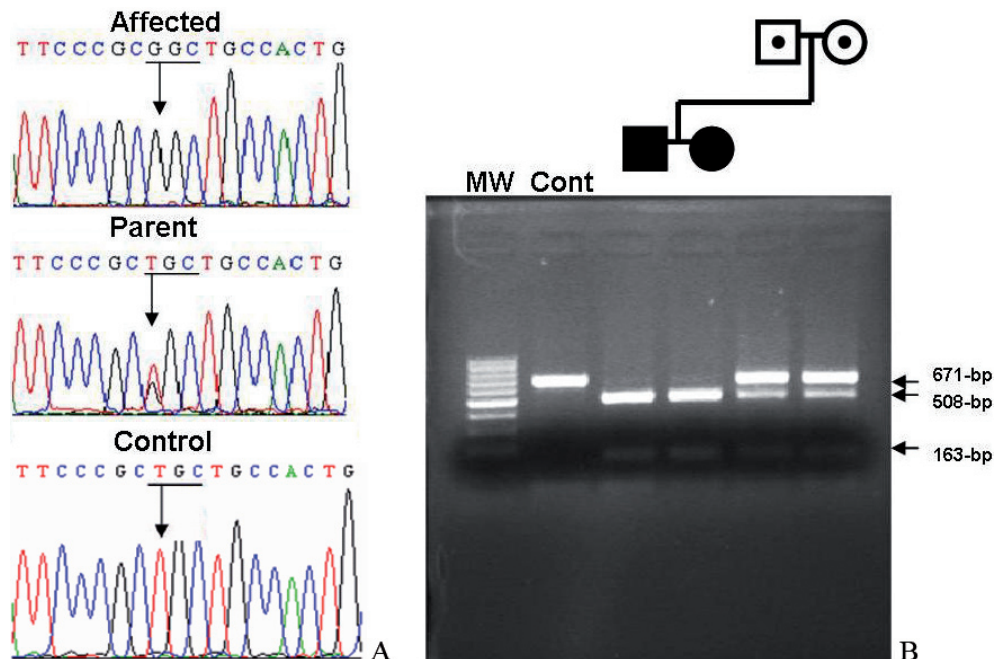
RESULTS

Direct sequencing of the PCR products amplified from the affected boy revealed a homozygous T>G transversion at nucleotide c.658 in exon 6, which changes a cysteine residue to glycine (TGC→GGC) at amino acid 220 (designated p.C220G). The same homozygous mutation was also found in his sister's genomic DNA gene. Both parents were heterozygous carriers of this mutation (Fig. 2A). The substitution c.658T>G generates a unique recognition site for the restriction endonuclease *Sac*II. This restriction endonuclease digests the PCR products of the affected individuals into 508 and 163 bp fragments, but leaves the wild-type sequence undigested with a product of 671 bp (Fig. 2B). We screened for this mutation in 50 unrelated control subjects, but identified no *Sac*II digestion for any amplified allele, thus making p.C220G unlikely as a non-pathogenic polymorphism.

DISCUSSION

LP is a rare autosomal recessive and clinically heterogeneous disorder. Clinical manifestations include varying degrees of skin scarring and infiltration, vocal hoarseness and respiratory distress, and some cases may be complicated by neurological abnormalities and dental problems. More than 250 cases of LP have been reported thus far. It occurs worldwide, but seems to be more common in some populations, such as South Africa, in which a founder effect has been demonstrated (3). Most cases of LP involve loss-of-function mutations in *ECM1*. The main function of the *ECM1* protein appears to involve protein-protein binding in

Fig. 2. (A) Direct sequencing of the PCR product amplified from exon 6 of *ECM1* gene. The 2 patients show a homozygous mutation of c.658T>G (arrow). His father and mother have a heterozygous mutation of c.658T>G. An unrelated healthy control shows the wild type (TGC) sequence. (B) Pedigree of the patients studied shows it is a non-consanguineous family, and the proband is the son. Both the son and daughter are patients, while the parents have the normal phenotype. The gel electrophoresis: MW: DNA size standard; Cont: Normal control; The male patient: The PCR product was completely cut into 508 and 163bp; The female patient: same as her brother; Their father: The PCR product was partially cut by *Sac* II and shows 508bp, 163bp and the intact band of 671bp; Their mother: same as their father.



the dermis. *ECMI* has been shown to bind to perlecan, fibulin and matrix metalloproteinase 9 and thereby it influences several aspects of dermal homeostasis (5, 6). Expression of *ECMI* has also been shown to alter in chronological ageing and photoageing (7). Nearly all the published *ECMI* mutations in LP are expected to lead to low or absent mRNA or protein expression and very few missense mutation have been reported. The missense mutations documented include F167I (8) and F167L (9), although only the latter was present on both alleles. Our case therefore represents the second report of a homozygous missense mutation as the molecular basis for individuals with LP. Interestingly, the same missense mutation, p.C220G, has recently been reported in another Chinese case of LP (10). In that report the affected individual was a compound heterozygote for the *ECMI* mutations p.C220G/p.R476X. That case emanated from Jinan, in the Shandong Province of China, which is more than 500 km from our family. Moreover, our family is not aware of any connection to the previously reported case. Nevertheless, it would be interesting to assess the *ECMI* haplotype in both families to see whether this might represent a common ancestral mutant Chinese allele, especially as this mutation has not been reported elsewhere (11). In summary, this paper describes 2 siblings with classical clinical features of LP, but with the unusual finding of a novel homozygous missense mutation in the *ECMI* gene.

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