Syndrome of the month

Zellweger syndrome and associated phenotypes

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Abstract

Until recently, the peroxisome was considered a "reactor chamber" for H_2O_2 producing oxidases, and it is now recognised as a versatile organelle performing complex catabolic and biosynthetic roles in the cell. Zellweger syndrome (ZS), the paradigm of human peroxisomal disorders, is characterised by neonatal hypotonia, severe neurodevelopmental delay, hepatomegaly, renal cysts, senorineural deafness, retinal dysfunction, and facial dysmorphism. It is now clear that ZS is at the severe end of a phenotypic spectrum of Zellweger-like syndromes which may present for diagnosis later in childhood and even in adult life. It is important that clinical geneticists are aware of these milder clinical variants as the availability of sensitive and specific biochemical assays of peroxisomal function (for example, serum VLCFA ratios, platelet DHAP-AT activity) makes their diagnosis relatively straightforward.

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Peroxisomes

In July 1995 an international symposium in Aspen marked the 30th anniversary of the naming and biochemical characterisation of peroxisomes by Baudhuin *et al*[1] as catalase containing microbodies on cell fractionation. These membrane bound metabolic compartments were probably acquired endosymbiotically[2] and are found throughout the eukaryotic kingdom. This review will concentrate on recent clinical, biochemical, and molecular developments in human disorders of assembly and function of this organelle.

Peroxisomes are roughly spherical organelles bound by a single lipid bilayer with a diameter of 0.1–1 μm. The exact nature and origin of the phospholipids which make up the peroxisomal membrane is not known although they are likely to be derived from the endoplasmic reticulum. The protein component of peroxisomes (integral membrane, membrane associated and matrix proteins) are translated on free polyribosomes and imported post–translationally[3] and stably folded[4] via specific peptide peroxisomal targeting sequences (PTS) (for review see Subramani[5]). Most matrix proteins appear to be imported using a C-terminal tripeptide sequence (-SKL) named PTS I.[6] PTS II is an N-terminal sequence (MHRLQVVLGHL-) found in mammalian peroxisomal 3-oxoacyl-CoA thiolase which, unlike PTS I, undergoes protease mediated cleavage after import.[7] It is likely that several other PTSs have yet to be identified.

The enzymatic abilities of human peroxisomes can be divided into five broad and overlapping categories: (1) simple oxidases (for example, D-amino acid oxidase, polyamine oxidase) producing heat and H_2O_2 which is decomposed by catalase; (2) β-oxidation cycles for degradation of very long chain fatty acids (VLCFA), pristanic acid, and bile acid intermediates; (3) the glyoxalate cycle which catalyses the conversion of acetyl-CoA to succinate (this is of uncertain significance in humans); (4) ether lipid synthesis pathway; (5) cholesterol and dolichol biosynthesis. These pathways have been comprehensively reviewed by Van den Bosch *et al*[8] and will be discussed in detail only as they relate to biochemical tests of peroxisomal function. An additional remarkable feature of peroxisomes is the induction of proliferation that occurs in response to exogenous agents (named peroxisome proliferators), such as the hypolipidaemic agent clofibrate in rodent hepatocytes[9] and methanol or oleic acid in yeast species.[10]

Clinical phenotypes

Zellweger (or cerebrohepatorenal) syndrome (ZS) was originally described as a lethal, multiple malformation syndrome of infancy.[11 12] The first indication that peroxisomes may be involved in human disease came in 1973 when Goldfischer *et al*[13] noted their apparent absence (combined with abnormalities in mitochondrial function) in the liver and kidney of a child with a clinical diagnosis of ZS. Since that report it has become clear that genetic mutations causing either a generalised disorder of peroxisomal function (that is, interrupting peroxisome assembly) or single matrix enzyme deficiencies can cause a similar spectrum of abnormalities.[14] In addition to ZS several different names have been used to describe these disorders, (neonatal adrenoleucodystrophy (NALD), infantile Refsum disease (IRD), hyperpipracolic acidaemia (HPA)); however, these generally denote differences in severity of the clinical phenotype (ZS > NALD > IRD (HPA is now obsolete))

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Table 1 Disorders of peroxisomal function excluding ZSAP

