Conventional and advanced MR imaging in infantile Refsum disease

Mustafa Kılıç[1], Kader Karlı-Oğuz[2], Göknur Haliloğlu[3], Meral Topçu[3], Ronald James Wanders[4], Turgay Coşkun[1]

Divisions of [1]Pediatric Nutrition and Metabolism and [3]Pediatric Neurology, Department of Pediatrics, and [2]Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, and [4]Department of Pediatrics, Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, the Netherlands
E-mail: kilickorkmaz@yahoo.com.tr
Received: 20 May 2014, Accepted: 29 July 2015

SUMMARY: Kılıç M, Karlı-Oğuz K, Haliloğlu G, Topçu M, Wanders RJ, Coşkun T. Conventional and advanced MR imaging in infantile Refsum disease. Turk J Pediatr 2015; 57: 294-299.

We report magnetic resonance (MR) imaging findings including diffusion-weighted imaging and proton MR spectroscopy findings in a patient with infantile Refsum disease. The initial diagnosis was made on the basis of history, clinical findings and biochemical studies. Bilateral and symmetrical involvement of the peritrigonal white matter, centrum semiovale, thalami, corpus callosum and corticospinal tracts as assessed by increased T2 signal was highly suggestive of a peroxisomal disorder. Facilitated diffusion was observed in diseased parenchyma. Long echo-time (TE: 270 ms) MRS showed decreased N-acetyl-aspartate/creatine and elevated choline/creatine and lactate; short echo-time MRS (TE: 30 ms) revealed increased myoinositol at 3.56 ppm and lipid peaks at 0.9 and 1.3 ppm. A major contribution to the differential diagnosis came from MR imaging and proton MRS, as discussed in this report.

Key words: infantile Refsum disease, cranial MRI, MRS.

The peroxisome biogenesis disorders comprise the Zellweger spectrum disorders (i.e., Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease) and rhizomelic chondrodysplasia punctata type 1. Peroxisome biogenesis disorders can be caused by mutations in any of 13 currently known PEX genes, which encode peroxins involved in peroxisomal protein import and/or assembly of the organelle[1]. Infantile Refsum disease is an autosomal recessive peroxisomopathy comprising retinitis pigmentosa, sensorineural hearing loss, psychomotor and mental developmental delays, hypotonia and cerebellar ataxia[2,3]. Biochemical findings of elevated levels of phytanic acid, pipecolic acid and very long chain fatty acids in plasma combined with multiple deficiencies of peroxisomal enzymes in fibroblasts affecting fatty acid alpha- and beta-oxidation as well as plasmalogen synthesis substantiated the classification of this disease as a multiple peroxisomal dysfunction disorder, similar to the more severe Zellweger syndrome[4,5]. Plasma levels of phytanic acid are consistently elevated in infantile Refsum disease. Although high docosahexaenoic acid and low phytanic acid diets can correct some of the biochemical defects, they do not consistently alter the progressive course of the disease[6]. Orthotopic liver transplantation has been shown to provide an efficient means of treatment in a mildly symptomatic patient[7]. Diagnosis may be difficult; however the MRI/MRS findings in our case suggested a metabolic disorder, and consequently we studied their significance in the diagnostic evaluation of these rare disorders.

Case Report

A 3-year-and-4-month-old girl with developmental delay and visual and hearing impairment was evaluated for the possibility of a multisystem genetic disorder (Fig. 1). She presented with developmental delay at the age of 8 months. Her medical history was otherwise unremarkable, with a normal

pregnancy and delivery. The family noticed that there was delay, especially in language, at the age of 1.5 years, and that she was unresponsive to her environment. She has been treated with L-thyroxine because of congenital hypothyroidism and was using a hearing aid for sensorineural hearing loss detected at another center. There was second-degree consanguinity between the parents, but family history was otherwise negative. Head control and sitting with support were achieved at the age of 8 months and 16 months, respectively. She was unresponsive to her name. On physical examination, her head circumference was 46.5 cm (-2SD), she had mild dysmorphism (round face, flat occiput, frontal bossing), severe developmental delay, generalized hypotonia, bilateral sensorineural hearing loss, retinitis pigmentosa and decreased vision, and deep tendon reflexes were decreased without pathological reflexes. Electroretinogram showed no response, indicating retinal involvement. She was unable to sit without help and was not able to recognize her parents.

