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Peroxisomal Disorders: A Review

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Summary: Until recently peroxisomal disorders were considered to be extremely rare and the diagnostic procedures available for postanatal and prenatal diagnosis were not widely known. At present, 17 human disorders are linked to peroxisomal dysfunction. The clinical, biochemical and morphological peroxisome heterogeneity described in the different diseases illustrate that only combined analysis of all the different approaches will lead to a correct diagnosis and a coherent pathophysiological model to guide ongoing research. With the study of human peroxisomal diseasese, advances have been gained as to the function of the peroxisome in normal and pathological conditions. Genetic analysis of peroxisome biogenesis and research on peroxisomal targeting signals are now in progress. Peroxisomal disorders are usually classified according to the degree of biochemical impairment. In this paper, a tentative classification of peroxisomal disorders will be proposed, based on the degree of biochemical abnormalities combined with new data obtained on whether or not defective peroxisome assembly is involved: (1) disorders with peroxisome assembly deficiencies; (2) disorders with single enzyme deficiencies.

The clinical onset and the major symptoms of the various disorders, and the recently discovered findings are discussed.

The essential role of peroxisomes in intermediary metabolism in man has been stressed by the existence of a variety of inherited peroxisomal disorders involving either a defect in the import of proteins into peroxisomes or a deficiency of one or more peroxisomal enzymes. The combined efforts of different disciplines have led to the development of sensitive and specific methods for the determination of peroxisomal functions. Both anabolic and catabolic pathways have been assigned to the peroxisome. These functions, which may be deficient in peroxisomal disorders, include biosynthesis of plasmalogens, bile acids and cholesterol, glyoxylate transamination, oxidation of very long-chain fatty acids, branched-chain fatty acids, dicarboxylic acids and polyunsaturated fatty acids, L-pipecolic acid oxidation and phytanic acid oxidation. Unlike mitochondria, peroxisomes do not contain DNA and must import all constituent proteins. Both peroxisomal matrix and membrane proteins are

synthesized on free polysomes in the cytosol and imported post-translationally into pre-existing peroxisomes, which enlarge and divide. Defects in the import mechanisms of matrix proteins are thought to form the basis of several peroxisomal disorders (Santos et al 1988; Balfe et al 1990; Walton et al 1992).

At least 17 human disorders are linked to peroxisomal dysfunction, 15 of which involve neurological defects. All but one disease, the X-linked adrenoleukodystrophy, are autosomal recessive disorders. The Zellweger cerebrohepatorenal syndrome (classical Zellweger) was the first disorder in which defects in numerous peroxisomal pathways were recognized. More recently, it has been shown that peroxisomal deficiencies are also found in less severe disease states. Despite all that has been learned about peroxisomal functions with the recognition of these disorders, the pathophysiology of these diseases remains poorly understood. Therefore, we emphasize the importance of combining analysis of clinical, biological and molecular markers for achieving an understanding of this intriguing organelle. In this paper, an overview of peroxisomal disorders will be provided, with emphasis on recently discovered findings and on the onset of clinical symptoms in the various disorders. A tentative classification of peroxisomal disorders will be discussed.

PEROXISOMES: MORPHOLOGY AND FUNCTIONS APT TO BE DEFICIENT IN PEROXISOMAL DISORDERS

The detection of peroxisomes is facilitated by using the diaminobenzidine staining procedure, which reacts with the marker enzyme catalase, and by immunochemical techniques with antibodies against matrix and membrane peroxisomal proteins (reviewed by Roels et al 1991). The abundance, size and shape of peroxisomes vary among different cell types. In man, peroxisomes are particularly abundant in liver and kidney and have an average diameter of 0.2–1 μm. In other tissues, including the brain and fibroblasts, they are less abundant and smaller. Patients with a peroxisomal disorder may present a discrepancy between the decrease in peroxisomal abundance in hepatocytes and cultured fibroblasts.

The most important peroxisomal functions in human pathology (reviewed by Van den Bosch et al 1992) are as follows.

Ether phospholipid biosynthesis: The key enzymes in ether phospholipid biosynthesis, acyl-CoA : dihydroxyacetone phosphate acyltransferase (DHAP-AT) and alkyldihydroxyacetone phosphate (alkyl-DHAP) synthase, are localized in peroxisomes (Figure 1). The main end-products of this anabolic pathway are plasmalogens, known to be present in cell membranes and particularly abundant in nervous tissue. Their physiological function has not yet been clarified, although they are known to be involved in platelet-activation and in the scavenging of free radicals.

β-Oxidation of fatty acids (reviewed by Osmundsen et al 1991): The peroxisomal β-oxidation system is not just a functional duplicate of the mitochondrial system. It is involved in the chain-shortening of a distinct variety of compounds, including saturated very long-chain fatty acids (VLCFA), long-chain dicarboxylic acids, polyunsaturated fatty acids and branched-chain fatty acids such as pristanic acid.

