Peroxisomal biogenesis disorders: A review about the molecular background, classification and recent developments

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Abstract

Peroxisomes are organelles found in virtually all eukaryotic organisms, fulfilling a variety of universal and also very species-specific tasks. In humans, peroxisomes accomplish essential functions, such as β-oxidation of very long chain fatty acids, plasmalogen synthesis and bile acid conjugation. The importance of this organelle in human physiology is emphasized by the multitude of peroxisomal disorders. In order to understand the physiological consequences of peroxisomal diseases in general and peroxisomal biogenesis disorders in particular, the most important aspects of peroxisomes are elucidated, concerning peroxisome biogenesis, proliferation and metabolic functions. The value of an effective classification is highlighted, progressively stimulating the identification of novel mutation causing severe peroxisomal dysfunctions. The very recent finding of a Pex11β dysfunction, surprisingly causing a mild Zellweger syndrome instead of a nonviable or neonatal lethal etiopathology as its subsequent mouse model, will be discussed as well as other substantial developments.

Introduction

Peroxisomes can be found in almost all eukaryotic cells.[1] They were first described as an organelle in 1969 by Christian de Duve.[2] Peroxisomes are highly flexible in abundance and size and by now, the only organelle that can have different functions according to the cell type and developmental and metabolic state of an organism. In plants for example, peroxisomes play a crucial role in anti-fungal defense, whereas certain yeast species use peroxisomes to oxidize and assimilate methanol and amines.[3] In human physiology, peroxisomes fulfill a variety of metabolic tasks.[4] They play a important role in β-oxidation of very long chain fatty acids (VLCFA) and branched chain fatty acids as well as in in the biosynthesis of plasmalogens.[5] There are numerous proteins (peroxins) known to be involved in peroxisome formation and proliferation, however the actual mechanism of the peroxisome biogenesis is still uncertain.

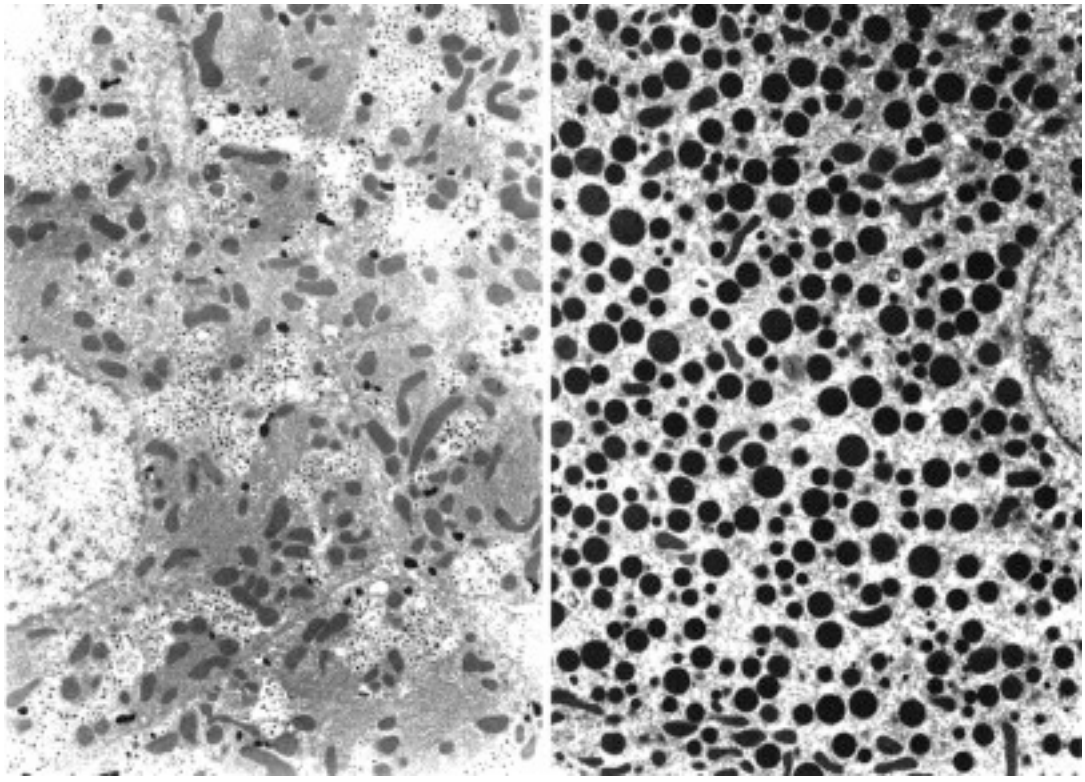

Figure 1: Electron microscopy image representing normal (left) and peroxisome proliferator treated (right) mouse livers cells, demonstrating the high flexibility of peroxisomes. Peroxisomes are stained as dark osmiophilic organelles.

