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Case Report

Chinese Boy with Normal Initial Peroxisomal Blood Assays: A Diagnostic Pitfall in the Workup for Infantile Refsum Disease

KL ***Cheung***, CH ***Ko***, HHC ***Lee*** , CM ***Mak***

Abstract The first Chinese case of Infantile Refsum disease (IRD), one of less severe form of peroxisome biogenesis disorders, is illustrated in this case report. The patient presented with global developmental delay without facial dysmorphism and his routine metabolic screening was negative. Initial very long chain fatty acids (VLCFA) assays showed marginally elevated C26/C22 ratio and pristanic and phytanic acid levels were within normal limits. Magnetic resonance imaging and nerve conduction velocity findings revealed abnormal results and VLCFA assays were subsequently repeated which demonstrated persistently elevated C26/C22 ratio and raised pristanic and phytanic acids. The diagnosis of IRD was confirmed with genetic study. The diagnostic approach and the corresponding investigations, in particular the use of serial VLCFA assays to improve the diagnostic yield, are highlighted.

Key words Fatty Acids; Genetic testing; Infantile; Peroxisomal biogenesis disorders; Refsum disease

Introduction

Infantile Refsum disease (IRD) is a rare neuro-metabolic disease which belongs to the group of peroxisomal biogenesis disorders (PBD) and is perceived to be a less severe form of Zellweger spectrum disorders (ZSD). Due to multiple defective peroxisomal enzymes, there are accumulation of phytanic acid and increased levels of very long chain fatty acids (VLCFA) in the blood and tissues leading to damage in brain parenchyma, peripheral nerves, liver and retina. The main manifestations include peripheral polyneuropathy, retinitis pigmentosa, cerebellar ataxia, developmental delay and growth retardation. Other clinical findings are craniofacial dysmorphism including high forehead, frontal bossing, antimongolian palpebral slant, sunken eyes, flat nasal root, elongated philtrum and bilateral simian creases, sensorineural deafness, seizures, hepatomegaly and malabsorption. The disease is inherited in autosomal recessive manner and confirmed by mutations in *PEX1* gene.

The current case illustrates the diagnostic approach to this rare disorder, and highlights the importance of repeating VLFCA assays to improve the diagnostic yield in suspected cases.

Department of Paediatrics and Adolescent Medicine, Caritas Medical Centre, 111 Wing Hong Street, Sham Shui Po, Kowloon, Hong Kong, China

KL **Cheung**（張國林） *MBChB(CUHK)*
CH **Ko**（高震雄） *FHKAM(Paediatrics), FRCP(Glasg)*

Department of Pathology, Princess Margaret Hospital, 2-10 Princess Margaret Hospital Road, Lai Chi Kok, Kowloon, Hong Kong, China

HHC **Lee**（李漢芝） *FRCPath, FHKAM(Pathology)*
CM **Mak**（麥苗） *MD, PhD*

Correspondence to: Dr KL **Cheung**
Email: ckwoklam@yahoo.com

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Case Report

A twenty-six-month-old Chinese boy was referred to us for global developmental delay. He was born at full term by uncomplicated spontaneous vaginal delivery with birth weight of 2.95 kg. Antenatal and postnatal histories were unremarkable. The parents were non-consanguineous and there was no known family history of genetic diseases. The

boy was the first child of the couple. Assessment at age 20 months revealed developmental age corresponding to 10 to 12 months in various aspects. Follow-up assessment at age 3 revealed milestones corresponding to 16 to 19 months of developmental age. He also had bilateral moderate to severe hearing loss and prominent sialorrhoea. General health was satisfactory and there was no recurrent seizure. Physical examination revealed prominent epicanthic folds and slightly low set ears with paucity of facial expression. No other craniofacial dysmorphic features were noted. He had generalised hypotonia and hyporeflexia. There was no skeletal abnormality. Systemic examination was otherwise unremarkable. Baseline investigations, including complete blood picture, liver and renal function, thyroid function, ammonia, lactate and pyruvate were unremarkable. Metabolic study revealed normal organic acid and amino acid profiles. Computed tomography of brain showed no definite abnormality.

