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SHORT REPORT

A new defect of peroxisomal function involving pristanic acid: a case report

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AN adult onset novel disorder of peroxisomal function is described, characterised by retinitis pigmentosa resulting in progressive visual failure, learning difficulties, a peripheral neuropathy, and hypogonadism. The defect results in accumulation of pristanic acid, and the bile acid intermediates, dihydroxycholestanoic and trihydroxycholestanoic acid, and is due to a deficiency of α-methylacyl-CoA racemase, making this the first fully characterised description of this defect. Screening of patients with retinitis pigmentosa should be extended to include pristanic acid and/or bile acid intermediate concentrations, as dietary measures offer a potential treatment for the disorder.

Peroxisomes are subcellular organelles found in all mammalian cell types, and are particularly abundant in oligodendrocytes with 40 times the amounts of neurons or astrocytes,[1] and in cells specialising in lipid metabolism. Their main function is H_2O_2 metabolism, ether-phospholipid biosynthesis, β-oxidation of fatty acids and other compounds (very long chain fatty acids (VLCFAs)), monounsaturated and polyunsaturated fatty acids, prostaglandins, dihydroxycholestanoic acid/trihydroxycholestanoic acid (DHCA/THCA), xenobiotics), glyoxylate metabolism, polyamine catabolism, cholesterol and dolichol synthesis, and pipecolic and phytanic acid degradation.[2]

Pristanic acid is derived from phytanic acid by α-oxidation, followed by decarboxylation, and also directly from exogenous dietary sources. Phytanic acid is derived from purely exogenous sources, mainly dairy products and ruminant fats.[3]

Peroxisomes contain more than 60 enzymes so disorders of peroxisomal function result in several syndromes combining neurological and systemic features.[4] Until 1999, 17 disorders had been described,[5] 16 with neurological involvement.

The first well defined disorder described in 1946 was a hereditary ataxia, later to be given the eponym Refsum's disease,[6] combining retinitis pigmentosa and a hypertrophic neuropathy associated with increased phytanic acid. It was, however, only in 1997 that the true peroxisomal localisation of the disorder was confirmed, with the enzyme phytanoyl-coenzyme A hydroxylase identified[7] and the gene cloned and located on chromosome 10p,[8 9] thus settling the controversy regarding a mitochondrial origin.[3 10] Until now the first "pure" peroxisomal disorder was considered to be Zellweger's syndrome of cerebrohepatorenal failure, described by Bowen *et al* in 1964.[11]

The incidence of peroxisomal disorders is in excess of 1:20 000, with X linked adrenoleukodystrophy being the most frequent at 1:50 000.[12] Peroxisomal disorders can be classified either according to loss of function being generalised (group 1), multiple (group 2), or single (group 3), or into two groups with the disorders of peroxisome biogenesis in one, and the single peroxisomal enzyme deficiencies in the other.[2 4 13–15]

In general, peroxisomal disorders present either at birth with deficits resulting in severe hypotonia and craniofacial dysmorphism or as later onset psychomotor retardation, seizures, and hepatomegaly. There is, however, a considerable range of clinical problems within a disorder and overlap between disorders, so that some can only be differentiated on biochemical grounds.

We present a case of adult onset neurological disease the features of which were reminiscent of a peroxisomal disorder, but in a novel combination.

The biochemical defect has been recently elucidated, and has been shown to be due to a deficiency of α-methylacyl-CoA racemase (AMACR) making our patient one of the first adults to be described with this condition.[16]

CASE REPORT

A 44 year old man presented with failing vision, having been suspected by his general practitioner of malingering.

He was born of non-consanguineous parents, one of six children, his brother and four sisters being in good health. He had left school at the age of 14. He had been a poor scholar with reading difficulties and after leaving had a succession of unskilled jobs from which he was invariably dismissed. At the age of 18 he presented with an encephalitic illness characterised by 3 days of severe headache, nausea, and photophobia, with a single blackout followed by progressive confusion, irrational behaviour, and resulting in coma.

