B.T. Poll-The, H. Clevers, J.M. Saudubray and B. Fournier

Deletion of the Acyl-CoA Oxidase Gene in Isolated Acyl-CoA Oxidase Deficiency (Pseudo-Neonatal Adrenoleukodystrophy)

Introduction

Disorders with defective peroxisome assembly such as the Zellweger cerebro-hepato-renal syndrome [2], neonatal adrenoleukodystrophy [3] and infantile phytanic acid storage disease [6], are characterized by a decreased number or an absence of morphologically detectable peroxisomes in liver and other tissues. Multiple enzymatic abnormalities, resulting in an accumulation of very long chain fatty acids (VLCFA), bile acids intermediates, pipecolic-, pristanic-, and phytanic acid, and impairment of plasmalogen biosynthesis, are observed in these disorders [8].

Peroxisomal β-oxidation of fatty acids is catalyzed by three enzymes that are immunologically distinct from the analogous mitochondrial enzymes [5]: 1. acyl-CoA oxidase; 2. trifunctional enzyme; 3. peroxisomal β-ketothiolase. An impairment of this pathway is responsible for the elevated plasma and tissue VLCFA values in patients.

Several patients with defective peroxisomal functions have been described in which peroxisomes were not decreased in number in various tissues. These patients were initially diagnosed with either Zellweger syndrome or neonatal adrenoleukodystrophy based on their clinical and pathological manifestations. However, in contrast to the disorders of peroxisomes assembly, these patients appeared to have an isolated defect of peroxisomal β-oxidation [1, 9].

We have described two siblings with pseudo-neonatal adrenoleukodystrophy who exhibited decreased VLCFA oxidation associated with an isolated deficiency of fatty acyl-CoA oxidase activity [7]. We cloned the human acyl-CoA oxidase cDNA and showed that the genetic lesion underlying the acyl-CoA oxidase deficiency in the two siblings involves a large DNA deletion.

Patients

Two previously reported siblings born to consanguineous healthy parents presented clinical manifestations very simular to those of patients affected by neonatal adrenoleukodystrophy, namely severe hypotonia, mental retardation, seizures and no dysmorphic features. Standard karyotypes were normal. In con-

trast to neonatal adrenoleukodystrophy, hepatic peroxisomes were enlarged in size and not decreased in number. A $C_{26:0}/C_{22:0}$ ratio of 0.076 and 0.16 was observed in the plasma of each patient as opposed to a ratio of 0.016 ($\pm$0.028) in control plasma, and a ratio of 1.577 and 1.047 in patients' fibroblasts compared to 0.08 ($\pm$0.03) in control fibroblasts. The parents' VLCFA ratios were comparable to those of controls. Immunoblotting experiments on liver tissue from the patients revealed no immunologically reactive material using anti-acyl-CoA oxidase antibodies. The accumulation of very long chain fatty acids appeared to be associated with an isolated deficiency of the fatty acyl-CoA oxidase, the enzyme that catalizes the first step of the peroxisomal β-oxidation. Plasmalogen biosynthe sis in cultured skin fibroblasts and plasma levels of di- and trihydroxycoprostanoic acid, phytanic acid and pipecolic acid were normal [7].

Strategies for isolation of the human acyl-CoA oxidase cDNA

In order to study the molecular defect in our patients with an acyl-CoA oxidase deficiency, we initiated to clone a full-length human acyl-CoA oxidase cDNA. A human liver cDNA library was screened with a probe representing the exon 13 of the rat acyl-CoA oxidase gene [4]. Two short human clones ($\pm$200 bp) obtained had an 84% homology to the rat cDNA sequence. Further screening of this library failed to give larger clones. We, therefore, amplified reverse transcribed human liver mRNA using primers based on the rat cDNA sequence. Most of the cDNA sequence was obtained in 3 amplification steps. This yielded overlapping fragments of 849, 843 and 774 bp respectively. The 5^1 end, however, remained elusive.

Screening of another human liver cDNA library was carried out using PCR fragments as probes and gave an incomplete 1.9 kb insert. Since the insert did not include the 5^1 end of the cDNA, a third library (a B cell line cDNA library) was screened. The 2.1 kb insert obtained from this last library, encoded the complete protein sequence.