Initial laboratory investigations, including electrolytes, urea, creatinine, aminotransferases, blood gases, glucose, ammonia, creatine kinase, complete blood count, vitamin B12 and TORCHES examinations, were normal. Metabolic investigations, including tandem mass spectrometry, urine organic acid analyses, serum lactate, pyruvate and transferrin isoelectric focusing were also normal. Biochemical abnormalities included elevation of very long chain fatty acid levels, with lower than normal long-chain polyunsaturated fatty acids and arachidonic and docosahexaenoic acid levels. There was elevation of C26:0 (2.52 μmol/L (N: 0.6-1.3)), phytanic acid (47.63 μmol/L (N: 0.42-3.77)) and pristanic acid (14.28 μmol/L (N: 0-1.5)), and an elevated C26/C22 ratio (0.11 μmol/L (N: 0.011-0.026)), pointing to either a defect in peroxisome biogenesis or a defect in the peroxisome beta-oxidation system. Phytanic acid oxidation in cultured cells was deficient. Furthermore, C26:0 and C26/C22 ratio were elevated in fibroblasts. Fibroblast levels of very long chain fatty acids, C26:0=0.54 (control: 0.18-0.38) and C26/C22=0.15 (control: 0.03-0.07) ratio were elevated. The activity of the peroxisomal enzyme dihydroxyacetonephosphate-acyltransferase, the first enzyme in the plasmalogen biosynthesis route, was only partially deficient (5.0 nmol/mg/2hr (control: 9.0±1.9)). Furthermore, immunofluorescence microscopy analysis revealed a marked deficiency of peroxisomes. Taken together, these data point to a disorder of peroxisome biogenesis. Sequence analyses of the *PEX 12* gene identified c.959C>T (p.Ser320Phe) homozygous mutation.

Cranial MR imaging was performed on a 1.5 T scanner (Symphony, Siemens, Erlangen, Germany). Imaging included T1-weighted (W) (TR/TE; 500/15ms) spin-echo (SE) in sagittal and axial, T2-W (TR/TE; 3900/100 ms) turbo SE in coronal and axial, fluid-attenuated inversion-recovery (FLAIR) (TR/TE/TI; 9000/100/2100 ms) in axial planes, and diffusion-weighted imaging (single-shot echoplanar; applied 3B values with a maximum of 1000 s/mm^2 and a TR/TE of 4800/120 ms; matrix (mtx) of 96-256). Apparent diffusion coefficient maps were obtained automatically on site, and measurements were done on an

