Chapter 2

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

E.G. van Grunsven and R.J.A Wanders

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INTRODUCTION

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 (4,6,10,14-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 chenodeoxycholicacid and cholic acid, respectively, which are then conjugated and excreted into bile.

The enzymatic organisation 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 (1) and branched chain fatty acyl-CoA esters (2). Until recently it was believed that the subsequent steps are catalysed by one bifunctional protein (3,4) and peroxisomal thiolase (5), but this view is not longer tenable (see (6)).

We have recently found that the bifunctional protein and thiolase as characterised 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 (7) contains 3-ketopristanoyl-CoA thiolase activity, whereas the classical thiolase lacks such activity (8).

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 the transport of very long chain acyl-CoA esters into the peroxisome (9). Other disorders with a defined defect in peroxisomal β-oxidation include acyl-CoA oxidase deficiency (10), bifunctional protein deficiency (11), and peroxisomal thiolase deficiency (12). Apart from these well-characterised patients, many have been reported with a defect in peroxisomal β-oxidation of unknown etiology (see (13) 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.* (14). The fused cellls 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.* (15).

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 measurements 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 (16)).

RESULTS

Complementation analysis is a powerfull 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

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

		Pristanic acid β-oxidation[a]	
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

[a]pmol/h per mg protein

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 (12), are not available, the only established cell line we could use for our complementation analysis was the one described by Watkins and collegues (11) 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 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 the 3-hydroxyacyl-CoA dehydrogenase activities, whereas in subgroups 2 and 3 only the enoyl-CoA hydratase or the 3-hydoxyacyl-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 candidates are the branched chain acyl-CoA oxidase accepting pristanoyl-CoA as a substrate and peroxisomal thiolase. We have recently found (8) that the protein discovered by Seedorf and coworkers (7) 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 collegues (5).

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