The nucleotide sequence of the human acyl-CoA oxidase cDNA was found to be highly homologous to its rat cDNA counterpart. The homology between the two sequences averaged 85% at the nucleotide level and 89% at the amino acid level. As in the rat [4], two species of acyl-CoA oxidase cDNA were found in the liver. These two regions had a 52% homology to each other at the nucleotide level and each had a 91% homology to their rat counterpart.

Southern blot analysis of the acyl-CoA oxidase gene in control and patients with an acyl-CoA oxidase deficiency

Southern blot analysis of total human genomic DNA digested with a number of restriction enzymes using the full length cDNA as the hybridization probe

indicated that the chromosomal acyl-CoA oxidase gene is at least 30–40 kb long and therefore contained multiple intervening sequences.

Total DNA, derived from either cultured skin fibroblasts or lymphoblastoid cell lines from the siblings with an acyl-CoA oxidase deficiency, their parents and controls was digested with restriction enzyme BglII, EcoRI and HindIII and probed with the cDNA. A partial deletion of the acyl-CoA oxidase gene was detected in the patients, regardless of the restriction enzyme used. Patients' DNA digested with restriction enzyme EcoRI lacked the 8.0, 5.5 and 2.4 kb fragments when probed with the 3′ fragment (Fig. 1A). The same blot was reprobed with a 5′ fragment from the human clone, homologous to the first two exons of the rat gene. A 16 kb fragment was now found to be present in patients as well as in controls (Fig. 1B). This observation was confirmed by PCR amplification of genomic DNA from patients and controls. A 500 bp fragment was found to be present both in the probands and in the controls. This fragment was sequenced and apparently included an intron at a position equivalent to the rat intron I.

Although the exact boundaries of the deletion remains to be determined, we conclude that the deletion in the patients spans most of the gene as observed by Southern blot analysis. However, the first 2 exons were left intact. These results indicate that the acyl-CoA oxidase deficiency in this family is due to a large deletion in the acyl-CoA oxidase gene.

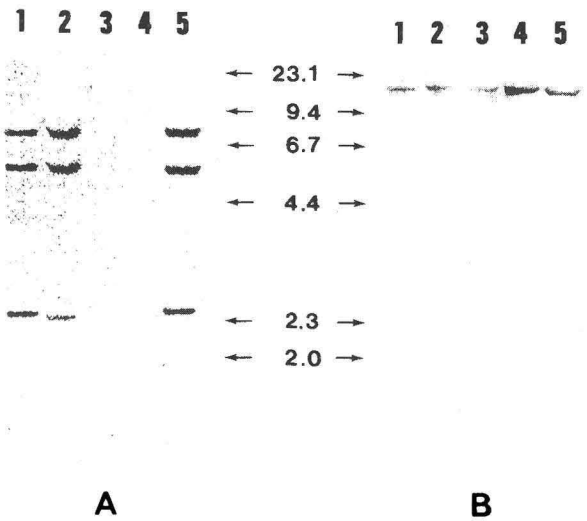

Fig. 1. Southern blot analysis of the affected family. DNA control (lane 1), the mother (lane 2), the two patients (lane 3 and 4) and a Zellweger patient (lane 5) was digested with the restriction enzyme EcoRI and probed with a 1793 bp cDNA fragment (A) or a 277 bp cDNA fragment which covers exons 1 and 2 (B). A 8.0, 5.5 and 2.4 kb fragments were found to be missing in patients' DNA (A) whereas a 16 kb fragment was present (B). The pattern observed for the Zellweger patient DNA was identical to control DNA. λ-DNA digested with HindIII is shown as a marker.

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Affiliations

University Children's Hospital 'Het Wilhelmina Kinderziekenhuis'
(Dr. B.T. Poll-Thé and Dr. B. Fournier) and University Hospital Utrecht
(Dr. H. Clevers) Nieuwe Gracht 137, 3512 LK Utrecht, The Netherlands
*Hôpital des Enfants-Malades, 75743 Paris, France (Dr. J.M. Saudubray).