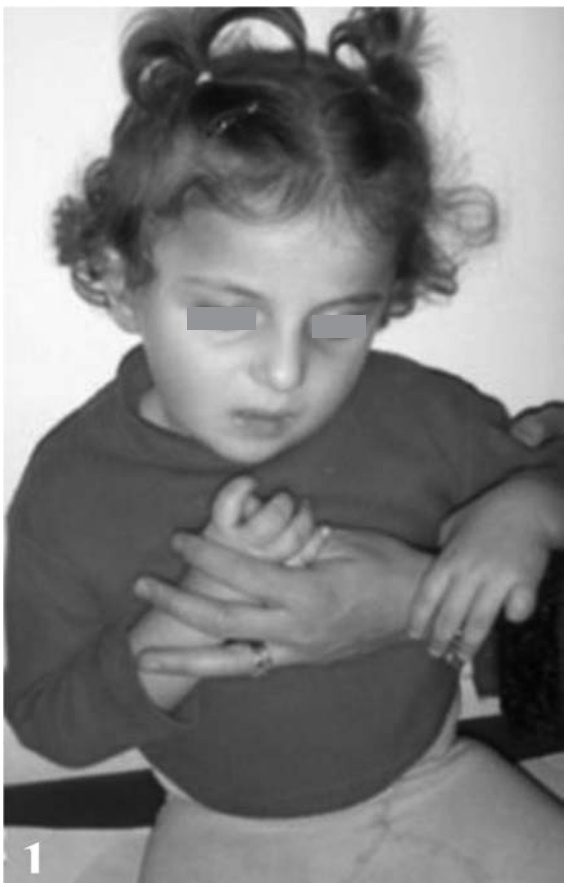
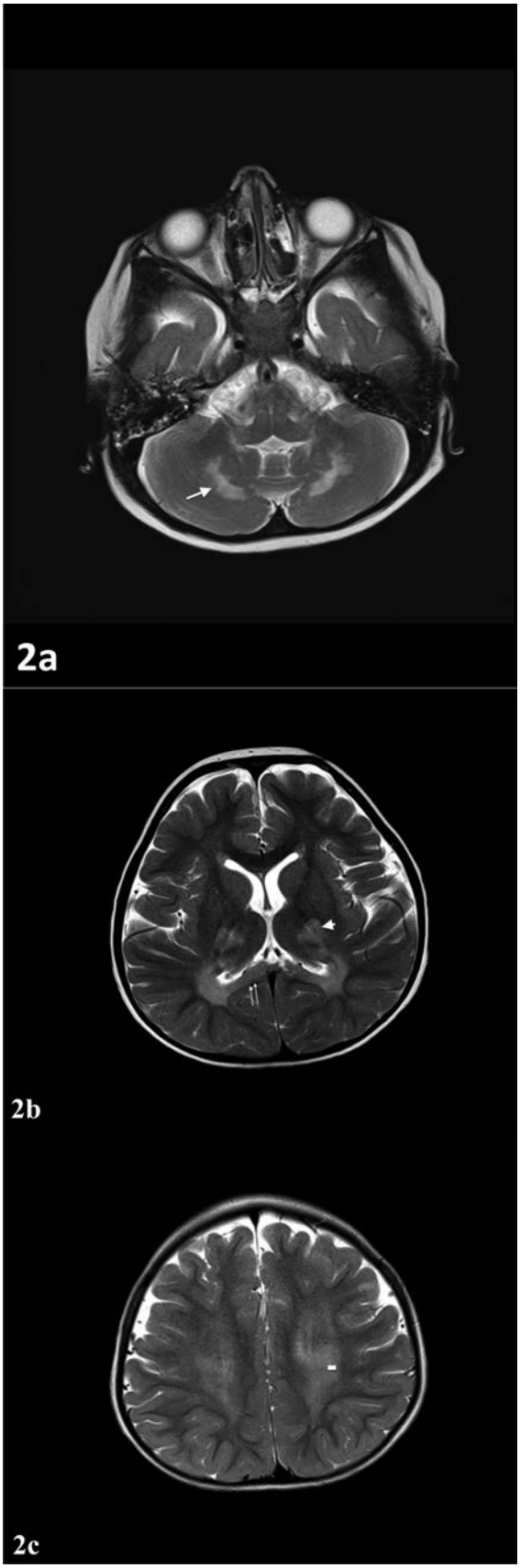
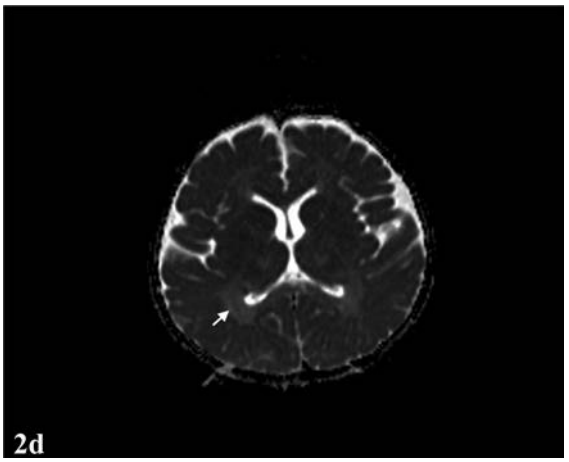
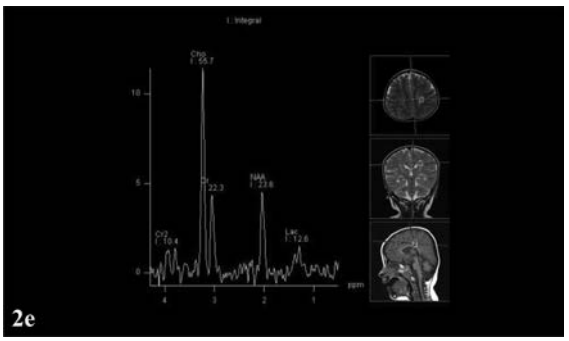


