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Short Communication

Genetic heterogeneity in patients with a disorder of peroxisomal β-oxidation: A complementation study based on pristanic acid β-oxidation suggesting different enzyme defects

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One of the most important functions of peroxisomes concerns the β-oxidation of fatty acids and fatty acid derivatives. Peroxisomes are incapable of oxidizing fatty acids to completion. Instead, fatty acids undergo only a few cycles of β-oxidation in the peroxisome and are then transported to the mitochondrion for complete oxidation to CO_2 and H_2O. This is true for very long-chain fatty acids like cerotic ($C_{26:0}$) and lignoceric ($C_{24:0}$) acid and pristanic acid (2,6,10,14-tetramethylpentadecanoic acid). Another important function of the peroxisomal β-oxidation system concerns its role in bile acid synthesis. Indeed, the CoA esters of di- and trihydroxycholestanoic acid which are formed from cholesterol are subjected to β-oxidation in the peroxisome, giving rise to propionyl-CoA and the CoA esters of chenodeoxycholic acid and cholic acid, respectively, which are then conjugated and excreted into bile.

The enzymatic organization of the peroxisomal β-oxidation system is as yet incompletely understood. It is clear that there are two acyl-CoA oxidases with specificity for straight-chain (Osumi et al 1980) and branched-chain fatty acyl-CoA esters (Vanhove et al 1993). Until recently it was believed that the subsequent steps are catalysed by one bifunctional protein (Osumi et al 1980) and peroxisomal thiolase (Miyazawa et al 1980), but this view is no longer tenable (see Novikov et al 1994).

We have recently found that the bifunctional protein and thiolase as characterized by Hashimoto and coworkers are *not* involved in pristanic acid β-oxidation. In collaboration with Seedorf and coworkers we have shown that the thiolase encoded by the sterol carrier protein X (SCPx) gene (Seedorf et al 1994) contains 3-ketopristanoyl-CoA thiolase activity, whereas the classical thiolase lacks such activity (Wanders et al 1996).

In the last few years an increasing number of patients have been described with a defect

in peroxisomal β-oxidation. In X-linked adrenoleukodystrophy the defect is at the level of an integral peroxisomal membrane protein (ALDP) probably involved in transport of very long-chain acyl-CoA esters into the peroxisome (Mosser et al 1993). Other disorders with a defined defect in peroxisomal β-oxidation include acyl-CoA oxidase deficiency (Poll-The et al 1988), bifunctional protein deficiency (Watkins et al 1989) and peroxisomal thiolase deficiency (Schram et al 1987). Apart from these well-characterized patients, many have been reported with a defect in peroxisomal β-oxidation of unknown aetiology (see Wanders et al 1995a for references).

We have studied 11 such patients using complementation analysis. Three distinct groups were found with strong over-representation of one particular group. The underlying basis for this genetic heterogeneity will be discussed.

MATERIAL AND METHODS

Procedure for complementation analysis: The cultured skin fibroblasts were fused essentially according to Brul et al (1988). The fused cells were cultured for 3 days on DMEM without FCS, after which the occurrence of complementation was tested by means of pristanic acid β-oxidation. This was done essentially as described by Wanders et al (1995b).

Patients: The patients studied in the work reported in this paper showed a wide variety of clinical abnormalities and displayed the biochemical features suggestive of a peroxisomal β-oxidation disorder. This was concluded from detailed studies in fibroblasts involving measurement of *de novo* plasmalogen biosynthesis; alkyl-DHAP synthase activity and DHAPAT activity; very long-chain fatty acid concentrations; $C_{26:0}$, pristanic and phytanic acid oxidation; immunoblot analysis; and catalase immunofluorescence (see Wanders et al 1993).

RESULTS

Complementation analysis is a powerful tool for resolving the genetic basis in patients sharing a particular clinical and/or biochemical phenotype. In this study we have applied this technique to study the genetic basis of the various disorders of peroxisomal β-oxidation. We selected 11 patients suffering from a disorder of peroxisomal β-oxidation as established from studies in fibroblasts. Only patients with a deficient pristanic acid oxidation activity were selected, thereby excluding X-linked adrenoleukodystrophy and acyl-CoA oxidase deficiency from this study.

Since fibroblasts from a peroxisomal thiolase-deficient patient, of which only one has been described (Schram et al 1987), are not available, the only established cell line we could use for our complementation analysis was the one described by Watkins and colleagues (1989) with bifunctional protein deficiency. The results in Table 1 show that 9 out of the 11 cell lines tested showed no restoration of pristanic acid β-oxidation after fusion with the bifunctional protein-deficient cell line. Care was taken to ensure that this was not an experimental artefact by visually inspecting the multinucleate cells to determine the fusion efficiency and by including a positive control in each experiment (fusion with a cell line from a Zellweger patient). Table 1 further shows that two cell lines did

Table 1 Results of complementation studies of patients with a defect in peroxisomal pristanic acid β-oxidation

		Pristanic acid β-oxidation (pmol/h per mg protein)	
Complementation group	*Patient*	*Cocultivated*	*Fused*
I	1	6	24
	2	7	17
	3	0	9
	4	0	0
	5	0	7
	6	0	12
	7	0	0
	8	0	2
	9	0	3
II	10	15	307
III	11	36	657

show complementation with the bifunctional protein-deficient cell line. Interestingly, these two cell lines complement one another, suggesting the involvement of different genes.

DISCUSSION

The results described in this paper show strong over-representation of one particular complementation group, with only single cell lines in the two other complementation groups. Interestingly, preliminary studies have revealed intragenic complementation within the large complementation group with three subgroups (van Grunsven et al, unpublished). The most likely explanation for this remarkable phenomenon is that in subgroup 1 the bifunctional protein is completely missing, with the functional loss of both the enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, whereas in subgroups 2 and 3 only the enoyl-CoA hydratase or the 3-hydroxyacyl-CoA dehydrogenase component is defective. This is now under active investigation.

Studies are also underway to determine the nature of the enzyme defect in complementation groups II and III. Obvious candidate enzymes are the branched-chain acyl-CoA oxidase accepting pristanoyl-CoA as substrate and peroxisomal thiolase. We have recently found (Wanders et al 1997) that the protein discovered by Seedorf and coworkers (1994) with both sterol carrier protein activity and 3-ketoacyl-CoA thiolase activity is involved in pristanic acid β-oxidation and not the classical peroxisomal thiolase identified by Miyazawa and colleagues (1980).

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