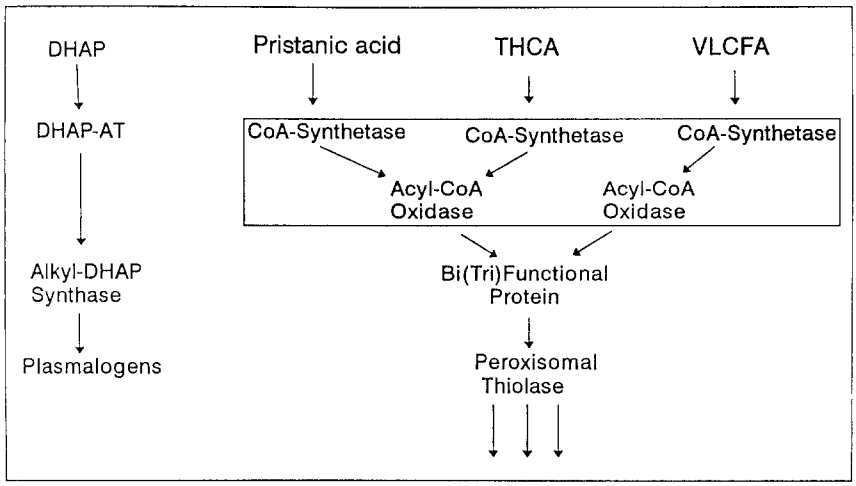

Figure 1 Schematic representation of the most important peroxisomal pathways apt to be deficient in peroxisomal disorders. Biosynthesis of plasmalogens with the peroxisomal enzymes dihydroxyacetonephosphate acyltransferase (DHAP-AT) and alkyldihydroxyacetonephosphate (DHAP) synthase. Peroxisomal oxidation of pristanic acid, trihydroxycholestanoic acid (THCA) and very long-chain fatty acids (VLCFA)

The β-oxidation of di- and trihydroxycholestanoic acid, normal intermediates in the synthesis of the primary bile acids, has been shown to be carried out in peroxisomes. Following activation to their corresponding acyl-CoA esters via membrane-bound acyl-CoA synthetases, fatty acyl-CoA esters are degraded in the peroxisomal matrix via the β-oxidation cycle, which consists of acyl-CoA oxidase, bi(tri)functional protein, and peroxisomal 3-oxoacyl-CoA thiolase. Recently, it was demonstrated that, in contrast to the situation in the rat, human liver peroxisomes contain only two acyl-CoA oxidases (Vanhove et al 1993): a palmitoyl-CoA oxidase with a function similar to that of the rat, and a branched-chain acyl-CoA oxidase, which oxidizes pristanoyl-CoA as well as di- and trihydroxycoprostanoyl-CoA (Figure 1). The enzyme known as the peroxisomal bifunctional protein, containing a hydratase domain on the amino-terminal half and a dehydrogenase domain on the carboxy-terminal half of the protein, appears to be a trifunctional protein as it also includes an enoyl-CoA isomerase (Palosaari et al 1990).

Cholesterol biosynthesis: Peroxisomes are involved not only in cholesterol oxidation to bile acids but also in cholesterol biosynthesis. Peroxisomes contain acetoacetyl-CoA thiolase, 3-hydroxy-3-methyl-glutaryl-CoA reductase, mevalonate kinase and the sterol carrier protein-2, at least in rat liver (reviewed by Krisans 1992).

Pipecolic acid oxidation: The first step in the degradation of L-pipecolic acid, an intermediate in lysine catabolism, is catalysed by the enzyme L-pipecolic acid oxidase, present in human peroxisomes.

Glyoxylate metabolism: In man, glyoxylate can be metabolized by its conversion to

glycine in a reaction catalysed by alanine : glyoxylate aminotransferase, a peroxisomal enzyme.

Glutaric acid oxidation: Glutaric acid is an intermediate in the catabolism of lysine as well as pipecolic acid. Apart from the mitochondrial glutaryl-CoA dehydrogenase, there also exists a peroxisomal glutaryl-CoA oxidase (Vamecq and Van Hoof 1984).

Phytanic acid α-oxidation: A difference in subcellular localization in different species has been demonstrated for the phytanic acid α-oxidation. Phytanic acid is primarily oxidized to pristanic acid in peroxisomes in humans and in mitochondria in rodents (Singh et al 1993).

PEROXISOME BIOSYNTHESIS - PROTEIN IMPORT

This section will focus on the known molecular defects involved in peroxisomal biogenesis in view of the possible classification of the disorders. Studies involving a genetic analysis of peroxisome biogenesis and research on peroxisomal targeting signals are now in progress.

