INVESTIGATIVE REPORT

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

Beibei HAN1, Xinglian ZHANG2, Qiang LIU2, Xixue CHEN1 and Xuejun ZHU1
Departments of Dermatology, 1Peking University First Hospital, and 2Children’s Hospital of Shanxi Province, China

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. 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; lipid proteinosis; LP; exon 6.

(Accepted March 12, 2007.)


Xixue Chen, Department of Dermatology, Peking University First Hospital, No. 8, XiShenKu Avenue, Western District, Beijing 100034, China. E-mail: xixue_Chen@yahoo.com.cn

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).
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 SacII. 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 SacII 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...
the dermis. *ECM1* 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 *ECM1* has also been shown to alter in chronological ageing and photo-ageing (7). Nearly all the published *ECM1* 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 *ECM1* 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 *ECM1* 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 *ECM1* gene.

**REFERENCES**