Fig. 1. Patient at the age of 3.5 years. She is unable to sit without help.







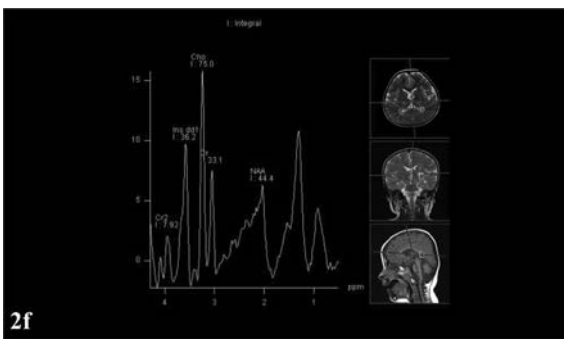


Fig. 2. T2 weighted (W) images (TR/TE: 4000/100 ms) show bilateral, symmetrical hyperintense lesions in the cerebellar white matter (arrow, a), along the corticospinal tracts (arrowhead, b), in the thalami and the splenium of the corpus callosum (double thin arrows, b) and in the centrum semiovale (rectangle, c). The apparent diffusion coefficient (ADC) map (d) derived from diffusion-weighted imaging displays increased values (0.95-1.1x10^{-3} mm^2/s), showing as hyperintense (arrow, d) in diseased parenchyma as compared to normal tissue (0.75-0.78x10^{-3} mm^2/s) in affected parenchyma. ^{1}H MRS with a long echo-time (TE: 270 ms) (e) shows decreased N-acetyl-aspartate (NAA), and increased choline (Cho) and lactate (lac). Short TE (30 ms) spectroscopic imaging (f) additionally shows increased myoinositol (Ins), and lipid and lactate (lac) peaks.

offline workstation (Leonardo workstation, VD 10B; Siemens). ^{1}H MRS with a long and short echo time (single-voxel spectroscopy; PRESS; TR/TE: 1500/270 and 30 ms respectively; number of acquisition: 192) was performed from the affected parenchyma. After data acquisition, an automated program provided by the manufacturer was used for offline post-processing on the same workstation, and NAA/Cr, Cho/Cr and Ino/Cr ratios were calculated.

On T2-W and FLAIR imaging, bilateral and symmetric T2 hyperintensity in the thalami and peritrigonal white matter continuing around the occipital horns and along the corticospinal tracts was observed. The splenium of the corpus callosum was diffusely involved. Deep cerebellar white matter contained similar bilateral lesions (Fig. 2a-c). All the lesions showed facilitated diffusion as assessed by higher apparent diffusion coefficient values (0.95-1.1x10^{-3} mm^2/s) in diseased parenchyma than in normal tissue (0.75-0.78x10^{-3} mm^2/s) on diffusion-weighted imaging (Fig. 2d). ^{1}H MRS with a long echo time (single-voxel spectroscopy; PRESS; TR/TE:1500/270 ms) from the affected parenchyma showed a moderate decrease in N-acetyl aspartate (NAA)/creatine (Cre) (1.06) and an increase in choline (Cho)/Cre (2.5) and lactate with a characteristic doublet at 1.33 ppm (Fig. 2e). Additional metabolic data were obtained with short echo-time ^{1}H MRS, showing increased myoinositol/Cre (1.08) and peaks at 0.9 and 1.3 ppm consistent with lipid and lactate (Fig. 2f).