At age two the child could walk on level ground by himself, but could not manage stairs. He gradually deteriorated by age 4.5 years, requiring one hand to be held on walking. He could build tower of three, obey simple command and speak 5-10 single words. Clinical examination showed persistent hypotonia, discrepant lower limb weakness with unsteady gait. Nerve conduction velocity (NCV) showed demyelinating motor neuropathy predominantly affecting bilateral lower limbs. Cranial magnetic resonance imaging (MRI) at age 4 years 10 months revealed T2 hyperintensity involving bilateral parieto-occipital regions & splenium of corpus callosum (Figure 1). Transferrin isoforms, acetylcholine receptor antibodies, carnitine profiles, morning cortisol level and lipid profiles were normal. Initial VLCFA assays showed marginally elevated C26/C22 ratio with normal C22:0, C24:0 and C26:0 levels; pristanic and phytanic acid levels were within normal limits. In view of abnormal MRI and NCV findings, assays were repeated four months later which demonstrated persistently elevated C26/C22 ratio and increasing C26:0 levels (but still within normal limits), with raised pristanic and phytanic acids. The pristanic to phytanic ratio was also elevated (Table 1). Genetic study revealed two heterozygous mutations for *PEX1* (NM_000466.2:c.2348A>G (p.Asp783Gly) and c.2783+2T>C), confirming the diagnosis of PBD (MIM #601539) with phenotype compatible with IRD.

The child was put on a phytanic acid restriction diet, with fat soluble vitamins and docosahexaenoic acid (DHA) supplementation. Despite initial transient improvement in self-care, he ran into relentless deterioration over the next 12 months. By age 6 he became minimally conscious, bedridden and required tube feeding. Repeat MRI brain at age 7 showed extensive leukodystrophic changes (Figure 2). There was persistent elevation of C26/C22 and C26:0 levels, with normalisation of pristanic and phytanic acids (Table 1).

Discussion

Peroxisomes are subcellular organelles present in all human tissues other than mature erythrocytes. They are mainly involved in lipid metabolisms. The reactions or

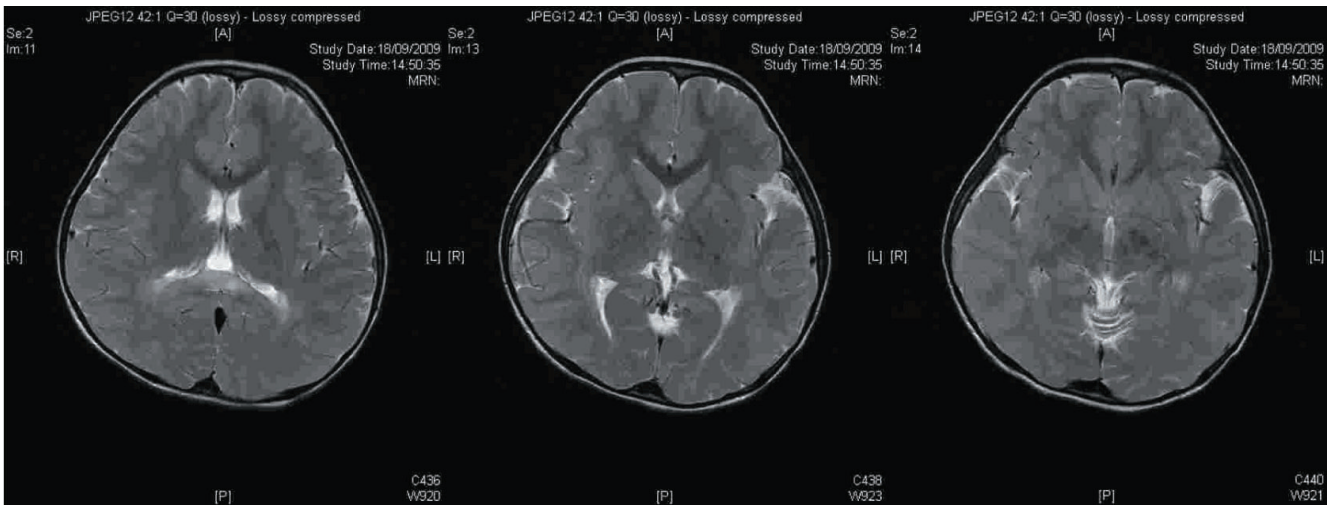

Figure 1 Cranial MRI at age 4 years 10 months showing T2 hyperintensity involving bilateral parieto-occipital regions and splenium of corpus callosum.