[6]Peroxisomal malfunction can cause multiple illnesses, which can be divided into two major groups due to their molecular cause - single enzyme deficiencies[7] and peroxisomal biogenesis disorders (PBD).[8] All of these peroxisomal diseases are genetically recessive. Whereas a single enzyme deficiency normally effects one certain step of a metabolic pathway, PDB exacerbates the entire peroxisome assembly.[9]
In this review, the main facts about peroxisomes as well as their physiological role in human metabolism will be highlighted to elucidate the molecular pathophysiology of different peroxisomal biogenesis disorders. In addition, recently solved mysteries and still unanswered questions about peroxisomal disorders will be discussed.

Peroxisome biogenesis and proliferation

As mentioned before peroxisomes are very flexible in size and abundance. Although the actual mechanism of peroxisome biogenesis is unknown the number of peroxisomes per individual cell is determined to four subunits: Inherited organelles, proliferation by fission, *de novo* formation and degradation. Peroxisome proliferation can be influenced by certain chemicals, so called peroxisome proliferators. As an illustration of the peroxisome flexibility, the relative peroxisome volume of liver cells changes from about 2% to 25% during peroxisome

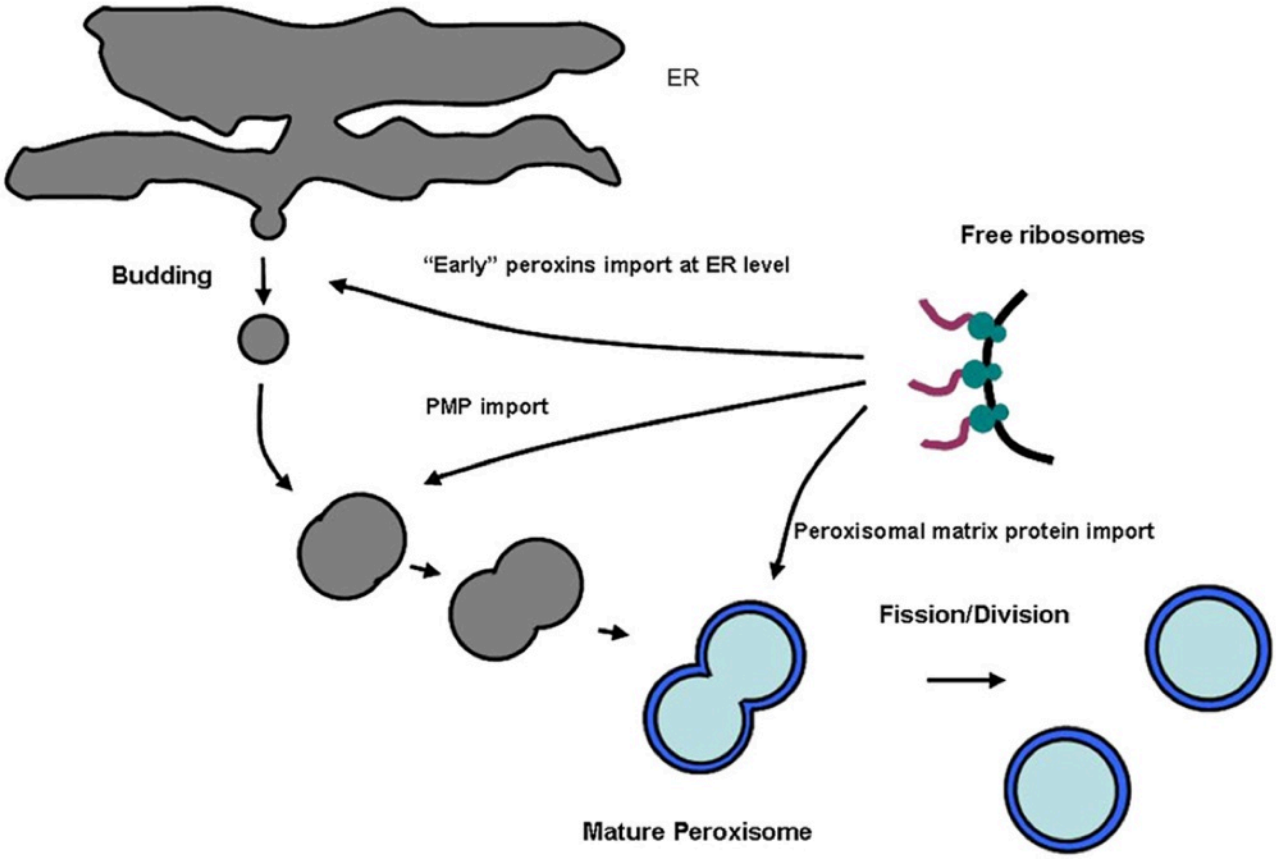

Figure 2: Theoretical model of peroxisome biogenesis. ER is involved in de novo biogenesis of peroxisome. Mature peroxisomes acquire proteins from free ribosomes and can proliferate by division.

proliferator medication, leading to a weight doubling of the entire liver (figure 1).[10]