The patient was diagnosed with infantile Refsum disease, and because the only source of phytanic acid in humans is exogeneous, i.e., from dietary sources, a phytanic acid-restricted and high docosahexaenoic acid diet was given. L-thyroxine was also given for congenital hypothroidism. The patient was followed at least 12 months after institution of the phytanic acid-restricted diet without any marked clinical improvement.

Discussion

Infantile Refsum disease is a peroxisome biogenesis disorder and clinically has an infantile onset with minor dysmorphism, mental retardation, hepatomegaly, sensorineural hearing loss, retinal pigmentary degeneration and hypocholesterolemia[8]. The most common clinical manifestation in this group of patients is the multisystem nature of the disease, with sensorineural hearing loss and retinitis pigmentosa[9]. This may occur in the first years of life. Affected individuals may initially be diagnosed as having a variant of Usher syndrome, but the additional manifestations such as hypotonia, developmental delay, mental retardation and failure to thrive provide clues to the appropriate diagnosis[3]. Differential diagnosis includes Leber's congenital amaurosis[10,11], congenital myopathies[12] and late onset leukodystrophies[13]. In our patient, due to the presence of congenital hypothyroidism and bilateral sensorineural hearing loss, Pendred syndrome, congenital disorders of glycosylation and intrauterine infections were included in the differential diagnosis.

An MRI-based approach and pattern recognition are extremely important in the diagnostic work-up of patients with metabolic diseases of the cerebral white matter[14-16]. Differential diagnosis of hypomyelinating leukoencephalopathies with normal to small cranial growth in children includes Krabbe disease, metachromatic leukodystrophy, multiple sulfatase deficiency, Pelizaeus–Merzbacher disease, childhood ataxia with diffuse CNS hypomyelination and peroxisomal disorders[14-17]. Previously described MR imaging findings in infantile Refsum disease include symmetrical increased T2 signal in the periventricular white matter at the level of the centrum semiovale, corticospinal tracts, corpus callosum and cerebellar dentate nuclei[8,18-20]. Autopsy findings in the brain that have been reported include mild diffuse reduction of axons and myelin within the corpus callosum, periventricular white matter, optic nerves and corticospinal tracts. These findings are also responsible for increased apparent diffusion coefficient values in affected tissue through increased extracellular space. Lipid-laden macrophages may be seen in the cerebral white matter. Severe hypoplasia of the cerebellar granule layer and ectopic Purkinje cells in the molecular layer have been shown[21]. Barth et al. suggested that peroxisome biogenesis disorders should be included in the differential diagnosis of post-infantile-onset cerebral white matter disease[22]. Serial MRI imaging is also helpful in terms of offering diagnostic clues and enabling an understanding of the natural history of neurodegeneration in peroxisomal disorders[23].

In one study, the sparing of gray matter early in the course of D-bifunctional protein deficiency in a patient with a neuroimaging pattern resembling X-linked adrenoleukodystrophy is described. The authors suggest that peroxisomal beta-oxidation studies in skin fibroblasts may be warranted in patients with undefined leukodystrophy, even when plasma very long chain acids are normal[23].

In the present case, conventional MR imaging findings suggested a peroxisomal disorder very similar to adrenoleukodystrophy. ^{1}H MRS has proven to be effective in predicting histopathologic changes occurring in the brain parenchyma in a wide spectrum of the diseases including inborn errors of metabolism[24]. In this patient, previously documented pathologic findings ranging from axonal degeneration to inflammatory demyelination were also predicted by ^{1}H MRS, which showed a decreased NAA/Cre ratio as well as an increased Cho/Cre ratio. Additionally, an elevated MI/Cre ratio suggested accompanying astrogliosis. MRS is able to identify alterations not only in the lesions visible on T2 W imaging, but also in normal-appearing white matter[25]. More peculiar for this group of disorders was the observation of peaks at 0.9 and 1.3 ppm in short TE sequences, which can be attributed to methylene and methyl groups of lipids respectively. Similar peaks have been reported in Zellweger syndrome[26,27] and Sjögren–Larsson syndrome[28,29], and were found to be consistent with long-chain fatty alcohols/aldehydes. By tracking metabolic and histologic alterations at the tissue level with MRS, it may be possible to monitor treatment during follow-up of such patients. Demonstrating increased myoinositol to creatine in various phenotypes of X-linked adrenoleukodystrophy and correlation of MI/Cre ratios with the severity of the symptoms, Ratai et al[30]. suggested this ratio as a meaningful biomarker. In line with these results, improvement of the clinical status of two children with X-linked adrenoleukodystrophy was also reflected in a reversal of the MRS findings following hematopoietic stem cell transplantation; i.e., an increase in the NAA/Cre- and reduction in the Cho/Cre ratios occurred[31]. In the present case, a peak in lactate was also observed; this is unexpected in normal parenchyma and may also suggest a possible mitochondrial dysfunction, anaerobic glycosylation or a mitochondrial disease. However, there was no brainstem or basal ganglia involvement in this patient. Turning to the possibility of lysosomal storage diseases, there was no thalamic signal change or prominent cortical atrophy on MR imaging; other clinical clues for these disorders were also absent.