The polypeptide composition of the peroxisomal membrane comprises two quantitative major (22 kDa and 70 kDa) and several minor membrane proteins. The cDNA for the rat 70 kDA peroxisomal membrane protein has been cloned and shown to be a member of the ATP-binding protein transporter family (Kamijo et al 1990). The topogenic signals for these membrane proteins are still unknown. Recent studies have demonstrated the existence of at least two distinct targeting signals in mammals that direct matrix proteins to the peroxisome: a carboxy-terminal SKL tripeptide and an amino-terminal presequence (for review see Roggenkamp 1992). In the case of at least the acyl-CoA oxidase, this import is ATP-dependent.

Initially, classical Zellweger patients were believed to be completely lacking peroxisomes, since peroxisomal matrix proteins were mislocalized to the cytosol. However, these patients were found to have peroxisomal 'ghosts' in cultured fibroblasts, which contain peroxisomal membrane proteins but do not contain most matrix proteins (Santos et al 1988). The primary defect in classical Zellweger syndrome may therefore be in the import machinery for peroxisomal matrix proteins rather than in the assembly of the membrane. Some classical Zellweger patients are unable to import matrix proteins containing the carboxy-terminal signal (Walton et al 1992) but do import the peroxisomal thiolase protein, which contains the amino-terminal presequence (Balfe et al 1990).

The defect in diseases in which peroxisomes fail to be formed normally and most peroxisomal functions are defective, as in classical Zellweger syndrome, remains to be shown. Particularly interesting is the question of whether or not there exist patients with a mislocalization of all peroxisomal matrix proteins to the cytosol.

Somatic cell fusion experiments with fibroblasts from different patients suggested that there are at least nine genes involved in the formation of normal peroxisomes and in the transport of peroxisomal enzymes (Brul et al 1988; Poll-The et al 1989; Roscher et al 1989; Yajima et al 1992; Shimozawa et al 1993). The different

complementation groups are thought to represent distinct phenotypes, each presumably involving a separate defect in the peroxisome import mechanism. The lack of correlation in these complementation groups between genotype and clinical phenotypes suggests that the currently used clinical denominations are not sufficiently distinctive.

As in classical Zellweger syndrome, the peroxisomal 3-oxoacyl-CoA thiolase protein is found to be present in its 44 kDa unprocessed form in liver and fibroblasts from patients with classical rhizomelic chondrodysplasia punctata (RCDP). The peroxisomal thiolase is one of the few peroxisomal proteins known to undergo proteolytic processing after synthesis. Although RCDP fibroblasts appeared to contain structures indistinguishable from control peroxisomes, the subcellular localization of the peroxisomal thiolase precursor is associated with peroxisome 'ghosts' rather than peroxisomes (Balfe et al 1990).

A defect in protein import has recently been suggested in X-linked adrenoleukodystrophy (ALD), a disease in which the principal biochemical abnormality is the accumulation of very long-chain fatty acids. X-linked ALD was thought to involve a defect in peroxisomal VLCFA-CoA synthetase since there is a normal oxidation of VLCFA-CoA in patients' fibroblasts. It now appears to be a defect in a protein involved in transport of VLCFA-CoA synthetase into the peroxisomal membrane or a protein that is associated with the VLCFA-CoA synthetase in the membrane (Mosser et al 1993).

Mistargeting of a peroxisomal protein to mitochondria has been demonstrated in some patients affected by primary hyperoxaluria type I (Danpure et al 1989).

PHENOTYPE AND GENOTYPE OF PEROXISOMAL DISORDERS

A coherent classification of peroxisomal disorders is not yet possible, since detailed molecular analysis is not available for most of the diseases. Additionally, clinical as well as biochemical heterogeneity exists in these disorders. Although a classification based on the various molecular defects would be the most accurate one, classification could also be based on the biochemical, pathological or clinical level. The disorders are usually classified according to the degree of biochemical impairment. Therefore, we propose a tentative classification based on the degree of biochemical abnormalities combined with the presently known data on whether or not a defect in peroxisome assembly is involved (Table 1).

Group 1: Disorders with defective peroxisome assembly

Classical Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, Zellweger-like syndrome: The disorders assigned to group 1 with generalized defects display a tremendous heterogeneity in their clinical severity. The classical Zellweger syndrome is the most severe, infantile Refsum disease the least severe, with neonatal ALD displaying intermediate severity (Poll-The et al 1987). Differences can be noted between the three disorders with respect to age of onset, severity of nervous system involvement and duration of survival. All patients present with diffuse encephalopathy, retinopathy or cataract, and sensorineural hearing loss. Infantile Refsum patients