Peroxisomes are not autonomous organelles as mitochondria and chloroplasts. As they are not able to synthesize their own membrane, it must be orchestrated elsewhere, potentially from the endoplasmic reticulum (ER).[11] Whether preexisting peroxisomes fuse with delivered membrane vesicles to grow and proliferate by fission or whether they derive directly from the ER is uncertain. Both theories have their supporters and opponents.[12] During the last years even more additional theories have been developed.[13] Nevertheless it has been proven, that a *de novo* formation exists under certain circumstances, without the need of any preexisting peroxisomes.[14] However this does not mean that *de novo* formations is indeed the predominant route of peroxisome proliferation.[15] The schematic illustration (figure 2) shows a theoretical model of the peroxisome biogenesis, including the *de novo* formation, proliferation by fission and peroxisomal membrane and matrix protein import.[16]

Peroxisomal matrix proteins are synthesized on free ribosomes in the cytosol. They are directed to peroxisomes by the peroxisomal targeting signal (PTS). The majority of all peroxisomal matrix proteins have the C-terminal SKL-tripeptide as targeting signal, the PTS1.[17] The

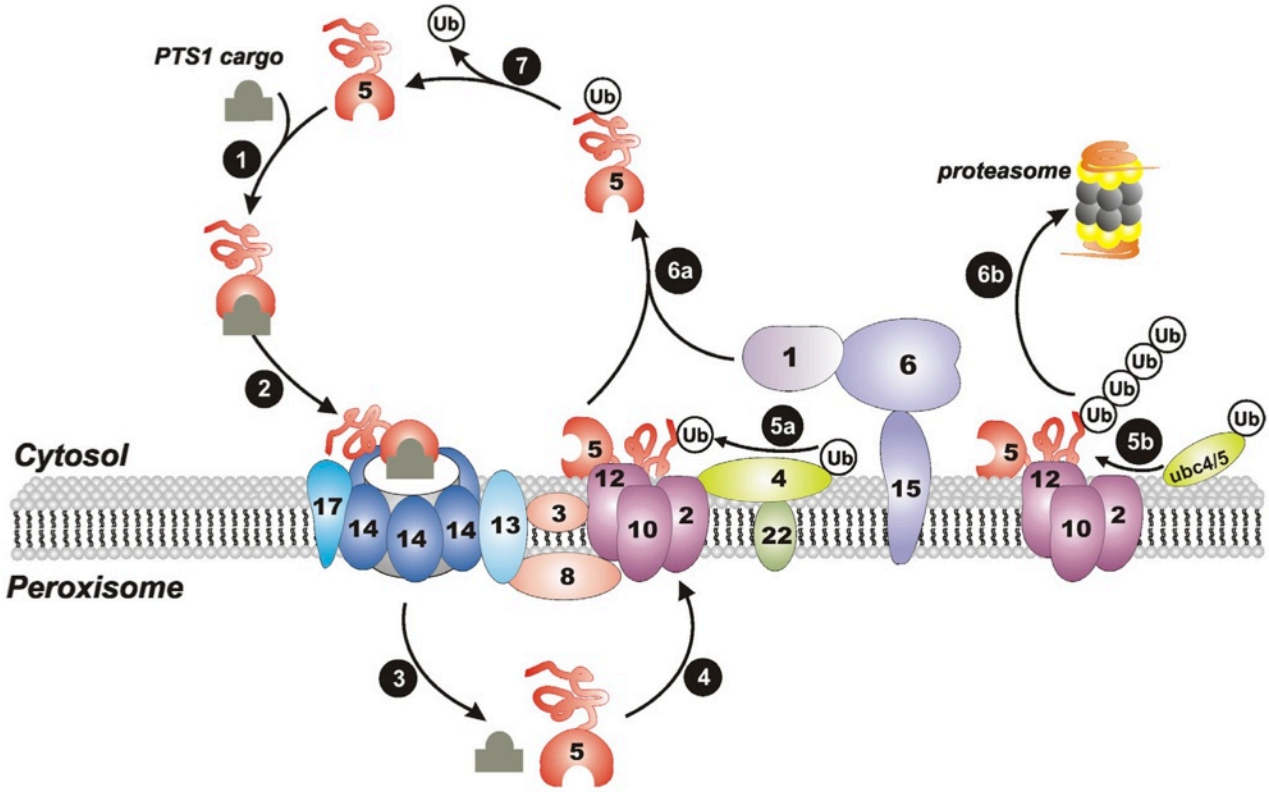

Figure 3: The import of peroxisomal matrix proteins. The process may be divided into distinct steps (white numbers in closed black circles). Bold numbers indicate corresponding Pex proteins. The steps are: (1) Receptor–cargo interaction in the cytosol (PTS2 pathway is not depicted). (2) Receptor–cargo docking at the peroxisomal membrane with the docking subcomplex, inducing the assembly of the translocon. (3) Translocation of the receptor–cargo complex across the membrane followed by the dissociation of the receptor–cargo complex; i.e., cargo release. (4) Export of cargo-free receptors from the peroxisome matrix to the membrane. (5a) Monoubiquitination of the receptor on a cysteine by Pex4 and Pex2 (for receptor recycling) or (5b) polyubiquitination of the receptor on a lysine by Ubc4/5 and Pex10/12 (for degradation by the RADAR pathway). (6a) Receptor recycling from the peroxisome membrane back to the cytosol by the action of the AAA ATPases (Pex1 and Pex6) and ATP hydrolysis, or (6b) degradation of a receptor that is blocked from recycling via the RADAR pathway involving the proteasome. (7) Deubiquitination of the receptor before the next round of import. The squiggly line on Pex5 denotes its disordered N-terminal segment.