Disease	*Gene*	*Locus*	*Lab findings*	*Phenotype*
Acatalasaemia (MIM 115500)	Catalase	11p13	Reduced H_2O_2 decomposition in red blood cell	Chronic oral ulceration, often asymptomatic
Adult Refsum disease (ARD)(MIM 266500)	Phytanic acid α-oxidase	?	High serum phytanic acid	Retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia
ARD with hyperpipracolic acidaemia[61] (no MIM number as yet)	?	10q	High serum phytanic acid and pipecolic acid	Same as ARD
Glutaric aciduria type III (MIM 231690)	Glutaryl CoA oxidase	?	Riboflavin responsive glutaric aciduria	Single case, failure to thrive
Hyperoxaluria type I (MIM 259900)	AGT	2q36-37	High serum and urinary oxalate	Urolithiasis, nephrocalcinosis, systemic oxalosis
Rhizomelic chondrodysplasia punctata (MIM 215100)	PTS II receptor or DHAP-AT	?	Reduced plasmalogen synth +/− cytoplasmic localised thiolase	Severe rhizomelia, cataracts, early lethality
Adrenoleucodystrophy (MIM 300100)	ALDP	Xq28	High VLCFA	Inflammatory adreno- and neurodegeneration

rather than differences in organ involvement, biochemical phenotype, or pathogenesis.

Pseudo-Zellweger syndrome (PZS) was originally used to describe a unique case with clinical features of ZS, structurally normal peroxisomes, and peroxisomal 3-oxoacyl CoA thiolase deficiency.[15 16] It is now clear that most often PZS is caused by acyl-CoA oxidase[17] or trifunctional enzyme[18] deficiency. For ease of discussion, all of the above disorders will be grouped under the heading of Zellweger Syndrome and Associated Phenotypes (ZSAP) in this review. The combined birth prevalence of these disorders is thought to be between 1: 25 000 and 1:50 000 live births.[19]

Rhizomelic chondrodysplasia punctata (RCDP), a peroxisomal disorder genetically and biochemically distinct from ZSAP, is characterised by severe proximal limb shortening, early onset symmetrical cataracts, retinal dysfunction, facial dysmorphism, ichthyosis, and early lethality.[20] The biochemical hallmarks of RCDP are disordered PTS II mediated protein import (that is, cytoplasmic localisation of thiolase[21]) or deficiency of plasmalogen biosynthesis[22] with normal localisation of PTS I proteins, or both. There is significant phenotypic overlap between RCDP and ZSAP suggesting that plasmalogens may have a critical role in, at least, lens development, retinal function, and the synchrony of normal ossification. Since RCDP has been the covered in a recent Syndrome of the month[23] it will not be considered in detail. Table 1 summarises the features of RCDP and several other disorders of peroxisomal function which result in clinical phenotypes distinct from ZSAP.

Clinical phenotype in ZSAP

NEURODEVELOPMENTAL DISORDER AND DYSMORPHISM

To date, global developmental delay has been a feature of all cases of ZSAP with many showing profound congenital hypotonia and no psychomotor development whatsoever. The basis of this severe cerebral dysfunction appears to be the premature arrest of migrating neuroblasts during development, resulting in site specific cerebral micro- and pachygyria with neuronal heterotopia (fig 1, top).[24] The cortical regions showing the most severe abnormalities are the perisylvian and frontoparietal areas.[25] Dysmyelination has been reported but this feature appears to be variable and poorly dis-

