Molecular, biochemical end clinical aspects of peroxisomes biogenesis disorders
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Citation for published version (APA):
Gootjes, J. (2004). Molecular, biochemical end clinical aspects of peroxisomes biogenesis disorders

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Summary
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The peroxisome biogenesis disorders (PBDs), which include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), represent a spectrum of disease severity with ZS being the most, and IRD the least severe disorder. Common to all three PBDs are liver disease, variable neurodevelopmental delay, retinopathy and perceptive deafness. Patients with ZS are severely hypotonic from birth and die before one year of age. Patients with NALD experience neonatal onset of hypotonia and seizures and suffer from progressive white matter disease, dying usually in late infancy. Patients with IRD may survive beyond infancy and some may even reach adulthood.

The absence of functional peroxisomes in patients with a PBD leads to a number of biochemical abnormalities among which 1) impaired synthesis of plasmalogens, due to a deficiency of the two enzymes dihydroxyacetonephosphate acyltransferase (DHAPAT) and alkyl-dihydroxyacetonephosphate synthase (alkyl-DHAP-synthase), 2) impaired peroxisomal fatty acid β-oxidation, leading to the accumulation of very-long-chain fatty acids (VLCFAs), notably C26:0, the branched-chain fatty acid pristanic acid and the bile acid intermediates di- and trihydroxycholestanolic acid (DHCA and THCA), and 3) impaired phytanic acid α-oxidation.

The PBDs are caused by genetic defects in PEX genes encoding proteins called peroxins, which are required for the biogenesis of peroxisomes and function in the assembly of the peroxisomal membrane or in the import of enzymes into the peroxisome. After synthesis on free polyribosomes, peroxisomal matrix proteins carrying either a carboxy-terminal peroxisomal targeting sequence 1 (PTS1) or a cleavable amino-terminal PTS2 signal are translocated across the peroxisomal membrane. A defect in one of the peroxins of the peroxisomal import machinery leads to failure of protein import via the PTS1- and/or PTS2-dependent import pathway and, consequently, to functional peroxisome deficiency. Cell fusion complementation studies using patient fibroblasts revealed the existence of at least 11 distinct genetic groups of which currently all corresponding PEX genes have been identified. Most complementation groups are associated with more than one clinical phenotype.

In this thesis some aspects of the peroxisome biogenesis and the PBDs have been studied. Chapter one reviews the current knowledge on peroxisome biogenesis, peroxisomal functions, the PBDs and their diagnosis, and therapy. Because of the marked genetic heterogeneity in PBDs, caused by the many complementation groups and the presence of many unique mutations among patients, genotype-phenotype studies are limited in giving a prognosis for new PBD patients. Therefore, in chapter two a different approach was used to predict the life expectancy of the patients: the effects of the defective genes on peroxisome function were examined, rather that the mutations themselves, by conducting a search for the biochemical parameters in fibroblasts, which would be best in predicting the severity of patients. DHAPAT activity and C26:0 β-oxidation turned out to be the best markers in predicting life expectancy of PBD patients. Combination of both markers gave an even better prediction. In chapter three and four, novel mutations in the PEX2 and PEX12 genes of PBD patients were identified. Both PEX2 and PEX12 contain a C-terminal zinc-binding domain, considered to be important for their interaction with other proteins.
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of the peroxisomal protein import machinery. The importance of the zinc-binding domain in PEX12 was underlined by the mutations found in our patients. However, patients lacking the zinc-binding domain of PEX2 displayed a mild phenotype, whereas a patient with a specific mutation within the domain was affected with the severe ZS phenotype. This makes the importance of this domain in PEX2 unclear. Chapter five describes eight PBD patients with a very unusual biochemical phenotype, characterized by abnormal peroxisomal plasma metabolites, but normal to very mildly abnormal parameters in cultured skin fibroblasts, including a mosaic catalase immunofluorescence pattern, which so far made complementation analysis impossible. We developed a novel complementation technique in which fibroblasts are grown at 40°C rather than 37°C, which exacerbated the defect in peroxisome biogenesis. Using this method, we assigned all patients to CG3, and subsequently identified a single homozygous S320F mutation in their PEX12 gene. We investigated various biochemical parameters in fibroblasts of these patients at 30°C, 37°C and 40°C and found a temperature-dependent behavior for all parameters. When compared to fibroblasts from patients homozygous for the G843D mutation in PEX1, well known for its mild biochemical and clinical phenotype, our patients displayed a milder biochemical temperature-sensitive phenotype for all parameters tested. Nevertheless, their clinical phenotype was more severe, suggesting the defect to be organ specific. In chapter six the reinvestigation of a unique patient is described, who was reported with a presumed deficiency in THCA-CoA oxidase, but instead was found to be suffering from a mild PBD, caused by two mutations in the PEX12 gene. The absence of clear peroxisomal abnormalities in the patient's cultured skin fibroblasts, including a normal peroxisomal localization of catalase, implied that even when, upon routine diagnostics, all peroxisomal functions in fibroblasts are normal, a PBD cannot be fully excluded and additional studies may be required. Chapter seven describes a rapid, non-invasive alternative technique to determine the presence of peroxisomes in patient cells: immunofluorescence microscopy analysis in lymphocytes, which can be isolated from the same blood samples as used for metabolite analyses. In some mild cases, immunofluorescence microscopy results in lymphocytes were less ambiguous than in cultured skin fibroblasts, which will aid in a more clear and firm diagnosis. In chapter eight, we report all mutations in the different PEX genes that have been determined in our laboratory so far, combined with those reported in literature, to present an overview of the mutational spectrum of the PBDs.