PTS2 has a less conserved consensus sequence and is less frequent.[18] The PTS1 and PTS2 tagged proteins bind to their subsequent receptors, Pex5 and Pex7 respectively. The receptor-cargo connects with the docking complex, consisting of Pex13, Pex14 and Pex17). Only if the RING-finger complex (Pex2, Pex10 and Pex12) is attached to docking complex, bridged by Pex8 and probably Pex3, the receptor-cargo can be translocated to the peroxisomal matrix.[19] This matrix protein translocation machinery is called the peroxisomal translocon (figure 3).[20] Remarkably, proteins can be translocated over the membrane in a folded or even in an oligomeric state.[21] The receptors, Pex5 and Pex20, are monoubiquitinated after the translocation for recycling them back into the cytosol or polyubiquitinated for degradation.[22]
The role of every single peroxisomal membrane protein (PMP) has not yet been elucidated in detail, but it has been indicated that a dysfunction of

any of these genes causes lethal peroxisomal biogenesis disorders.[23]

Peroxisomes in human physiology

Mammalian peroxisomes contain approximately 50 different catalytic active proteins.[24] In addition, there are proteins with unknown enzymatic function, but also enzymatic activities that are not yet linked to a specific peroxisomal protein.[25] Some of the peroxisomal located proteins can also be found in other cellular compartments as mitochondria or the cytosol. This can be explained by multiple isoforms of the protein with a different targeting signal.[26]
During the last two decades, our understanding of peroxisomal functions in human physiology and pathophysiology has increased dramatically. This has been triggered by two major developments: The intensive study of *pex gene deletion* strains in yeast and identification of numerous mutations in those genes in peroxisomal biogenesis disorder (PDB) patients.

The role of peroxisomes in metabolic pathways

One of the best-known functions of peroxisome is oxygen metabolism and the production and decomposition of reactive oxygen species (ROS).[27] Due to this catalase and peroxidase activity, breaking down hydrogen peroxide, the organelle got its name, the peroxisome.

Apart from hydrogen peroxide, there are other ROS generated and degraded in peroxisomes, like superoxide anions and peroxynitrite.[28] The ROS levels are kept relatively constant by the sum of all ROS-producing oxidases and ROS-scavenging enzymes.[29] It has been shown that peroxisomes not only regulate their own matrix ROS-levels, but also influence the entire cell.[30] This process contributes to cellular signaling pathways, involving hydrogen peroxide as well as nitric oxide. It is also been stated that these metabolites play an important role in cellular aging.[31]

