Zellweger Spectrum Disorder with Mild Phenotype Caused by PEX2 Gene Mutations

Andrea Mignarri • Claudia Vinciguerra • Antonio Giorgio • Sacha Ferdinandusse •
Hans Waterham • Ronald Wanders • Enrico Bertini • Maria Teresa Dotti •
Antonio Federico

Received: 27 July 2011 / Revised: 30 September 2011 / Accepted: 30 September 2011 / Published online: 29 January 2012
© SSIEM and Springer-Verlag Berlin Heidelberg 2012

Abstract The Zellweger spectrum disorders (ZSDs) are known to be severe disorders with onset in the newborn period or later in childhood, frequently resulting in death during childhood or adolescence. Here, we report a case of ZSD due to mutations in the PEX2 gene, with very mild phenotype. A 51-year-old Italian man was referred to us because of a clinical picture characterized by ataxia, areflexia, nystagmus, and strabismus, with childhood onset and slowly progressive course. The patient showed no cognitive impairment. Neurological examination revealed gait ataxia, dysarthria, dysmetria, areflexia, and bilateral pes cavus. Nerve conduction studies indicated a severe axonal sensorimotor polyneuropathy. Brain MRI showed marked cerebellar atrophy and absence of white matter involvement. MR spectroscopy uncovered a decreased N-acetyl aspartate peak. Biochemical analyses suggested a mild peroxisomal defect. Sequence analysis of the PEX2 gene identified two heterozygous mutations. The clinical phenotype of our patient differs from previously reported ZSD

patients with PEX2 gene mutations and suggests that genetic screening of PEX2 is warranted in children and adults with otherwise unexplained autosomal recessive ataxia. MRI findings diverged from the “classic” spectrum observed in ZSDs. The moderate impairment in peroxisome biogenesis seems to affect predominantly neuronal cells in cerebellum, leading to cerebellar atrophy.

Introduction

Peroxisome biogenesis disorders (PBDs) comprise a wide spectrum of diseases characterized by the loss of multiple peroxisomal metabolic functions due to mutations in genes (PEX) coding for different peroxisome biogenesis factors involved in the import of peroxisomal membrane and matrix proteins (Steinberg et al. 2006). PBDs include the Zellweger spectrum disorders (ZSDs) and rhizomelic chondrodysplasia punctata type I. The ZSDs are autosomal recessive diseases caused by mutations in any of at least 12 different PEX genes (Ebberink et al. 2011) and are represented by a continuum of three phenotypes including Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease. Despite the current classification, clinical, biochemical, and genetic overlap is present among the three phenotypes. Moreover, only limited sources are available to serve as background for the prognosis. All of the ZSDs are known to be severe disorders with onset in the newborn period or later in childhood and death during childhood or adolescence. Cases of ZSDs with prolonged survival (up to 24 years of age) have been described among the Northern-Western European population, with the majority of these patients (21/31) having mutations in the PEX1 gene (Poll-The et al. 2004). Other unusual ZSDs with a relatively mild clinical phenotype caused by PEX2, PEX10,

Communicated by: Robert Steiner

Competing interests: None declared

A. Mignarri • C. Vinciguerra • A. Giorgio • M.T. Dotti •
A. Federico (✉)

Department of Neurological, Neurosurgical and Behavioural
Sciences, Medical School, University of Siena, Viale Bracci 2,
Siena 53100, Italy
e-mail: federico@unisi.it

S. Ferdinandusse • H. Waterham • R. Wanders
University of Amsterdam, Academic Medical Centre,
Departments of Clinical Chemistry and Pediatrics, Laboratory
Genetic Metabolic Diseases, Amsterdam, The Netherlands

E. Bertini
Unit of Molecular Medicine, Department of Neurosciences,
Bambino Gesù Pediatric Hospital, Rome, Italy

PEX12, and PEX16 mutations have been reported (Ebberink et al. 2010; Régál et al. 2010; Sevin et al. 2011; Zeharia et al. 2007). So far, the term “mild” has been used mostly to make a difference from classical ZS. Here, we report a case of ZSD due to mutations in the PEX2 gene, with very mild phenotype and unusually prolonged survival.