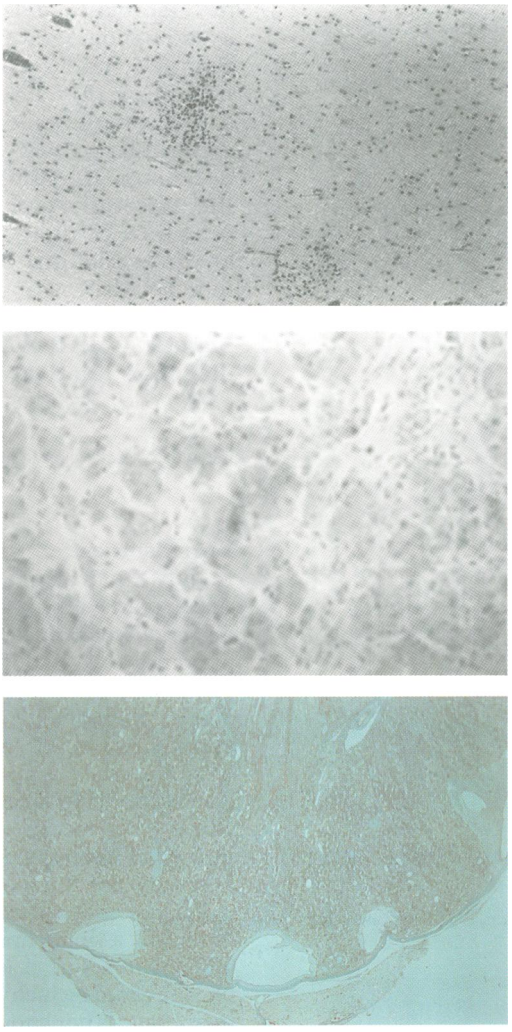

Figure 1 (Top) The subcortical cerebral white matter in a 6 week old male case of ZSAP showing two irregular collections of ectopic neurones. Haematoxylin and eosin (H&E). (Middle) A high powered H&E section from postmortem liver biopsy in a 3 month old child with ZSAP showing characteristic giant cell formation and marked hepatic fibrosis. (Bottom) A low powered H&E section from a postmortem renal biopsy of the same child as in the top photograph showing subcapsular cortical cyst formation.

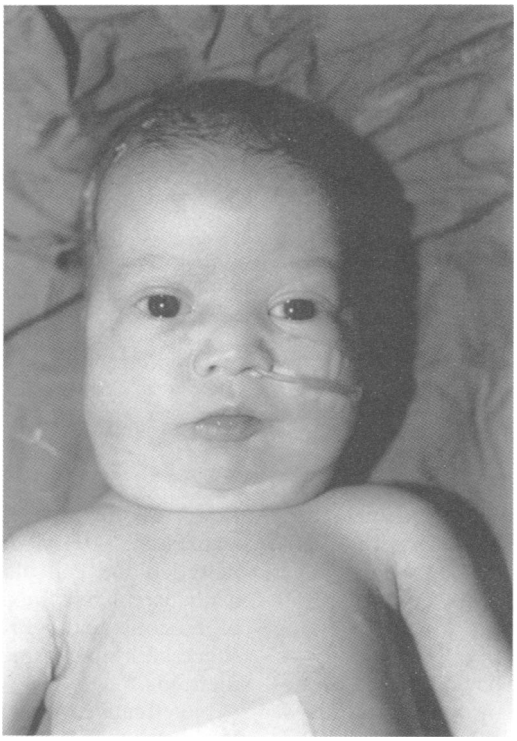

Figure 2 A clinical photograph of Lauren at 3 weeks showing hypoplastic supraorbital ridges and a low and broad nasal bridge, which constitute the typical craniofacial features of ZSAP. (Photograph reproduced with permission.)

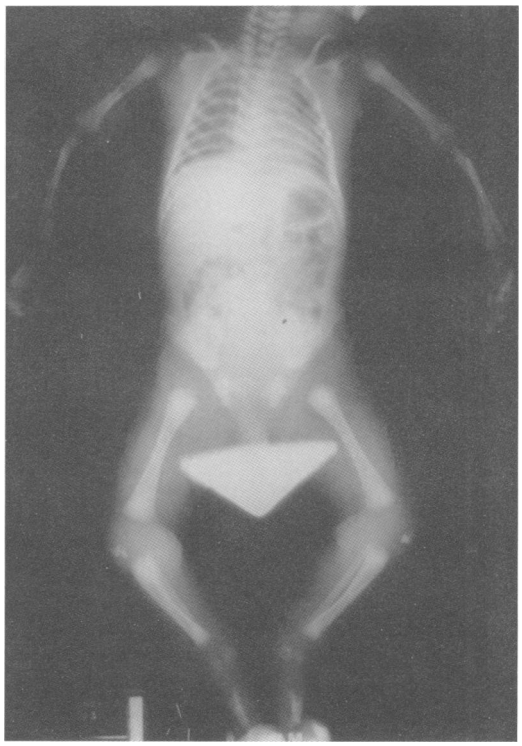

Figure 3 Whole body x ray of a male infant with ZSAP showing calcific stippling of the patellae bilaterally.

criminant.[26] There are very few published reports of detailed neuroimaging in ZSAP. These would, obviously, be useful data to determine if a specific cortical phenotype could be detected in living subjects. The craniofacial features of ZS are striking and memorable (fig 2). These are characterised by marked paucity of facial movement with a large anterior fontanelle, prominent forehead, hypoplastic supraorbital ridges, and broad nasal root.