Table 1 Classification of peroxisomal disorders

Enzyme defect[a]	*Disorder*
1. Peroxisome assembly deficiencies	
Generalized	Classical Zellweger syndrome Neonatal adrenoleukodystrophy Infantile Refsum disease
VLCFA oxidation, THCA oxidation, DHAP-AT, phytanic acid oxidation	Zellweger-like syndrome
DHAP-AT, alkyl-DHAP synthase, phytanic acid oxidase, unprocessed peroxisomal thiolase	Rhizomelic chondrodysplasia punctata (classical/atypical phenotype)
2. Single peroxisomal enzyme deficiencies	
Isolated DHAP-AT or alkyl-DHAP synthase	Rhizomelic chondrodysplasia punctata
VLCFA-CoA synthetase transport	X-linked adrenoelukodystrophy
Acyl-CoA oxidase	Pseudo-neonatal adrenoleukodystrophy
Bi(tri)functional enzyme	Bifunctional enzyme deficiency
Peroxisomal thiolase	Pseudo-Zellweger syndrome
THCA-CoA oxidase	Trihydroxycholestanoic acidaemia
Pipecolic acid oxidase	Isolated pipecolic acidaemia
Phytanic acid oxidase	Classical Refsum disease
Peroxisomal glutaryl-CoA oxidase	Glutaric aciduria type III
Alanine: glyoxylate aminotransferase	Hyperoxaluria type I
Catalase	Acatalasaemia

[a]DHAP-AT, dihydroxyacetonephosphate acyltransferase; DHAP, dihydroxyacetonephosphate; VLCFA, very long-chain fatty acids; THCA, trihydroxycholestanoyl

may present with predominant digestive problems associated with hepatomegaly, hypocholesterolaemia and failure to thrive during the first 6 months of life. Patients with classical Zellweger syndrome show typical dysmorphic features, severe psychomotor retardation, profound hypotonia, seizures, ocular abnormalities, and often hepatomegaly, renal cysts and chondrodysplasia punctata. They usually do not survive beyond the first year of life. The typical craniofacial dysmorphia in classical Zellweger patients may become less characteristic when the patient survives beyond the first year of life. Hepatic peroxisomes are virtually absent or severely diminished in number and size.

In classical Zellweger patients, errors of morphogenesis (e.g. facial dysmorphia, defective neural migration, renal cysts) are prominent, whereas in neonatal ALD degenerative changes (brain white matter demyelination) dominate. In the only described autopsied infantile Refsum patient, the brain showed no malformations of the cortex and no heterotopias of the cerebral or cerebellar white matter (Lazarow and Moser 1989).

Two infants with a 'Zellweger-like' syndrome presented with clinical and biochemical features consistent with the classical Zellweger syndrome (Paturneau-Jouas et al 1987; Suzuki et al 1988). Unlike in Zellweger syndrome, hepatic peroxisomes were morphologically normal in these patients.

An increasing number of patients have also been reported with 'mild variants of

Zellweger syndrome' (Bleeker-Wagemakers et al 1986, Barth et al 1987). Multiple peroxisomal dysfunction in all these disorders results in the accumulation of bile acid intermediates, VLCFA, pipecolic, pristanic and phytanic acid (age dependent), and severe impairment of plasmalogen biosynthesis. In addition, patients may show elevated transaminases, conjugated hyperbilirubinaemia, hypocholesterolaemia and decreased clotting factors. Recently, in three classical Zellweger patients, genetic mutations have been shown to exist in two peroxisomal integral membrane proteins: a point mutation in the cDNA of the 35 kDa membrane protein in one patient, and a splice site or a missense mutation in the gene of the 70 kDa membrane protein in the two others (Shimozawa et al 1992; Gärtner et al 1992). Chromosomal abnormalities involving a deletion or an inversion in region 7q11.23 have also been demonstrated in two classical Zellweger patients (Naritomi et al 1989,1990).

Rhizomelic chondrodysplasia punctata (RCDP): Classical RCDP is an autosomal recessive disorder, characterized by the presence of rhizomelic dwarfing, facial dysmorphia, cataracts, psychomotor retardation, and stippled foci of calcification of the epiphyses in infancy, which may disappear after the age of 2 years. Peroxisomal structures appear to be intact in RCDP fibroblasts, whereas in liver the organelles may be fewer or absent in some hepatocytes and enlarged in size in others (Roels et al 1991). Three peroxisomal functions are impaired in classical RCDP (Hoefler et al 1988): (1) impairment in plasmalogen biosynthesis due to a deficiency of DHAP-AT and alkyl-DHAP synthase; (2) impaired oxidation of phytanic acid; (3) failure to process the mature form of peroxisomal thiolase as in classical Zellweger syndrome, but without an accumulation of VLCFA. In addition, the absence of pristanic acid accumulation in RCDP also indicates that the peroxisomal β-oxiation is not defective. Fractionation studies have revealed that, in classical RCDP fibroblasts, the peroxisomal thiolase is present in the peroxisomal 'ghosts' fraction, while other peroxisomal β-oxidation proteins are in their normal peroxisomal location. In this view classical RCDP is assumed to be due to defective protein import (Balfe et al 1990).