He developed focal seizures, with eye deviation to the left and jerking of the neck muscles, which on one occasion generalised. He had tonic deviation of his eyes to the right, sometimes with slow deviation, bilateral papilloedema, but no other focal neurological signs. He had a mild pyrexia and a neutrophil leucocytosis (13×10^9/l). He underwent cerebral angiography and CSF analysis, including protein estimation, both of which were normal. An EEG showed gross disturbances with generalised slowing and loss of α activity. He required ventilation, but then underwent spontaneous recovery, whereupon he was found to be blind. This was initially suspected to be due to occipital lobe infarction, but pigmentary retinal changes were seen extensively in the periphery of both fundi, and a neuroretinitis was proposed. His EEG improved, but did not return to normal, remaining slowed. Vision slowly recovered, initially with perception of light, then colours, and finally acuity, achieving a distance acuity of 6/12 bilaterally and a reading acuity of N24 right N18 left. A neuropsychometric assessment showed a premorbid verbal IQ of 86, a reading age of 7.5 years, and a long term problem with vision was suspected.

At the age of 22 he developed generalised seizures, only partially controlled with phenobarbital and phenytoin. An

Abbreviations: VLCFA, very long chain fatty acids; DHCA/THCA, dihydroxycholestanoic/trihydroxycholestanoic acid; AMACR, α-methylacyl-CoA racemase

Table 1 Results of fatty acid and bile acid analysis from serum

	Value found	Normal
VCLFA profile:		
C26 (μmol/l)	0.53	(0.33–1.39)
C26/C22	0.008	(<0.030)
C24//C22	0.58	(0.32–0.92)
Phytanic acid (μmol/l)	20	(<12.8)
Pristanic acid (μmol/l)	105	(<3.0)
Pristanic/phytanic	5.25	(0.05–0.40)
Bile acid profile (μmol/l):		
Deoxycholic acid	0.02	(<4.4)
Chenodeoxycholic acid	0.22	(0.22–12.4)
Cholic acid	0.44	(0.05–4.60)
Ursodeoxycholic acid	0.00	(<2.1)
Hyocholic acid	0.00	(<1.0)
Dihydroxycholestanoic acid	0.11	(not detectable)
Trihydroxycholestanoic acid	2.90	(not detectable)
Dihydroxycholestenoic acid	0.00	(not detectable)
C29 dicarboxylic acid	0.01	(not detectable)

EEG showed excessive slowing with a right temporal focus and a photoconvulsive response.

At the age of 25 he had an episode of status epilepticus, by which time his vision had declined to 6/36 right and 6/60 left uncorrected.

At the age of 34 he was involved in a road traffic accident and sustained a small right frontal extradural haematoma with confusion, not requiring surgery. After this he developed drop attacks and frequent headaches.

At the age of 41 he became aware of declining vision, and was found to have VA 1/18 L+R, constricted fields, and a generalised "retinopathy".

When he presented at the age of 44, he was complaining of migrainous headaches daily from his accident, and a recent episode of amnesia with automatic behaviour. He had not been employed since his encephalopathy.

There was a family history of ischaemic heart disease, his father dying aged 67 of a heart attack. He was single without children, and taking only phenytoin and phenobarbital. There was a previous episode of depression with an overdose, and he was known to have a low renal glucose threshold.

His examination showed bilateral gynaecomastia with sparse body hair and a feminine habitus. Both testes were atrophied. He had micrognathia with a high arched palate, but no other dysmorphic features. He had posterior subcapsular lens opacities, with extensive retinal pigmentation, optic disc atrophy, and vascular attenuation all typical of retinitis pigmentosa. There was marked peripheral field loss and distance VA 1/24 right, 1/18 left, N36 L+R reading. There were afferent pupillary defects the remaining cranial nerves were normal. He had depressed reflexes generally with impaired pin prick and temperature below the knee but with flexor plantars, and no other limb signs, or cerebellar and extrapyramidal features.

RESULTS

Full blood count, differential count, urea, electrolytes, liver and bone profiles, thyroid function, glucose, B12 and folate, VDRL, TPHA, protein electrophoresis, and immunoglobulin concentrations were all normal or negative. Cholesterol was borderline low at 3.4 nmol/l (normal 3.5 – 5.2).

Testosterone was 3.2 nmol/l (normal 9–38), estradiol <30 pmol/l (normal 40–185), FSH 34.1 IU/l (normal 1.1–7.8), and LH 10.8 IU/l (1.1–9.4) confirming primary gonadal failure. Prolactin was normal.

Chromosomal analysis showed a normal male karyotype. Mitochondrial DNA analysis was negative for MELAS 3243, 3271, 8344 deletions.