In conclusion, infantile Refsum disease is a very rare neurometabolic disorder and should be considered in differential diagnosis in the presence of appropriate clinical, biochemical, molecular and radiological findings. MR imaging showing symmetrical signal intensity abnormalities in the cerebellum, thalami, periventricular white matter, corticospinal tracts and corpus callosum appears to be highly suggestive of infantile Refsum disease. Underlying pathological (axonal degeneration, demyelination and astrogliosis) and metabolic (presence of lactate and lipid consistent with long-chain fatty acids along with increased choline, decreased NAA and increased MI) alterations can be traced with diffusion-weighted imaging and magnetic resonance spectroscopy; moreover, these modalities can be used in evaluating response to therapy. Typically, the diagnosis is suspected based upon clinical and biochemical findings; MR may serve to solidify the diagnosis.

REFERENCES

1. Steinberg SJ, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW. Peroxisome biogenesis disorders. Biochim Biophys Acta 2006; 1763: 1733-1748.
2. Poll-The BT, Saudubray JM, Ogier H, et al. Infantile Refsum's disease: biochemical findings suggesting multiple peroxisomal dysfunction. J Inherit Metab Dis 1986; 9: 169-174.
3. Lazarow PB, Moser HW. Disorders in peroxisome biogenesis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill; 1995; 2287-2324.
4. Poll-The BT, Saudubray JM, Ogier HA, et al. Infantile Refsum disease: an inherited peroxisomal disorder. Comparison with Zellweger syndrome and neonatal adrenoleukodystrophy. Eur J Pediatr 1987; 146: 477-483.
5. Wanders RJ, Schutgens RB, Schrakamp G, et al. Infantile Refsum disease: deficiency of catalase-containing particles (peroxisomes), alkyldihydroxyacetone phosphate synthase and peroxisomal beta-oxidation enzyme proteins. Eur J Pediatr 1986; 145: 172-175.