As in the peroxisomal disorders with generalized defects, there exists heterogeneity and an absence of correlation between phenotype and genotype in RCDP patients. Recently, patients were described with a new variant of chondrodysplasia punctata associated with the characteristic biochemical abnormalities observed in classical RCDP, but in which rhizomelic shortening of the extremities was absent (Pike et al 1990; Poll-The et al 1991; Smeitink et al 1992). Conversely, patients have been identified who have the clinical phenotype of classical RCDP but whose biochemical abnormalities were limited to a deficiency of DHAP-AT (Wanders et al 1992b), or alkyl-DHAP synthase (Wanders et al 1993). Patients with the clinical and biochemical phenotype of classical RCDP were found to belong to a single complementation group, whereas the patient with an isolated DHAP-AT deficiency appeared to belong to a different complementation group, indicating that mutations in at least two different genes can lead to the clinical phenotype of RCDP (Heikoop et al 1992). The RCDP patients with an isolated deficiency of DHAP-AT or alkyl-DHAP synthase belong to the group 2 disorders with single enzyme deficiency.

Group 2: Disorders with single enzyme deficiencies

Rhizomelic chondrodysplasia punctata: An isolated deficiency of DHAP-AT or alkyl-DHAP synthase has been described (already discussed in group 1).

X-linked adrenoleukodystrophy (ALD): The most common peroxisomal disorder is X-linked ALD. As already mentioned, the underlying molecular defect in this disorder presumably involves a defective transport of the VLCFA-CoA synthetase into the peroxisomal membrane rather than a deficiency in peroxisomal VLCFA-CoA synthetase (Mosser et al 1993). The putative X-linked ALD gene shows significant homology to the peroxisomal 70 kDa membrane protein and the gene was found to be partially deleted in 7% of 85 X-linked ALD patients (Aubourg et al 1993b).

The clinical presentation of X-ALD varies considerably (Moser and Moser 1989). The childhood form is the most common and the most severe phenotype, with onset of neurological involvement usually between 5 and 10 years of age, leading to a vegetative state and death within a few years. School failure or attention deficit disorder are common early symptoms, followed by visual and auditory disturbances and quadriplegia, whereas seizures are usually a late manifestation. The progressive central nervous system demyelination is associated with an inflammatory response in brain. The second most common form is adrenomyeloneuropathy, a form with onset in adulthood and a course of decades. In this phenotype patients present progressive paraparesis and sphincter dysfunction due to spinal cord involvement. In both phenotypes symptoms of adrenal insufficiency may precede, coincide with, or follow the onset of neurological involvement.

Other phenotypes of the disease include adolescent ALD, adult cerebral ALD, Addision disease without neurological symptoms and biochemically affected individuals who are asymptomatic. The various phenotypes may occur within the same pedigree. The principal biochemical abnormality of X-linked ALD is the accumulation of VLCFA due to impaired β-oxidation in peroxisomes. Liver peroxisomes are considered to be microscopically normal. Female heterozygotes for X-linked ALD may develop neurological symptoms resembling a chronic non-progressive spinal cord involvement.

Peroxisomal acyl-CoA oxidase deficiency (pseudo-neonatal ALD): This disease is characterized by an absence of dysmorphic features, early-onset seizures, and severe psychomotor retardation. Clinical manifestations resemble those observed in patients with neonatal ALD. In contrast to neonatal ALD, this disorder is associated with enlarged hepatic peroxisomes, normal bile acids, normal pristanic acid and an isolated VLCFA accumulation due to a deficiency of acyl-CoA oxidase, as shown by immunoblotting analysis (Poll-The et al 1988a). The primary defect in the first two patients described involves a large deletion of the acyl-CoA oxidase gene (Fournier et al 1993). Subsequently, other patients have been identified by using complementation analysis between different cell lines from patients suspected of having a single enzyme defect in the peroxisomal β-oxidation pathway, and cell lines from known specific enzyme defects in the VLCFA β-oxidation (Suzuki et al 1993; Watkins et al 1993).

Bi(tri)functional protein deficiency: The first patient described with a bi(tri)functional protein deficiency presented the clinical features of neonatal ALD without retinal changes and dysmorphia, but with the presence of polymicrogyria, and normal liver peroxisomes (Watkins et al 1989). The accumulation of the bile acid intermediate trihydroxycoprostanoic acid and VLCFA in this patient appeared to be due to an isolated deficiency of the bi(tri)functional protein as shown by immunoblotting analysis of peroxisomal β-oxidation enzymes. Other patients have been identified by the complementation analysis technique as used for the diagnosis of patients with isolated acyl-CoA oxidase deficiency (Wanders et al 1992a; McGuinness et al 1993). In contrast to the patient described by Watkins and colleagues (1989), some of these patients presented dysmorphic features and ocular abnormalities indicating clinical heterogeneity among patients with bi(tri)functional protein deficiency.