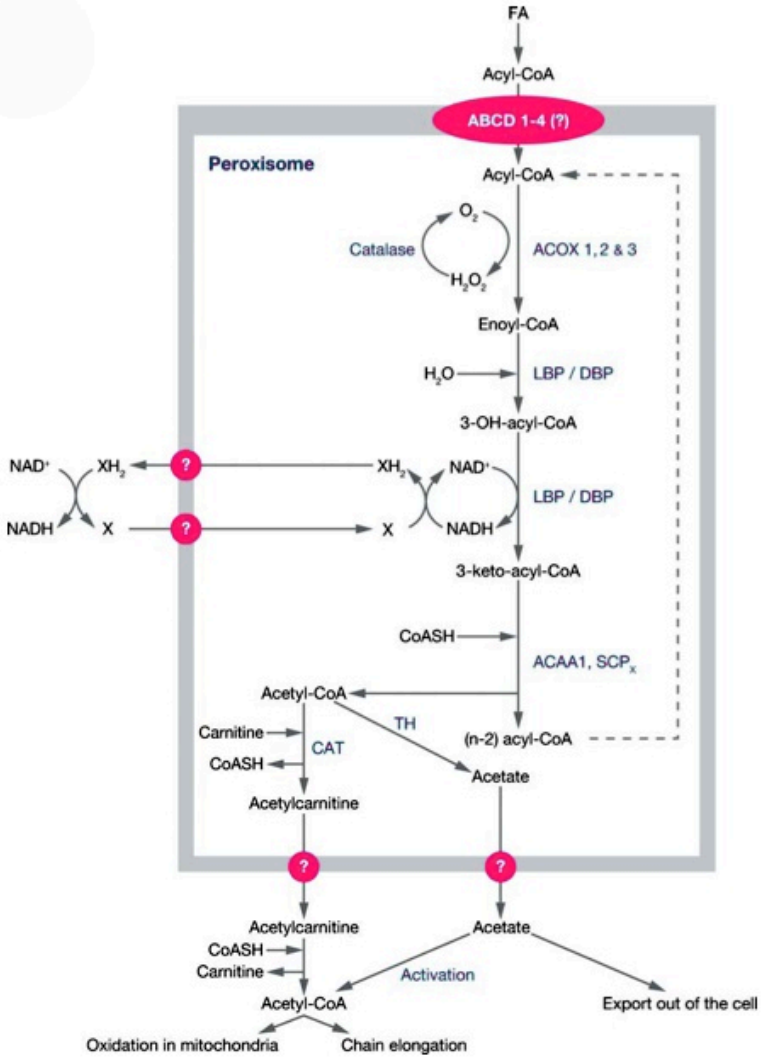

Figure 4: Schematic representation of the peroxisomal β-oxidation system in humans. In peroxisomes, molecular oxygen is the electron acceptor in the first step of beta-oxidation, resulting in the formation of H2O2, which is reconverted into O2 by catalase. The NADH generated in the third step of peroxisomal beta-oxidation is reoxidized via a NAD(H)-redox shuttle, involving the cytosolic and peroxisomal isoforms of lactate dehydrogenase.

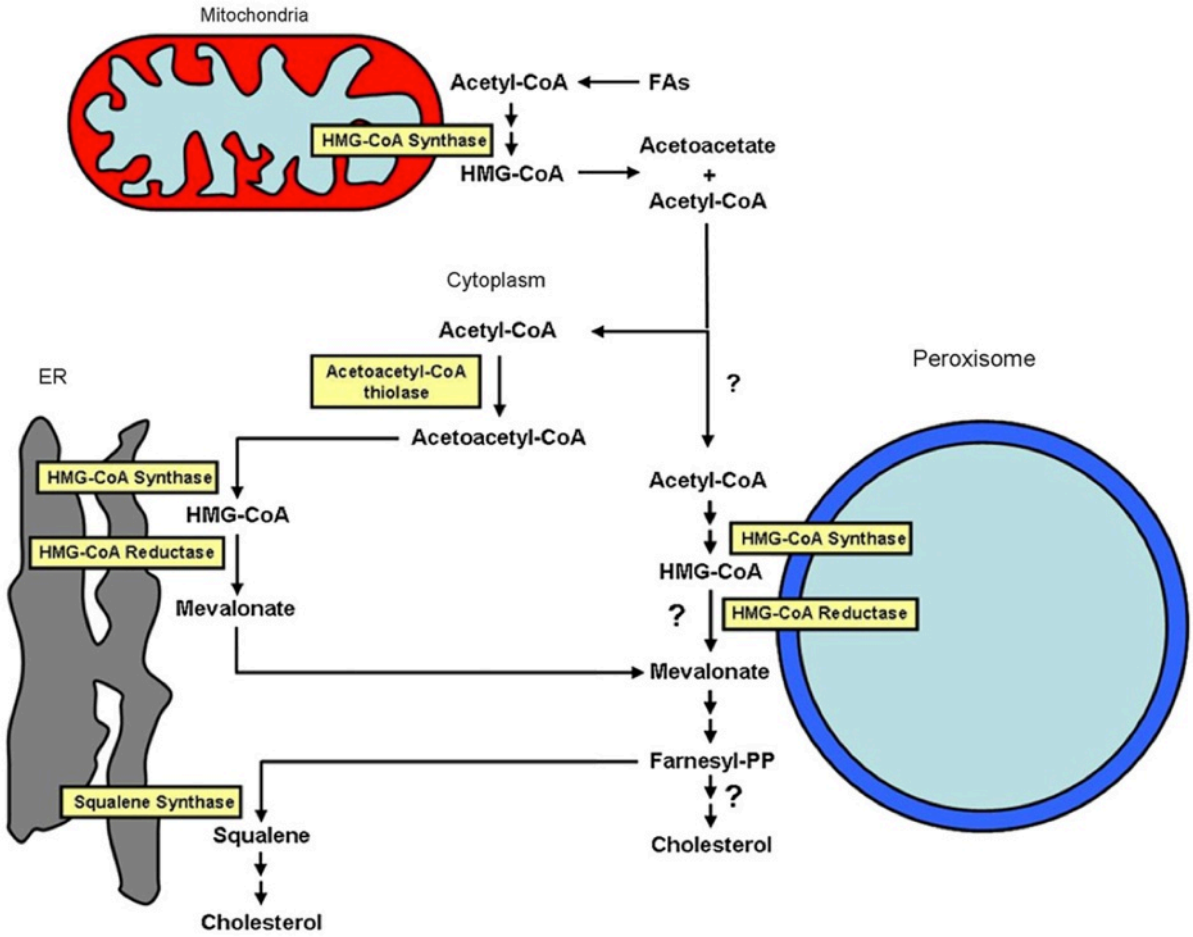

Figure 5: Cholesterol biosynthesis. The synthesis of cholesterol is carried out in different cell organelles, i.e. mitochondria, peroxisome and ER. The question marks indicate the probably pathways not yet confirmed.