6. Paker AM, Sunness JS, Brereton NH, et al. Docosahexaenoic acid therapy in peroxisomal diseases: results of a double-blind, randomized trial. Neurology 2010; 75: 826-830.
7. van Maldergem L, Moser AB, Vincent MF, et al. Orthotopic liver transplantation from a living-related donor in an infant with a peroxisome biogenesis defect of the infantile Refsum disease type. J Inherit Metab Dis 2005; 28: 593-600.
8. Naidu S, Moser H. Infantile Refsum disease. AJNR Am J Neuroradiol 1991; 12: 1161-1163.
9. Bader PI, Dougherty S, Cangany N, Raymond G, Jackson CE. Infantile Refsum disease in four Amish sibs. Am J Med Genet 2000; 90: 110-114.
10. Ek J, Kase BF, Reith A, Björkhem I, Pedersen JI. Peroxisomal dysfunction in a boy with neurologic symptoms and amaurosis (Leber disease): clinical and biochemical findings similar to those observed in Zellweger syndrome. J Pediatr 1986; 108: 19-24.
11. Michelakakis HM, Zafeiriou DI, Moraitou MS, Gootjes J, Wanders RJ. PEX1 deficiency presenting as Leber congenital amaurosis. Pediatr Neurol 2004; 31: 146-149.
12. Baumgartner M, Verhoeven N, Jacobs C, et al. Defective peroxisome biogenesis with a neuromuscular disorder resembling Werdnig-Hoffmann disease. Neurology 1998; 51: 1427-1432.
13. Moser AB, Rasmussen M, Naidu S, et al. Phenotype of patients with peroxisomal disorders subdivided into sixteen complementation groups. J Pediatr 1995; 127: 13-22.
14. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. Neurology 2009; 72: 750-759.
15. Patay Z. Diffusion-weighted MR imaging in leukodystrophies. Eur Radiol 2005; 15: 2284-2303.
16. Vargas MI, Merlini L, Haller S, et al. Magnetic resonance imaging of metabolic diseases of the cerebral white matter. Top Magn Reson Imaging 2009; 20: 333-341.
17. Steenweg ME, Vanderver A, Blaser S, et al. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. Brain 2010; 133: 2971-2982.
18. Choksi V, Hoeffner E, Karaarslan E, Yalcinkaya C, Cakirer S. Infantile Refsum disease: case report. Am J Neuroradiol 2003; 24: 2082-2084.
19. Cakirer S, Savas MR. Infantile Refsum disease: serial evaluation with MRI. Pediatric Radiol 2005; 35: 212-215.
20. Dubois J, Sebag G, Argyropoulou M, Brunelle F. MR findings in infantile Refsum disease: case report of two family members. AJNR Am J Neuroradiol 1991; 12: 1159-1160.
21. Torvik A, Torp S, Kase BF, Ek J, Skjeldal O, Stokke O. Infantile Refsum's disease: a generalized peroxisomal disorder. Case report with postmortem examination. J Neurol Sci 1988; 85: 39-53.
22. Barth PG, Gootjes J, Bode H, Vreken P, Majoie CB, Wanders RJ. Late onset white matter disease in peroxisome biogenesis disorder. Neurology 2001; 57: 1949-1955.
23. Khan A, Wei XC, Snyder FF, Mah JK, Waterham H, Wanders RJ. Neurodegeneration in D-bifunctional protein deficiency: diagnostic clues and natural history using serial magnetic resonance imaging. Neuroradiology 2010; 52: 1163-1166.
24. Phelan JA, Lowe LH, Glasier CM. Pediatric neurodegenerative white matter processes: leukodystrophies and beyond. Pediatric Radiol 2008; 38: 729-749.
25. Eichler FS, Barker PB, Cox C, et al. Proton MR spectroscopic imaging predicts lesion progression on MRI in X-linked adrenoleukodystrophy. Neurology 2002; 58: 901-907.
26. Bruhn H, Kruse B, Korenke GC, et al. Proton NMR spectroscopy of cerebral metabolic alterations in infantile peroxisomal disorders. J Comput Assist Tomogr 1992; 16: 335-344.
27. Groenendaal F, Bianchi MC, Battini R, et al. Proton magnetic resonance spectroscopy (1H-MRS) of the cerebrum in two young infants with Zellweger syndrome. Neuropediatrics 2001; 32: 23-27.
28. Mano T, Ono J, Kaminaga T, et al. Proton MR spectroscopy of Sjögren-Larsson's syndrome. AJNR Am J Neuroradiol 1999; 20: 1671-1673.
29. Willemsen MA, Van Der Graaf M, Van Der Knaap MS, et al. MR imaging and proton MR spectroscopic studies in Sjögren-Larsson syndrome: characterization of the leukoencephalopathy. AJNR Am J Neuroradiol 2004; 25: 649-657.
30. Ratai E, Kok T, Wiggins C, et al. Seven-Tesla proton magnetic resonance spectroscopic imaging in adult X-linked adrenoleukodystrophy. Arch Neurol 2008; 65: 1488-1494.
31. Warren DJ, Connolly DJ, Wilkinson ID, Sharrard MJ, Griffiths PD. Magnetic resonance spectroscopy changes following haemopoietic stem cell transplantation in children with cerebral adrenoleukodystrophy. Dev Med Child Neurol 2007; 49: 135-139.