Peroxisomal 3-oxoacyl-CoA thiolase deficiency (pseudo-Zellweger syndrome): This condition was first described in a patient with all the clinical and pathological features of classical Zellweger syndrome, including the typical facial dysmorphia, neuronal heterotopia, and renal cortical cysts (Goldfischer et al 1986). However, peroxisomes in hepatocytes were found to be abundant and enlarged in size. Biochemical studies revealed increased levels of VLCFA and bile acid intermediates due to a deficient peroxisomal thiolase, as shown by immunoblotting (Schram et al 1987).

Di- and trihydroxycholestanoic acidaemia: Four patients have been reported with an accumulation of di- and trihydroxycholestanoic acid without increased VLCFA levels, but associated with or without pristanic acidaemia (Christensen et al 1990; Przyrembel et al 1990; Wanders et al 1991). Clinical as well as biochemical heterogeneity exists in these patients. Three of the patients presented neurological involvement, facial dysmorphia and hepatomegaly from the neonatal period, and an absence of increased phytanic acid and pristanic acid. Although the trihydroxycholestanoyl-CoA oxidase activity was found to be deficient in post-mortem liver from one of these three patients, the accuracy of this result is doubtful owing to the poor quality of the liver (Przyrembel et al 1990). The patient described by Christensen and colleagues (1990) was normal until the age of 18 months, after which she developed ataxia, hypotonia, absent reflexes and impaired hearing. Recently it was found that in this patient the accumulation of bile acid intermediates and phytanic acid was associated with an increased level of pristanic acid (Ten Brink et al 1994). No enzyme activity measurements were performed in this patient. However, the recent results that the first step in the β-oxidation of pristanoyl-CoA and di- and trihydroxycholestanoyl-CoA is catalysed by a single oxidase may suggest that this patient will have a defect at the level of cholestanoyl-CoA oxidase (Vanhove et al 1993).

Hyperpipecolic acidaemia: The term hyperpipecolic acidaemia was assigned to this disease on the basis of the observation of an increase in pipecolic acid prior to the discovery of the generalized peroxisomal defects (Wanders et al 1988b; H.W. Moser, personal communication). Four patients were diagnosed as having hyperpipecolic acidaemia (Gatfield et al 1968; Thomas et al 1975; Burton et al 1981). In fact, the

diagnosis hyperpipecolic acidaemia should only be assigned to the three siblings described with isolated increased pipecolic acid in body fluids (Poll-The et al 1988b). These three patients presented a combination of clinical features of a generalized peroxisomal disorder and Joubert's syndrome, including abnormal liver peroxisomes, abnormal respiration pattern and cerebellar vermis dysplasia.

Classical Refsum disease (phytanic acid storage disease): The clinical presentation is mainly characterized by retinitis pigmentosa, peripheral polyneuropathy, cerebellar ataxia, and elevated cerebrospinal fluid protein concentrations. Less constant features are nerve deafness, anosmia, ichthyosis, and skeletal and cardiac abnormalities. There is an absence of mental retardation, hepatomegaly and dysmorphia in classical Refsum disease. The onset of clinical symptoms varies from childhood to the fifth decade. Classical Refsum disease is associated with the accumulation in blood and tissues of phytanic acid due to an isolated phytanic acid α-hydroxylase deficiency (for review see Steinberg 1989).

Glutaric aciduria type III: This condition has so far been described in only one patient. A girl presented failure to thrive and postprandial vomiting in infancy. She was found to have a significantly increased urinary excretion of glutaric acid and to be homozygous for β-thalassaemia. Riboflavin therapy was started. Subsequently she has remained well except for a facial palsy at the age of 2.5 years that slowly resolved and did not appear to be associated with a metabolic decompensation. A healthy sibling was shown to have a small but persistent excretion of glutaric acid (Bennett et al 1991). The elevated glutaric acid excretion in the patient appeared to be caused by a deficiency of peroxisomal glutaryl-CoA oxidase rather than a mitochondrial dysfunction at the level of glutaryl-CoA dehydrogenase (glutaric aciduria type I) or a deficiency of either the electron-transfer flavoprotein or of the electron-transfer flavoprotein–ubiquinone oxidoreductase (glutaric aciduria type II).

Primary hyperoxaluria type I: Primary hyperoxaluria type I is an autosomal recessive disorder characterized by nephrolithiasis and nephrocalcinosis. It is the subject of another paper in this volume.

Acatalasaemia: Acatalasaemia is an autosomal recessive disorder due to a deficiency of catalase activity, especially in the erythrocyte. In general, acatalasaemia is a relatively benign disease characterized by oral gangrene and ulcerations (Eaton 1989).