components of such reactions, namely plasmalogen, bile acid and DHA biosynthesis, and oxidation of pipecolic acid and VLCFA take place exclusively in the perioxisomes.[1] Peroxisomal matrix and membrane proteins are initially synthesised in free polyribosomes, enter cytoplasm and are directed to the preexisting peroxisomes from which new peroxisomes arise by budding. The targeting mechanism to the organelle is guided by peroxisomal targeting sequences (PTS1 and PTS2). Complementation analysis identifies up to 23 PEX genes coding for peroxins, which facilitate importation of targeted proteins into the peroxisomes through receptor docking, stability and translocation. PEX1 gene encodes AAA - ATPases for normal import of PTS1 proteins; PEX1 mutations are found in majority of cases with ZSD.[2]

Peroxisomal disorders are genetically heterogeneous group of disorders, classified into two categories. The first category is PBD, in which peroxisome fails to form normally. PBD are further sub-classified into ZSD and rhizomelic chondrodysplasia punctate (RCDP).[1] The spectrum of ZSD ranges from severe Zellweger syndrome to more indolent neonatal adrenoleukodystrophy (NALD) and IRD, which share similar clinical and biochemical features without distinct phenotypes. RCDP is characterised by severe skeletal abnormalities, cataracts and facial dysmorphism resulting from impairment in plasmalogen synthesis.[2] The second category is disorders of single peroxisomal protein in which peroxisome structure is intact but with defect of single peroxisomal enzymes; classical conditions include X-linked adrenoleukodystrophy (X-ALD) and D-bifunctional protein (DBP) deficiency.[1]

Historical cohorts demonstrate nearly half of presumed genetic leukodystrophies remain unresolved. Peroxisomal disorders are rare; the collective estimated frequency is 1 in 5000 to 10000 live births. X-ALD is the most commonly identified subtype, with an estimated incidence of 1:17000. Detection of elevated VLCFA in plasma suggests possibility of PBD and biochemical studies on fibroblasts and genetic test are necessary for proper diagnosis. Newborn screening for PBD utilising liquid chromatography and tandem mass spectrometry to detect elevated VLCFA in blood spots is rarely included in newborn metabolic screening worldwide. Pilot expanded newborn metabolic screening programme in Hong Kong offers screening for multiple aminoacidopathies, organic acidurias, and fatty acid oxidation disorders but does not

Table 1 Patient's plasma levels of very long chain fatty acids

	Age 4y 8m	Age 5	Age 7	Reference	SI unit
C22:0	31.5	39.1	53.1	<96.3	umol/L
C24:0	33.5	38.7	49.4	<91.4	umol/L
C26:0	0.87	1.03	**1.67**↑	<1.30	umol/L
C24:0/C22:0	1.06	0.99	0.93	<1.39	
C26:0/C22:0	**0.028**↑	**0.026**↑	**0.032**↑	<0.023	
Pristanic acid	2.23	**6.7**↑	0.33	<2.98	umol/L
Phytanic acid	5.69	**13.7**↑	1.28	<9.88	umol/L
Pristanic/Phytanic	0.39	**0.49**↑	0.26	<0.39	