Another well-known function of peroxisomes is the β-oxidation of very long chain fatty acids (VLCFA). This peroxisomal function is universal, present in all organisms examined so far.[32] In yeast and plants, peroxisomes are the only site of β-oxidation, whereas in human and other higher eukaryotes, β-oxidation also takes place in mitochondria.[33] Shorter chain fatty acids are solely oxidized in mitochondria. Long chain fatty acids can be oxidized in either mitochondria or in peroxisomes. The essential role of peroxisomes in β-oxidation is the breakdown of very long chain fatty acids (VLCFA). [34] Another unique task of peroxisomes in human metabolism is the α-oxidation of VLCFA. If the reaction site for β-oxidation is blocked by a methyl group, the metabolite can be oxidized by three specific enzymes, localized in peroxisomes only.[35] All these fatty acids chains with aliphatic tails longer than 22 carbon residues are exclusively oxidized in peroxisomes (figure 4).[36] After activation with CoA by the acetyl-CoA synthetases, the VLCFAs are imported by ATP-binding cassette transporters (ABC transporters).[37] The products of the peroxisomal β-oxidation, acetyl-CoA and octanoyl-CoA are exported back into the cytosol.[38]

The role of peroxisomes in cholesterol biosynthesis is highly controversial. It is suggested that an interplay between the ER, mitochondria and peroxisomes results in the production of cholesterol, in which every step may take place at more than just one

location. Only one single conversion of mevalonate to farnesyl diphosphate has been assigned predominantly to the peroxisome (figure 5).[39] Whether peroxisomes are indispensable in the cholesterol pathway is still uncertain.

However, numerous steps of the bile acid pathway are peroxisomal located. Bile acids are the main product of cholesterol degradation.[40] After formation, bile acids are conjugated to glycine or taurine in hepatocytes and stored in the gal bladder to promote processing of dietary fat when secreted.[41]
Another function of peroxisomes that has been proven to be essential in human physiology is the synthesis of plasmalogens. Plasmalogens are phospholipids, characterized by a vinyl ether group at the sn1 position and an ether at the first carbon of the glycerol.[42] Plasmalogens are the main component of myelin sheats, the electric insulating material surrounding axonos of a neuron.[43] Important enzymes for the plasmalogen synthesis have also been found in the ER, but the importance of the peroxisome in this pathway is unquestioned.[44]

Peroxin	Phenotypes	Ghost peroxisomes
Pex1	ZS, NALD, IRD	+
Pex2	ZS, IRD	+
Pex3	ZS	-
Pex5	ZS, NALD, IRD	+
Pex6	ZS, NALD	+
Pex7	RCPD	+
Pex10	ZS, NALD	+
Pex12	ZS, NALD, IRD	+
Pex13	ZS, NALD	+
Pex14	ZS	+
Pex16	ZS	-
Pex19	ZS	-
Pex26	ZS, NALD, IRD	+

Table 1: List of peroxins involved in peroxisomal biogenesis disorders with their corresponding clinical phenotype, including the presence of ghost peroxisomes. ZS = Zellweger syndrome; NALD = neonatal adrenoleukodystrophy; IRD = infantile Refsum disease; RCDP = rhizomelic chondrodysplasia punctata.

Peroxisomal Diseases

Peroxisomal diseases can be divided into three main groups. The first group are the peroxisomal biogenesis disorders (PBD). This disorder is caused by dysfunction of one or more peroxins (Pex genes), leading to the disturbance of the entire peroxisome.[45] Secondly, there are single enzyme/ transporter deficiencies (SED). The lack or dysfunction of a single peroxisomal protein causes a blockade of a sole step in a metabolic pathway leading to the accumulation or absence of a certain metabolite. Contra-intuitively, such a dysfunction of only one enzyme can be even severe than a PBD (see van Veldhoven PP 2012 for review).[46] The third group of the classification of peroxisomal disorders, X-linked adrenoleukodystrophy, has only one known member so far. It is caused by a contiguous deletion of the ABCD1 gene and its upstream gene, DXS1357E (see Corzo D et al 2002 for detailed description).[47]

Peroxisomal biogenesis disorders

The majority of peroxisomal biogenesis disorders is caused by a heterogeneous nonsense point mutation in one single peroxin. By now, 13 peroxins are known to cause PDBs (Table 1).[48] PDBs are further categorized into four clinical phenotypes: The Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), all three representing the continuum of the Zellweger Syndrome spectrum (ZSS). The fourth PBD categoryis called rhizomelic chondrodysplasia punctata (RCDP).

The Zellweger syndrome, also called cerebro-hepato-renal syndrome, is associated with all 13 known peroxins, except for Pex7. Patients with ZS are lacking functional peroxisomes in brain, liver and kidney cells, which leads to the accumulation of VLCFA and bile acid metabolites, as well as a decreased level of plasmalogens in blood.[49] [50] It is the most severe form of peroxisomal biogenesis disorders with numerous serious symptoms: jaundice (because of liver dysfunction), ventricular enlargement in the brain, abnormal stippling of multiple joints, severe hypotonia, facial dysmorphism and psychomotor retardation. Patients usually do not survive their first year of life.[51]