HEPATO-ADRENO-RENAL PHENOTYPE

Hepatomegaly is seen in ~80% of infants with ZSAP associated with raised levels of liver enzymes and bilirubin in the serum. Liver biopsy may show a micronodular cirrhosis and giant cell formation with or without hepatic fibrosis (fig 1, middle).[27] Prenatal onset renal cortical cysts of variable size are seen in ~70% of cases (fig 1, bottom).[28] Many of the cases also have adrenal hypoplasia with striated reticularis cells very similar to those seen in adrenoleucodystrophy.[29]

SENSORY ORGANS

Around 90% of ZSAP children will have congenital sensorineural hearing impairment.[30] Ocular findings include abnormal electroretinogram (ERG) (~85%), cataracts (~70%), peripheral pigmentary retinopathy (~40%), and optic nerve hypoplasia (~40%).[31]

OTHER FEATURES

The main radiological finding in ZSAP is calcific stippling of the patellae (fig 3) with synchondrosis of the acetabulum.[32] Of particular interest is the association of thymic aplasia and congenital outflow tract anomalies of the heart,[33] suggesting some phenotypic overlap with DiGeorge syndrome (DGS). No peroxisomal genes are known to map in the DGS critical region although the peroxisome proliferator activated receptor (PPARa) gene has been localised to chromosome 22q12-13.[34]

INHERITANCE AND VARIABILITY

Numerous sib pairs[35,36] and examples of parental consanguinity have been reported in ZSAP and autosomal recessive inheritance is assumed in most cases. There have, however, been two reports of ZSAP with karyotype abnormalities involving 7q11.23.[37,38] It is not clear if these represent examples of haploinsufficiency or unmasking a recessive mutation.

It should be noted that although most cases of ZSAP are lethal in early childhood there are now several reports of affected subjects surviving into late childhood and adulthood.[30,38,39] In these subjects the neurodevelopmental and craniofacial phenotypes tend to be less distinctive[30,38] and the diagnosis should be considered in any developmentally delayed child with sensorineural deafness, retinal dysfunction, or hepatomegaly.[30] Facial features such as low/broad nasal bridge, large anterior fontanelle, and shallow orbital ridges also appear to be relatively discriminant. It is important that we consider ZSAP in the differential diagnosis of such cases, as relatively straightforward, inexpensive biochemical diag-

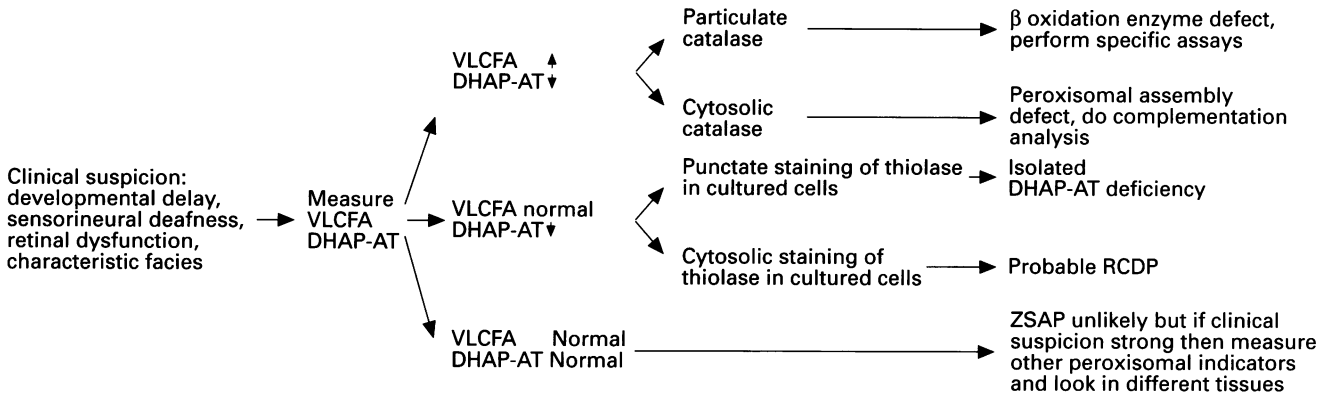

Figure 4 Clinical algorithm for the diagnosis and investigation of a child suspected of having ZSAP.

nostic tests are available. A simple clinical diagnostic algorithm is presented in fig 4 and discussed below.

DIAGNOSIS AND TREATMENT

All patients suspected of having ZSAP on neurodevelopmental or dysmorphic grounds or both should have clinical photographs, ERG, brainstem auditory evoked potentials (BAER), and skeletal survey performed as first line investigations (fig 4). Histological examination of biopsied material can be used to identify peroxisomes using either electron microscopy combined with diaminobenzene cytochemical staining[40] or immunofluorescence. However, with the development of simple biochemical tests of peroxisome assembly, such as the particulate catalase assay[41] (this indicates if catalase activity is organellar or cytosolic after centrifugation of permeabilised cells), morphological diagnosis has become less important.