Case Report

A 51-year-old Italian man was recently referred to us because of a complex clinical picture characterized by ataxia, areflexia, nystagmus and strabismus, with childhood onset and slowly progressive course. He was the first child of nonconsanguineous healthy parents. Family history was apparently negative for neurological disturbances. The patient, born at term with normal birth weight and Apgar scores, developed normally until 3 years of age, when unsteady walking and frequent falls were noticed. Motor disturbances progressed very slowly during childhood and adolescence without impairing his daily activities. Dysmorphic features, cognitive impairment, and seizures were absent. Between the third and the fifth decades, bilateral hypoacusia and initial retinopathy were detected. On admission to our Department, neurological examination showed gait ataxia, lateral and vertical gaze-evoked nystagmus, hypoacusia, mild dysarthria, slight dysmetria, generalized areflexia, and bilateral pes cavus. Electromyography and nerve conduction studies indicated a severe axonal sensorimotor polyneuropathy. Brain MRI revealed marked atrophy of the subtentorial regions including cerebellum, cerebellar peduncles, and bulbar olives, and showed moderate atrophy of the supratentorial regions of the brain (Fig. 1). There were no signal abnormalities in the white matter. Proton MR spectroscopy imaging (MRSI) of the brain showed decreased values (2.55; normal values = 3.0 ± 0.2) of N-acetyl-aspartate/Creatine (NAA/Cr) in the supratentorial and periventricular volume of interest. Analysis of plasma peroxisomal parameters revealed increased levels of very long chain fatty acids (VLCFAs), branched-chain fatty acids (pristanic and phytanic acid), and bile acid intermediates, prompting us to suspect a peroxisomal disorder. Plasmalogens in erythrocytes were normal. Further metabolic studies performed in fibroblasts showed normal dihydroxyacetonephosphate acyltransferase (DHAPAT) activity, abnormal VLCFAs profile, normal rates of beta-oxidation of VLCFAs and pristanic acid, and partial deficiency of phytanic acid alpha-oxidation. Immunofluorescence microscopy analysis using antibodies raised against catalase, a peroxisomal matrix enzyme, revealed a heterogeneous pattern: some cells showed normal peroxisomes whereas in other cells peroxisomes were absent, and

Fig. 1 Axial brain magnetic resonance (MR) images of fluid attenuated inversion recovery (FLAIR) sequence show a moderate atrophy of the supratentorial regions of the brain (a, b) and a marked atrophy of the subtentorial regions such as pons (b) and cerebellum (c, d – white arrows). Note the absence of signal changes in the white matter

in yet other cells a mixed picture was observed. The mosaicism was observed both at 37°C and at 40°C. In addition, immunoblot analysis showed that the peroxisomal enzymes acyl-coenzyme A oxidase (ACOX) and thiolase I were normally processed. Peroxisomal tests results (Table 1) pointed toward a mild peroxisomal defect, but did not clearly indicate at which level. Sequence analysis of the PXPMP3 (PEX2) gene (reference sequence NM_000318.2) identified two heterozygous mutations, c.355°C>T (p.Arg119X) and c.865_866insA (p.Ser289-LysfsX36), confirming the diagnosis of ZSD.

Discussion

We describe a patient affected by a ZSD with very mild phenotypic and biochemical expression, caused by two heterozygous mutations in PEX2 gene. One mutation, c.355°C>T (p.Arg119X), was previously reported in patients with ZS (Gootjes et al. 2004). The second mutation, c.865_866insA introducing a frameshift 17 codons upstream of the stop codon and resulting in a PEX2 protein with an altered C-terminus (p.Ser289-LysfsX36), has been recently reported in two brothers who developed isolated progressive cerebellar ataxia at 3½ and 18 years of age, respectively (Sevin et al. 2011). The latter mutation is most probably responsible for the mild clinical presentation and the unexpectedly prolonged survival. The clinical phenotype of our patient, mainly

Table 1 Peroxisomal tests results

Peroxisomal test	Patient	Control	Zellweger
<i>Plasma</i>			
C26:0, $\mu\text{mol/l}$	2.03	0.45–1.32	1.80–8.10
C26/C22	0.036	0–0.020	0.069–0.045
C24/C22	0.80	0.57–0.92	0.92–2.53
Pristanic acid, $\mu\text{mol/l}$	10.6	0–4.0	0.5–30.3
Phytanic acid, $\mu\text{mol/l}$	10.3	0–9.0	1.6–115.7
THCA, $\mu\text{mol/l}$	0.10	0–0.08	↑
DHCA, $\mu\text{mol/l}$	0.5	0–0.2	↑
<i>Cultured fibroblasts</i>			
C22:0, nmol/mg	5.72	3.84–10.20	Abnormal
C24:0, nmol/mg	13.27	7.76–17.66	Abnormal
C26:0, nmol/mg	0.66	0.18–0.38	Abnormal
C26/C22	0.12	0.03–0.07	Abnormal
α -Oxidation (phytanic acid), pmol/mg/h	24	68 $\pm$ 29	≤ 10
β -Oxidation (C26:0), pmol/mg/h	1.434	1.300 $\pm$ 475	≤ 350
β -Oxidation (pristanic acid), pmol/mg/h	850	1.145 $\pm$ 356	≤ 30
DHAPAT activity, nmol/mg/2 h	6.6	5.3–12.7	≤ 0.9
<i>Thiolase immunoblot</i>			
41 kDa	+	++	–
44 kDa	–	–	+
<i>Catalase immunofluorescence</i>			
Catalase	$\pm$	+	–
<i>ACOX immunoblot</i>			
72 kDa	+	+	+
52 kDa	+	+	–
20 kDa	+	++	–

Abnormal values detected in our patient are indicated in bold. Control values are 5–95% ranges or mean $\pm$ standard deviation