Neonatal adrenoleukodystrophy (NALD) is a clinically less severe form of PDB, because of a certain degree of peroxisomal functionality left (ghost peroxisomes). Patients generally die during young childhood.[52] There is no chondodysplasia in NALD and the facial dysmorphism is much less than in ZS, but the etiopathology of the disease is inexorably progressive. Patients suffer from severe epileptic seizures, probably because of an increasing demyelination and cortical atrophy in the brain.[53]

Infantile Refsum disease (IRD) is the least severe form of the Zellweger syndrome spectrum PDBs. Even all the dysfunctional genes causing IRD can also cause ZS, the clinical phenotype is very different. The symptoms are loss of hearing, little psychomotor retardation and retinal degeneration. Patients normally die in their mid twenties.[54]

Dysfunction of Pex7, the cytosolic receptor of the PTS2 signal, does not influence the formation or functioning of the entire peroxisome, but only the import of PTS2 tagged peroxisomal matrix proteins. Therefore, rhizomelic chondrodysplasia punctata (RCDP), caused by a mutation in Pex7, differs from the other twelve known peroxin dysfunctions of the Zellweger spectrum.[55] Since there are only a very few peroxisomal matrix proteins with a PTS2 signal peptide, the number of

metabolic routes affected is limited. [56]Nevertheless the damage to the human metabolism is not negligible. Two pathways being affected are the plasmalogen synthesis, leading to a lower plasmalogen blood level, and the α-oxidation of branched fatty acids such as phytanic acid, leading to accumulation of this metabolite.[57] In contrast, the oxidation of VLCFA is not disrupted. The diagnose is made by measuring the blood concentration of these three metabolites: plasmalogens (decreased), phytanic acid (increased) and VLCFA (not affected).[58] The aftermaths of this dysfunctions are calcific stippling and shortening of proximal bones, severe growth and mental retardation and facial dysmorphism, leading to death usually within the first two years of life.[59]

Recent findings and unanswered questions

During the last few years, numerous new mutations of all PBD subcategories have been identified.[60] Although hundreds of different mutations and their individual phenotypes have been described, a general genotype-phenotype correlation remains difficult.[61]

Very recently another peroxin has been found to cause disease in humans, Pex11β.[62] It is the first gene of the *pex11* family known to cause a peroxisomal biogenesis disorder, even the symptoms are quite untypical. Hereby, it is proven, that a dysfunction of any of the 13 peroxins in human causes PBD. The patient, a 26-year old Dutchman, suffered from some symptoms typical for mitochondrial or peroxisomal dysfunction, such as hearing loss, sensory nerve involvement and recurring migraine-like attacks, but all biochemical indicator parameters were within the normal range. All known genes that could cause the mild ZSS symptoms have been checked with negative results. After sequencing of all

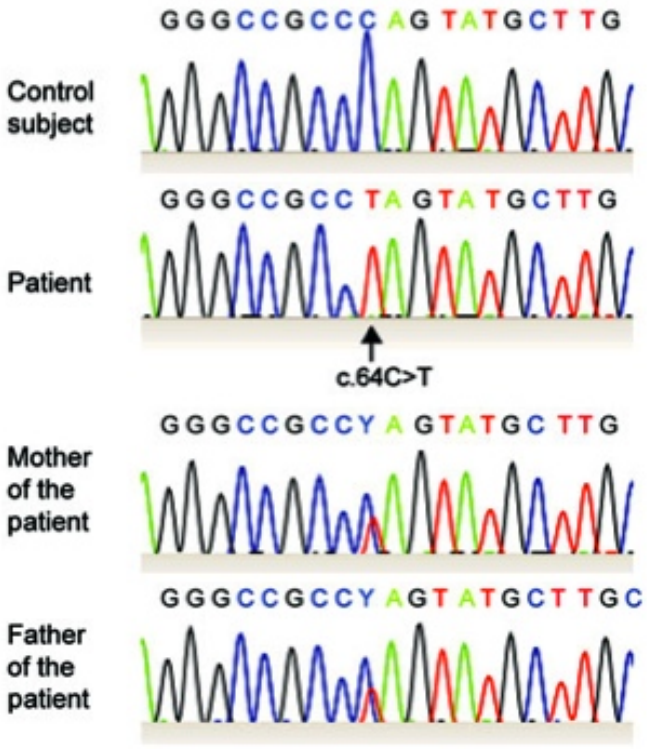

Figure 6: Sequence analysis of the coding region of the *pex11β* gene of the patient identified a homozygous mutation, c.64C>T (A), which changes the glutamine located at amino acid position 22 into a non-sense mutation (p.Q22X).

three *pex11* family members, *Pex11α*, *β* and *γ*, a nonsense mutation of *Pex11β* has been found (figure 6). An Pex11β immunoblot analysis has indicated the complete absence of this peroxin. Microscopic analysis of patient's fibroblast showed elongated and enlarged peroxisomes (figure 7), which has been seen in several yeast *pex11 deletion* strains.[63 64]

These findings are remarkable, since the mouse model of an Pex11β dysfunction, a *pex11β deletion* mouse, showed severe ZS syndromes and neonatal lethality.[65] Interestingly, the *pex11β* mouse was also only mildly affected in peroxisomal metabolism.[66] It remains uncertain how these significant differences in seriousness of the symptoms can be explained. More knowledge about the detailed molecular mechanisms and consequential damages is required to fully understand the pathophysiology of this dysfunction.