DIAGNOSIS OF PEROXISOMAL DISORDERS WITH NEUROLOGICAL INVOLVEMENT

Except for X-linked ALD, classical Refsum disease, glutaric aciduria type III, and some patients with trihydroxycholestanoic acidaemia, all the peroxisomal disorders with neurological involvement present clinical abnormalities from the neonatal period. The clinical symptomatology is very much dependent upon the age of the patient at presentation (Table 2). In the neonatal period, the clinical picture may be dominated by severe hypotonia and craniofacial dysmorphia, whereas in patients older than 6

Table 2 Clinical onset of peroxisomal disorders with neurological involvement

Symptoms	*Disorder*[a]
Neonatal period	
Hypotonia, areactivity, seizures	ZS, ZS variants
Craniofacial dysmorphia	Neonatal ALD
Skeletal abnormalities	Pseudo-neonatal ALD (acyl-CoA oxidase deficiency)
Conjugated hyperbilirubinaemia	Bifunctional enzyme deficiency
	RCDP (typical/atypical)
	THC acidaemia
	Pipecolic acidaemia
First 6 months of life	
Hepatomegaly, prolonged jaundice	IRD
Digestive problems, hypocholesterolaemia	Pipecolic acidaemia, neonatal ALD, milder forms of ZS
Vitamin E deficiency	
Failure to thrive	Atypical chondrodysplasia
Beyond 4 years of age	
Behaviour changes	X-linked ALD
Deterioration of intellectual functions	
White matter demyelination	
Visual and hearing impairment (ERG, BAEP)	Classical Refsum
Peripheral neuropathy, gait abnormality	

[a]ZS, Zellweger syndrome; ALD, adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata; THC, trihydroxycholestanoic; IRD, infantile Refsum disease; ZS variants, including ZS-like, pseudo-ZS (peroxisomal thiolase); ERG, electroretinogram; BAEP, brain auditory evoked potentials

months of age, the major symptoms are usually psychomotor retardation, visual and hearing deficit, seizures and failure to thrive. Ophthalmological examination including an electroretinogram should be performed since peroxisomal disorders are frequently associated with retinitis pigmentosa or cataract. Clinically, it may be difficult to differentiate between a mitochondrial respiratory chain disorder and a peroxisomal disease. An important difference is that peroxisomal disorders are not associated with metabolic acidosis and/or lactic acidaemia.

Table 3 lists the diagnostic assays that may be used for the diagnosis of peroxisomal disorders. Only urinary pipecolic excretion can be detected by an 'overall metabolic screening' (analysis of amino acids). Eight of the 15 peroxisomal disorders with neurological involvement are associated with an accumulation of VLCFA, which suggests that VLCFA analysis should be used as primary test. However, phytanic acid and bile acid intermediates should also be performed in view of the recent reports of atypical chondrodysplasia variants and isolated trihydroxycholestanoic aciduria. The clinical presentation of the typical phenotype of rhizomelic chondrodysplasia punctata and classical Refsum are distinct from the other disorders and should not cause difficulties in their diagnosis. In order to elucidate whether the accumulation of VLCFA in a patient's plasma results from a defect in peroxisome biogenesis or is caused by a defect in one of the peroxisomal β-oxidation enzyme activities, additional assay procedures must be carried out, especially plasmalogen concentrations and

Table 3 Diagnostic assays in peroxisomal disorders

Disease	*Material*	*Type of assay*[a]
	Plasma	VLCFA, bile acids, phytanic acid, pristanic acid, pipecolic acid, polyunsaturated fatty acids
Classical ZS	RBCs[a]	Plasmalogens
Neonatal ALD Infantile Refsum Zellweger-like	Fibroblasts	Plasmalogens biosynthesis, DHAP-AT, alkyl-DHAP synthase, particle-bound catalase, VLCFA β-oxidation, immunoblotting β-oxidation proteins, phytanic acid oxidation
Rhizomelic chondrodysplasia punctata (classical/atypical phenotypes)	Plasma RBCs Fibroblasts	Phytanic acid Plasmalogens DHAP-AT, alkyl-DHAP synthase, phytanic acid oxidation
Isolated peroxisomal β-oxidation defects	Plasma Fibroblasts	VLCFA, bile acids VLCFA β-oxidation, immunoblotting β-oxidation proteins
Isolated defect of bile acid synthesis	Plasma Liver	Bile acids THCA-CoA oxidase
Isolated pipecolic acidaemia	Plasma Liver	Pipecolic acid Pipecolic acid oxidase
Classical Refsum	Plasma Fibroblasts	Phytanic acid Phytanic acid oxidation
Glutaric aciduria type III	Urine Liver	Organic acids Glutaryl-CoA oxidase
Hyperoxaluria type I	Urine Liver	Organic acids AGT
Acatalasaemia	RBCs	Catalase

[a]VLCFA, very long-chain fatty acids; DHAP-AT, dihydroxyacetonephosphate acyltransferase; DHAP, dihydroxyacetonephosphate; THCA, trihydroxycholestanoyl; AGT, alanine:glyoxylate aminotransferase; RBCs, red blood cells

immunoblotting of peroxisomal β-oxidation proteins. Figure 2 summarizes the peroxisomal disorders associated with an accumulation of VLCFA. In view of the fact that new phenotypes continue to be detected, it would be advisable to carry out all the biochemical investigations and morphological study of liver peroxisomes before excluding the diagnosis of peroxisomal disorder.