THCA trihydroxycholestanoic acid, DHCA dihydroxycholestanoic acid, dma dimethyl acetal, DHAPAT dihydroxyacetone phosphate acyltransferase, ACOX acyl-coenzyme A oxidase

characterized by ataxia and axonal sensorimotor neuropathy with childhood onset and slow progression, strongly differs from previously reported ZSD patients with PEX2 gene mutations who may display cerebellar symptoms in addition to other severe neurological and systemic signs, and further suggests that genetic screening of PEX2 is warranted in children and adults with otherwise unexplained autosomal recessive ataxia. The PEX2, PEX10, and PEX12 genes encode for integral peroxisomal membrane proteins with a cytosolic carboxy-terminal RING finger domain that act as ubiquitin ligases required for the ubiquitination of the PTS1-receptor (PEX5) in the peroxisomal membrane (Girzalsky et al. 2010). Both PEX2 and PEX10 mutations have been reported to cause autosomal recessive ataxia and cerebellar atrophy (Régál et al. 2010;

Sevin et al. 2011). In our patient, some peroxisomes in the fibroblasts contained catalase: this fact suggests that the mutated PEX2 is localized correctly in the peroxisomal membrane and still is partly active. Peroxisomal mosaicism with a mixed population of fibroblasts with and without peroxisomes persisted at 40°C, making systematic sequencing of the different PEX genes necessary. DHAPAT activity and C26:0 beta-oxidation, which are considered the best biochemical markers in predicting life expectancy of patients with PBDs (Gootjes et al. 2002), were normal. In our patient, the moderate impairment in peroxisome biogenesis affects predominantly neuronal cells in the cerebellum. Indeed, brain MRI diverged from the “classic” ZSD pattern (Barth et al. 2004; Weller et al. 2008), showing cerebellar atrophy and absence of white matter abnormalities. MRI findings were similar to those observed in other patients with autosomal recessive ataxia due to PEX2 and PEX10 mutations (Régál et al. 2010; Sevin et al. 2011). Moreover, proton MRSI confirmed the presence of neuro-axonal damage by showing decreased levels of NAA (De Stefano et al. 2001). In summary, our case report further suggests to search for PBDs in patients of different ages with unexplained autosomal recessive cerebellar ataxia and decreased tendon reflexes. Furthermore, it demonstrates that PEX2 mutations can lead to ZSDs with mild phenotypic and biochemical alterations.

Take-Home Message

PEX2 gene mutations can cause Zellweger spectrum disorders with mild phenotype resulting in cerebellar ataxia.

Conflict of Interest

All authors declare no conflict of interest.

References

- Barth PG, Majoie CB, Gootjes J et al (2004) Neuroimaging of peroxisome biogenesis disorders (Zellweger spectrum) with prolonged survival. *Neurology* 62:439–444
- De Stefano N, Narayanan S, Francis GS et al (2001) Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. *Arch Neurol* 58:65–70
- Ebberink MS, Csanyi B, Chong WK et al (2010) Identification of an unusual variant peroxisome biogenesis disorder caused by mutations in the PEX16 gene. *J Med Genet* 47:608–615
- Ebberink MS, Mooijer PA, Gootjes J, Koster J, Wanders RJ, Waterham HR (2011) Genetic classification and mutational spectrum of more than 600 patients with a Zellweger syndrome spectrum disorder. *Hum Mutat* 32:59–69

- Girzalsky W, Saffian D, Erdmann R (2010) Peroxisomal protein translocation. *Biochim Biophys Acta* 1803:724–731
- Gootjes J, Mooijer PA, Dekker C et al (2002) Biochemical markers predicting survival in peroxisome biogenesis disorders. *Neurology* 59:1746–1749
- Gootjes J, Elpeleg O, Eyskens F et al (2004) Novel mutations in the PEX2 gene of four unrelated patients with a peroxisome biogenesis disorder. *Pediatr Res* 55:431–436
- Poll-The BT, Gootjes J, Duran M et al (2004) Peroxisome biogenesis disorders with prolonged survival: phenotypic expression in a cohort of 31 patients. *Am J Med Genet A* 126:333–338
- Régál L, Ebberink MS, Goemans N et al (2010) Mutations in PEX10 are a cause of autosomal recessive ataxia. *Ann Neurol* 68:259–263
- Sevin C, Ferdinandusse S, Waterham HR, Wanders RJ, Aubourg P (2011) Autosomal recessive cerebellar ataxia caused by mutations in the PEX2 gene. *Orphanet J Rare Dis* 6:8
- Steinberg SJ, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW (2006) Peroxisome biogenesis disorders. *Biochim Biophys Acta* 1763:1733–1748
- Weller S, Rosewich H, Gärtner J (2008) Cerebral MRI as a valuable diagnostic tool in Zellweger spectrum patients. *J Inher Metab Dis* 31:270–280
- Zeharia A, Ebberink MS, Wanders RJ et al (2007) A novel PEX12 mutation identified as the cause of a peroxisomal biogenesis disorder with mild clinical phenotype, mild biochemical abnormalities in fibroblasts and a mosaic catalase immunofluorescence pattern, even at 40 degrees C. *J Hum Genet* 52:599–606