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Short Communication

Molecular basis of rhizomelic chondrodysplasia punctata type I: High frequency of the Leu-292 Stop mutation in 38 patients

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Rhizomelic chondrodysplasia punctata (RCDP; Mckusick 215100 is an autosomal recessive disease characterized by a disproportionate stature, typical facial appearance, congenital contractures, eye abnormalities and severe growth and mental retardation, although patients have been described with a much milder clinical presentation (see for instance Smeitink et al 1992). Apart from the clinical heterogeneity there is also biochemical heterogeneity, suggesting the involvement of at least three distinct genes. In most RCDP patients (>90%) there is a tetrad of biochemical abnormalities including a deficiency of dihydroxyacetonephosphate acyltransferase (DHAPAT), alkyldihydroxyacetonephosphate synthase (alkyl DHAP synthase), phytanoyl-CoA hydroxylase, and peroxisomal thiolase I (see Wanders et al 1996 for review).

A minority of patients lack this tetrad of abnormalities and suffer from an isolated deficiency of DHAPAT or alkyl DHAP synthase deficiency. We propose to call these three biochemical phenotypes RCDP type I, type II and type III, respectively.

Genetic complementation studies have revealed that all type I RCDP patients characterized by the tetrad of biochemical abnormalities belong to a single complementation group, suggesting the involvement of a single gene (Heikoop et al 1992). Obviously, the product of this gene is of crucial importance, being required for the correct expression of all four enzymes. The gene involved, *PEX7*, was recently identified simultaneously by three groups of investigators including our own (Braverman et al 1997; Motley et al 1997; Purdue et al 1997). It encodes the peroxisomal PTS2 receptor which is involved in the recognition of peroxisomal proteins containing a certain peroxisome targeting signal (PTS2) in the cytosol and

their subsequent delivery to the peroxisome (Rachubinski and Subramani 1995). Recent studies have shown that type I RCDP is caused by mutations in the *PEX7* gene encoding the PTS2 receptor.

We now report on the frequency of the Leu-292 Stop mutation which was earlier identified in some patients (Braverman et al 1997; Motley et al 1997). In a series of 38 patients we found a very high frequency of the Leu-292 Stop mutation with important implications for carrier-detection and prenatal diagnosis.

MATERIALS AND METHODS

The patients studied show all the clinical and biochemical abnormalities described for type I RCDP including a deficiency of DHAPAT, alkyl DHAP synthase, phytanoyl-CoA hydroxylase and peroxisomal thiolase I as concluded from detailed studies in fibroblasts (see Wanders et al 1996).

Mutation analysis was performed exactly as described previously (Motley et al 1997).

RESULTS AND DISCUSSION

Previous studies by Braverman et al 1997 and ourselves (Motley et al 1997) have led to the identification of the Leu-292 Stop mutation leading to a truncated protein with no biological activity. We have now studied the occurrence of the Leu-292 Stop mutation in a series of 38 patients. The results presented in Table 1 show high frequency of the Leu-292 Stop mutation with homozygosity in 20 and heterozygosity in 7 out the 38 patients analysed, showing an allelic frequency of the Leu-292 Stop mutation of 62% (47/76). Current efforts are directed to identifying the other mutations.

The finding that the Leu-292 Stop mutation is frequent among type I RCDP patients has a number of important implications. Firstly, in families in which the index patient is homozygous for the Leu-292 Stop mutation, carrier detection can now be done without difficulty. Secondly, these new results may also be of great use in prenatal diagnosis. Until now prenatal diagnosis of type I RCDP as done in our laboratory rests upon the analysis of two parameters which includes immunoblot analysis of peroxisomal thiolase and measurement of dihydroxyacetonephosphate acyltransferase activity and/or plasmalogen levels in chorionic villus biopsy specimens (see Wanders et al 1996). Immunoblot analysis requires the availability of specific antibodies against peroxisomal thiolase which are not generally available and DHAPAT activity measurements are difficult to perform. Accordingly, the

Table 1 Analysis of the Leu-292 Stop mutation in 38 patients with type I rhizomelic chondrodysplasia punctata

Leu-292 Stop mutation	*Frequency*	
Homozygous(−/−)	20/38	(53%)
Heterozygous (+/−)	7/38	(18%)
Homozygous normal (+/+)	11/38	(29%)

development of DNA-based techniques is important for future prenatal diagnosis. So far, identification of the Leu-292 Stop mutation has only been achieved at the cDNA level and future studies are aimed at identifying the Leu-292 Stop and other mutations at the genomic level. As soon as these techniques are available, we will apply DNA-based methods in future requests for prenatal diagnosis of type I RCDP.

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