The EEG showed occipital slowing with no seizure activity or photoconvulsive response. Visually evoked potentials were abnormal bilaterally with low amplitude disrupted wave forms. Brain stem auditory evoked potentials were normal with N1-N5 latencies of 3.3 ms left, 3.96 ms right. Brain MRI showed minor cerebral atrophy only with normal white matter. Chest radiography was normal. Nerve conduction studies shows a peripheral symmetric sensory motor axonal neuropathy.

Table 1 lists the results of fatty acid and bile acid analyses in serum from the patient. The results show normal very long chain fatty acids, a slightly increased phytanic acid concentration, and profoundly increased pristanic acid concentration. Furthermore, both dihydroxycholestanoic acid and trihydroxycholestanoic acid are greatly increased.

These data pointed to a defect in the β-oxidation system which was supported by the finding of a reduced pristanic acid β-oxidation capacity (table 2). Subsequent studies showed that the primary defect in this patient was not at the level of one of the β-oxidation enzymes themselves but rather in one of the auxiliary enzymes involved in β-oxidation called α-methyl-acyl -CoA-racemase.

DISCUSSION

Our patient had learning difficulties and his psychometric testing as an adult after his encephalitic illness suggested a longstanding premorbid problem with functional and language skills. Encephalitic illnesses have not been noted as a feature of peroxisomal disorders, although Goldman *et al*[17] described a patient with Refsum's disease who had an acute onset of ataxia after a viral illness—our patient was initially thought to have had a viral illness with pyrexia. Minor surgery may precipitate deterioration, so it could be postulated that oxidative stresses triggered decompensation or release of phytanic acid from fat stores as a result of catabolic stress. The association with encephalopathy we presume to be genuine, but coincidence cannot be excluded.

A comparison of his clinical features with those found in the other peroxisomal disorders shows a general similarity to those of late onset, particularly Refsum's disease and in common with the single enzyme deficits, there are mild dysmorphic features, he has a seizure disorder with a peripheral neuropathy, no ataxia, a retinopathy and hypogonadism. His brain MRI did not show white matter abnormalities, as

Table 2 Results from fibroblast studies

Patient	De novo plasmalogen biosynthesis:	Normal
	DHAP-AT activity:	Normal
	Pristanic acid β- oxidation activity*	284
	AMACR activity†	ND
Controls	Pristanic acid β- oxidation activity*	1147 (SD 325) (n=30)
	AMACR activity†	92 (SD 30) (n=11)

*pmol/h/mg protein; †pmol/min/mg protein.
ND, Not detectable; AMACR, α-methylacyl-CoA racemase.
For methods see Ferdinandusse[16].