The mainstay of biochemical diagnosis of ZSAP, however, is the measurement of saturated VLCFA.[42] VLCFA have a chain length of 22 carbon atoms or greater and are derived from both dietary sources and chain elongation processes (predominantly microsomal) within the cell. Mitochondria are able to metabolise VLCFA but the flux through this pathway is considerably less than peroxisomal β-oxidation. Several different methods for the measurement of VLCFA in plasma, red blood cells, leucocytes, fibroblasts, and tissue specimens have been described.[43] Normal levels of C26:0 in plasma are ~0.33 μg/ml with levels in ZSAP more than five times this level with markedly raised C24:0/C22:0 and C26:0/C22:0 ratios. Plasmalogen biosynthesis can be assessed by assay of dihydroxyacetone phosphate acyl transferase (DHAP-AT) activity or by analysis of red blood cell plasmalogens.[43] Other clinically useful assays exist for phytanic acid[44] and piperocolic acid.[45] Prenatal diagnosis has been successfully performed using several of these methods.[46]

No effective treatment is available for ZSAP. Recently, partial biochemical normalisation of the VLCFA profiles in patients with ZSAP has been achieved using dietary supplementation with glyceral trioleate (GTO is thought to inhibit microsomal chain elongation systems)[47] or ether lipids[48] with no apparent alteration in the clinical course of the disease. There is one interesting report of clinical improvement in a 6 year old boy with ZSAP after administration of oral docosohexaenoic acid (DHA).[49] The rationale for this therapy is the importance of this fatty acid in neuronal and photoreceptor membranes and the severe deficiency of DHA in ZSAP. Control trials of these therapies are currently under way.

Table 2 Genes associated with the ZSAP phenotype

Gene	*Locus*	*Structure and function*	*Complement*[n] *Gp*
PAF1	8q21.1	35 kDa, cysteine rich RING-finger	10
PAF2	?	ATPase (AAA proteins)	?4
PXR1	?	PTS import receptor	2
PMP70	1	ATP binding cassette, 6 transmembrane domains	?1
ACOX	17q25	Acyl CoA oxidase	OXIDASE*
ECH/HACD	3q26–28	Peroxisomal β-oxidation trifunctional enzyme	BIFUNCTIONAL 1 & 2*
OACT	3p23	Peroxisomal 3-oxyacyl-CoA thiolase	?

From McGuiness *et al.*[55]

GENETIC PATHOLOGY AND MODEL SYSTEMS

Complementation assays using patient fibroblast cell lines suggest that there are at least 10 (probably more[50]) different human genes involved in peroxisome assembly. The genes defining two of these complementation groups (CG) have been unequivocally identified (PAF1[CG4][51] and PXR1[CG2][52]) and one other gene (PXMP1) is mutated in one allele of two patients from CG1.[53] In ZSAP patients with isolated disorders of peroxisomal β-oxidation there are at least four complementation groups owing to loss of function mutations in the genes encoding acyl CoA oxidase, 3-oxoacyl-thiolase, and the enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase domains of the peroxisomal trifunctional enzyme.[54] Details of all these genes are given in table 2.

Studies of peroxisomal assembly mutants in lower eukaryotic organisms such as *Saccharomyces cerevisiae*[55] and *Pichia pastoris*[56] have enabled cloning of many genes involved in organelle biogenesis. Indeed this work led directly to the identification of one of the disease causing human genes mentioned above (PXR1) by sequence homology searching of the public database of expressed sequence tags

from a fetal brain cDNA library. Although mammalian model systems have been more difficult to generate, cDNA complementation of chinese hamster ovary (CHO) cell peroxisomal mutants has been successful in identifying the PAF1 gene and has recently enabled cloning of another gene involved in peroxisome assembly, PAF2.[57] Other approaches to cloning mammalian peroxisomal genes have involved making subtracted libraries from cells induced with peroxisomal proliferators,[58] developing new mutant selection and complementation assays,[59] and cDNA cloning using antibodies raised against proteins purified from induced peroxisomes.[60 61]

Conclusions

ZSAP are fascinating disorders from many points of view. They were among the first dysmorphic syndromes subsequently shown to result from an inborn error of metabolism and are currently the only human diseases caused by agenesis of an intracellular organelle. Advances in our understanding of ZSAP have been the result of international collaborations between paediatric neurologists, clinical biochemists, and human and yeast geneticists, and the quest for a full understanding of the biology of peroxisomes continues apace. Continued cloning of genes involved in human peroxisomal disease combined with the knock out technology in mice will elucidate the role that these organelles play in development, particularly of the nervous system, and enable us to assess the effect of different treatment strategies. Clinical geneticists will play important roles in further clinical delineation of ZSAP and working towards a sensible and effective therapeutic plan for affected subjects.

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