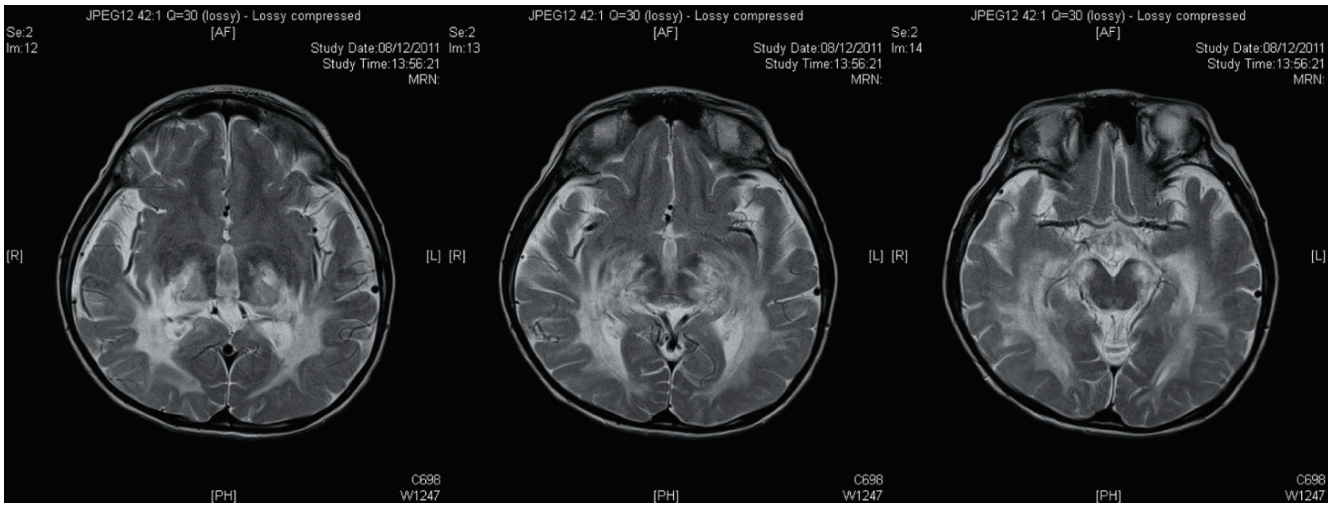

Figure 2 Cranial MRI at age of 6 years old and 1 month showing markedly increased extensive leukodystrophic changes.

include peroxisomal disorders.[3] In contrast, legislation for newborn screening of X-ALD has been passed in some states of United States including New York; this allows early detection and treatment of peroxisomal disorders.[4]

To our knowledge, this is the first reported case of IRD in Hong Kong. One local Chinese case of DBP deficiency with features mimicking Zellweger syndrome has been reported in 2009.[5] As IRD runs a more indolent course than classic Zellweger syndrome, detection in early stage may provide a window for therapeutic intervention. Early diagnosis of IRD is challenging; the initial signs can be subtle and nonspecific, with emerging intellectual deficits, progressive sensorineural deafness and motor deterioration. Dysmorphic features can be subtle with absence of hepatomegaly and the diagnosis relies on identification of a constellation of clinical, radiological and electrophysiological features. The findings of NCV abnormalities and MRI leukodystrophic changes should prompt further biochemical confirmation. However, initial VLFCA, pristanic and phytanic acid assays could yield false negative results. Van der Knapp et al retrospectively reviewed the pattern of six unclassified leukoencephalopathies; sequential MRI abnormalities started from dentate nucleus and superior cerebellar peduncles, subsequently affecting cerebellar white matter and brainstem, followed by parietal occipital white matter, splenium and internal capsule posterior limbs, with eventual diffuse involvement. Initial peroxisomal blood tests were notably all inconclusive. Diagnoses were eventually substantiated by fibroblast perosixomal immunofluorescence studies, complementation analysis and molecular studies, confirming three cases of ZSD and 3 DBP deficiencies.[6] Berendse et al reviewed and followed up nineteen patients for duration of mean 16 years. Three out of nineteen patients had insignificant peroxisomal blood assays and were diagnosed by complete analysis in skin fibroblasts.[7]

Ratbi et al identified six cases of Heimler syndrome confirmed by bi-allelic mutations in PEX1 or PEX6. This is defined as a mild variant of PBD with unremarkable blood and skin fibroblast analyses.[8] As illustrated in the present case, negative initial peroxisomal blood assays do not rule out the diagnosis. Plasmalogen and complementation studies are not available locally. In cases with concordant phenotypic and radiological findings, it is mandatory to repeat biochemical assays to improve the diagnostic yield and guide further targeted molecular studies.

Treatment of IRD is mainly supportive and phytanic acid-restricted diet remains the mainstay therapy. DHA is polyunsaturated fatty acid that has important functions in retina and brain synapses. DHA synthesis takes place in the peroxisome and is deficient in PBD patients. Anecdotal reports revealed improvement in clinical status from DHA supplementation.[9] However, in a randomised controlled trial of DHA at 100 mg/kg/day for PBD patients, there was no significant difference in biochemical function, electroretinogram and growth after one year.[10] In the present case, despite normalisation of pristanic/phytanic acid levels on phytanic acid-restricted diet and DHA supplementation, the relentless degenerative course had not been altered.

Declaration of Interest

The authors declare that they have no financial or other conflicts of interest in relation to this publication.

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