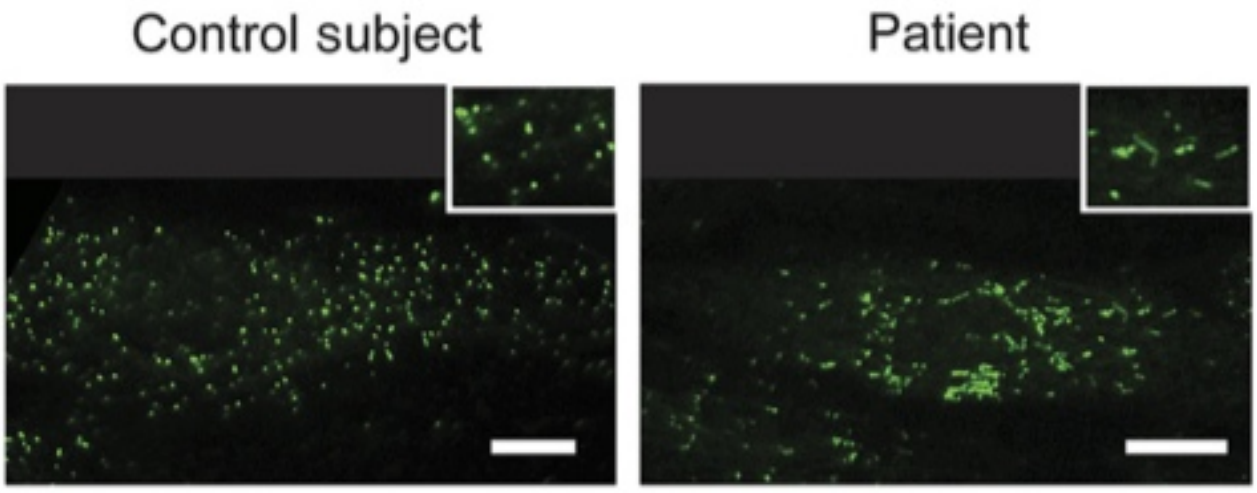

Figure 7: Microscopical analysis of cultured primary skin fibroblasts. Immunofluorescence microscopical analysis of fibroblasts using a specific antiserum against peroxisomal catalase shows elongated and enlarged peroxisomes in the patient's fibroblasts when compared with the typical punctuated appearance in control skin fibroblasts (A). Blowups are a 1.6 times enlargement of the original picture.

Another recent major achievement in the field of peroxisomal biogenesis disorders is the systematical genetic classification and mutation spectrum of hundreds of ZSS patients (see Ebberink MS *et al.* 2010 for review). Human fibroblast cell lines of more than 600 ZSS patients are available for genetic and biochemical analysis. Such a large quantity of data can be helpful for statistical approaches to discover new genotype-phenotype correlations. However, it has been concluded wrongly, that all possible peroxins causing PBDs are currently known, because there were no additional peroxin mutations found among the 613 ZSS patient‘s cell lines. Since the *pex11β* mutation was discovered a few month ago, this statement needs to be updated.

The biggest question about peroxisomal biogenesis disorders is relating to the treatment of symptoms and the disease itself. By now, there is only little that can be done to reduce symptoms.[67]

Discussion

The knowledge about peroxisomes in general and peroxisomal biogenesis disorders in particular has increased immensely. Novel mutations causing PDBs are found regularly, further increasing our understanding of the molecular malfunctioning.

The organization and categorization of all so far known mutations causing PBDs is a powerful tool to effectively categorize and distinguish new genotypes as well as their clinical phenotypes. It will help to diagnose already described mutations and novel ones, making it much easier to identify similar symptoms as well as untypical phenotypes in PBDs.

The novel Pex11β defect widens the spectrum of peroxisomal biogenesis disorders and adds a new clinical phenotype to the Zellweger syndrome spectrum, which was undetectable with the standard diagnostic procedure. Now this new phenotype and the way to

diagnose it has been described, it is very likely that more patients with a *pex11* family dysfunction can be identified. This could help to gain further insights of this medical condition.

Still, in most cases early diagnosis is the only way to alleviate, when possible, disease pain. Prenatal testing for patients with family history is possible via cells from amniotic fluid or chorionic villi, whereas postnatal screening is carried out by blood analysis.[68] The only way to guarantee the health of an unborn child up to now is by preimplantation genetic diagnosis (PGD).[69] Unfortunately, obligate carriers do not express any partial symptoms and thus unaware couples usually do not make use of prenatal counseling. In my opinion PGD would be the most effective way to prevent PBDs and I am positive it will become a common procedure due to the increasing clinical use of next-generation sequencing techniques.[70] Nevertheless, any effort invested into understanding the underlying principles of peroxisomal biogenesis disorders is worth it in order to increase our knowledge about the functioning and malfunctioning of this organelle.

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