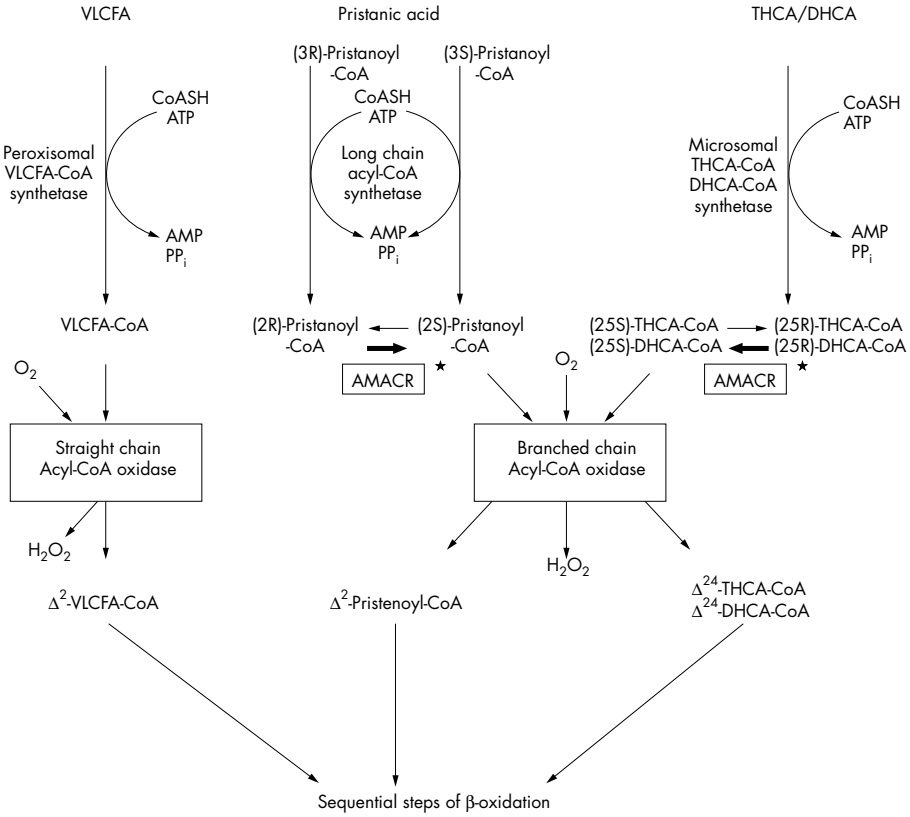

Figure 1 The peroxisomal β-oxidation pathway, showing the steps involved in the oxidation of pristanic acid and THCA/DHCA. The site of activity of AMACR, where the defect occurs in this disorder, is indicated. *

seen in adrenomyeloneuropathy, nor neuronal migration deficits as seen in a postmortem of infantile Refsum's disease.[18]

The biochemical defect causing Refsum's disease lies "upstream", yet the clinical phenotype of the disorders differ, although with considerable overlap. Why there should be this distinction is uncertain, but there may be a differential effect on tissues depending on the proportions of product accumulation (phytanic and pristanic acid and bile acid intermediates), or loss of lipid functions "downstream".

In humans, the only peroxisomal disorders of β-oxidation so far identified are those relating to VLCFAs, DHCA/THCA, and pristanic acid. Multiple enzymes are involved (fig 1) and abnormalities of pristanic acid metabolism were first associated with generalised peroxisomal disorders.[19]

This currently described patient showed highly increased pristanic acid concentrations and mildly raised phytanic acid and VLCFA concentrations. Had the pristanic acid concentrations not been measured, the condition would have been missed, as peroxisomal disorders have always been assumed to cause abnormalities of VLCFA or phytanic acid.[20]

The biochemical defect in this case has only recently been characterised as an absence of the α-methylacyl-CoA racemase.[16] There is stereoselectivity of the α-methyl branched acyl CoA esters and the bile acid intermediates, and these must be converted to their *S* forms before degradation by peroxisomal β-oxidation. Absence of the racemase has the same consequences as a deficiency of the branched chain acyl-CoA oxidase, although in the second *R* and *S* stereoisomers accumulate, and in racemase deficiency only *R* isomers accumulate. Analysis of both enzymes is required to establish the precise defect.

He therefore has a unique combination of features, distinct from the other peroxisomal disorders, but with many features in common, particularly with Refsum's disease. His disease course has been relatively benign.

Presumably this is autosomal recessive as are most of the other peroxisomal disorders, but his family have refused blood testing and skin biopsy. The presence of hypogonadism does raise the possibility of an X linked disorder, but we have been made aware of a woman with the condition (personal communication), so this seems unlikely.

Given that Refsum's disease responds to dietary elimination of phytanic acid,[3] therapy for this disorder was attempted using a pristanic acid and phytanic acid depleted diet, but he would not tolerate the dietary change. His seizures have remained controlled on phenytoin alone, and there has been no significant progression in his visual failure or neuropathy over 2 years.

As biochemical and molecular biological techniques advance, further peroxisomal disorders are likely to emerge. Recently, another novel disorder of peroxisomes has been described, with multiple enzyme deficiencies (reduced lignoceric acid oxidation, cylosol catalase only, reduced di-hydroxyacetone phosphate acyl transferase, and reduced phytanic acid oxidation) and normal peroxisomes as in type 2 disorders, but clinically a mixed type 1 and 3 (an adult with facial deformity, cognitive impairment, retinal pigmentation, seizures, and deafness without liver problems).[21]

These overlapping clinical syndromes highlight the importance of wider screening of biochemical function using plasma and fibroblasts. We recommend that any patient presenting with retinal pigmentation resulting in visual failure, and neurological disturbances, particularly seizures and a peripheral neuropathy, be screened not only for VLCFA/C26 ratio, but should also have pristanic acid concentrations assayed.

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