All of the peroxisomal disorders can be identified prenatally, either by chorionic villus biopsy or amniocentesis (Wanders et al 1988a). Various methods are available for prenatal diagnosis of peroxisomal disorders. Depending on the peroxisomal disorder suspected, measurement of VLCFA and/or assay of plasmalogen synthesis are the most useful methods today, except for the THCA-CoA oxidase deficiency, isolated pipecolic acidaemia, glutaric aciduria type III and hyperoxaluria type I.

At present heterozygote detection is only possible in X-linked ALD using VLCFA analysis or restriction fragment polymorphism (Aubourg et al 1987).

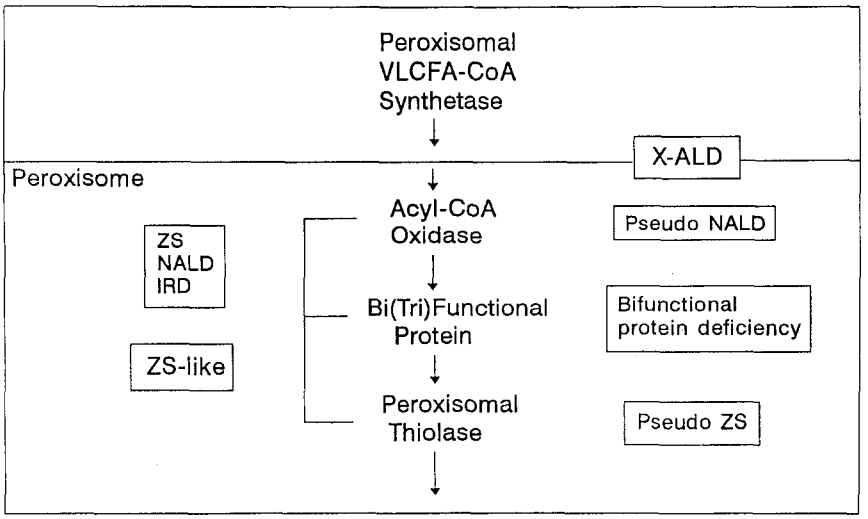

Figure 2 Schematic representation of the peroxisomal disorders associated with an accumulation of very long-chain fatty acids (VLCFA). Zellweger syndrome and variants (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD), X-linked adrenoleukodystrophy (X-ALD), pseudo-NALD, bifunctional protein deficiency and pseudo ZS

THERAPY OF PEROXISOMAL DISORDERS

In classical Refsum, treatment with diets low in phytanic acid, combined or not with plasmapheresis, reduces plasma phytanic acid levels and brings about clinical improvement (Steinberg 1989).

In X-linked ALD patients it has been demonstrated that it is possible to normalize the plasma VLCFA levels by a regimen that combines dietary restriction of VLCFA with the oral intake of monounsaturated fatty acids (oleic and erucic). However, the clinical benefit of dietary therapy has been rather disappointing (reviewed by Moser et al 1992; Aubourg et al 1993a). Final conclusions on the efficiency of these treatments will hopefully be available in the near future. The same considerations apply to the usefulness of bone marrow transplantation, which has been encouraging in a clinically mildly affected patient with the childhood form of X-ALD (Aubourg et al 1990).

Therapeutic measures, such as supplementation of docosahexaenoic acid (Martinez 1992) or other regimens (Holmes et al 1987; Robertson et al 1988; Smeitink et al 1992), have also been tried in patients with multiple peroxisomal dysfunction.

CONCLUDING REMARKS

Peroxisomal disorders provide a model for research into how metabolic disturbances may lead to embryopathy and/or severe neurological dysfunction. It is evident that combined analysis of clinical, biological, pathological, morphological and genetic markers is necessary for the understanding of underlying pathogenetic processes.

One of the intriguing questions for the clinician is the link between the various phenotypes, the type of organ involved, and the severity of neurological dysfunction.

It should also be considered that the metabolic perturbations observed in the peroxisomal disorders might be an epiphenomenon of an as yet unidentified key element substantially involved in the expression of a phenotype.

NOTE ADDED IN PROOF

Recently, patients with pseudo-infantile Refsum (*Pediatr Res* (1993) **34**: 270–276) and atypical Refsum (*Neurology* (1993) **43**: 2040–2044) have been described.

Mevalonic aciduria should be considered a peroxisomal disorder since mevalonate kinase is predominantly localized in peroxisomes (*J Biol Chem* (1994) **269**: 1197–1205).

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