Mucopolysaccharidosis Type VII: Characterization of Mutations and Molecular Heterogeneity

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Mucopolysaccharidosis type VII (MPS VII) is a genetic disorder as an autosomal recessive trait characterized by accumulation of undegraded glycosaminoglycans with the lysosomes due to markedly decreased activity of $\beta$-glucuronidase ($\beta$-Gl). We have identified several point mutations in three Japanese cases with MPS VII. These mutations reduce the enzyme activity in COS cells transfected with mutant cDNA. A family study was also possible with the PCR technique and direct sequencing procedures.

MATERIALS AND METHODS

Three Japanese patients were studied (cases 1, 2, and 3). There was a consanguinity in case 1 family. Northern blot analysis was performed after extracting total RNA from cultured patients' fibroblasts. Mutated cDNA clones including the entire coding sequence were isolated from a cDNA library in case 1 and PCR (polymerase chain reaction) products in cases 2 and 3 derived from cultured fibroblasts. All the coding sequences were determined by the dideoxynucleotide chain termination method. $\beta$-Gl activity was measured in COS cells transfected with mutant cDNA. The PCR technique and direct sequencing procedures were available for confirmation of mutations, and a family study was performed in two cases.
RESULTS AND DISCUSSION

Northern blot analysis of patients revealed normal transcripts which showed that a promoter mutation was ruled out. Sequencing of the full-length mutated cDNA revealed C→T transitions, an event causing a single Ala$^{615}$→Val change (cases 1 and 2) and Arg$^{982}$→Cys and Pro$^{649}$→Leu changes (case 3). The former change is detected by loss of the cleavage site for the enzyme Fnu 4HI in the mutated cDNA. On the basis of the loss of the Fnu 4HI restriction site, the patients (case 1 and case 2) were shown to be homozygotes with this mutation and the parents and brother in case 1 were heterozygotes (Fig.1). The latter change was confirmed by direct sequencing to be a homozygote in the patient and heterozygotes in her parents (Fig.2). The Ala$^{619}$→Val and Arg$^{982}$→Cys mutations reduce the enzyme activity, as tested by transfection of COS cells with expression vectors harboring the mutated cDNA. But the Pro$^{649}$→Leu mutation does not reduce the activity (Fig.3).

Fig.1  Fig.2  Fig.3