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MRI as diagnostic tool in early-onset peroxisomal disorders

M.S. van der Knaap,
MD, PhD
E. Wassmer, MD
N.I. Wolf, MD, PhD
P. Ferreira, MD
M. Topçu, MD
R.J.A. Wanders, PhD
H.R. Waterham, PhD
S. Ferdinandusse, PhD

Correspondence & reprint requests to Dr. van der Knaap: ms.vanderknaap@vumc.nl

ABSTRACT

Objective: Peroxisomal blood tests are generally considered to be conclusive. We observed several patients with a clinical and MRI phenotype suggestive of an infantile onset peroxisomal defect, but no convincing abnormalities in initial peroxisomal blood tests. Brain MRI showed typical abnormalities as observed in the neonatal adrenoleukodystrophy variant of infantile peroxisomal disorders. Our aim was to evaluate the accuracy of this MRI diagnosis with further peroxisomal testing.

Methods: We searched our database of unclassified leukoencephalopathies and found 6 such patients. We collected clinical data and scored available MRIs of these patients. We performed further peroxisomal studies in fibroblasts, including immunofluorescence microscopy analysis with antibodies against catalase, a peroxisomal matrix enzyme. We performed complementation analysis and analyzed the suspected genes.

Results: We confirmed the diagnosis of Zellweger spectrum disorder in 3 patients and D-bifunctional protein deficiency in the others. The clinical findings were within the spectrum known for these diagnoses. Sequential MRIs showed that the abnormalities started in the hilus of the dentate nucleus and superior cerebellar peduncles. Subsequently, the cerebellar white matter and brainstem tracts were affected, followed by the parieto-occipital white matter, splenium of the corpus callosum, and posterior limb of the internal capsule. Eventually, all cerebral white matter became abnormal. The thalamus was typically affected as well.

Conclusions: If MRI reveals abnormalities suggestive of infantile onset peroxisomal defects, negative peroxisomal blood tests do not exclude the diagnosis. Further tests in fibroblasts should be performed, most importantly immunofluorescence microscopy analysis with antibodies against catalase to stain peroxisomes. *Neurology*® 2012;78:1304-1308

GLOSSARY

DBP = D-bifunctional protein; **DHAPAT** = dihydroxyacetonephosphate-acyltransferase; **DHCA** = dihydroxycholestanic acid; **IRD** = infantile Refsum disease; **NALD** = neonatal adrenoleukodystrophy; **THCA** = trihydroxycholestanic acid; **VLCFA** = very long-chain fatty acid; **ZS** = Zellweger syndrome; **ZSD** = Zellweger spectrum disorder.

Clinically, Zellweger spectrum disorders (ZSDs) and the single peroxisomal β -oxidation defects acyl-CoA oxidase 1 (ACOX1) and D-bifunctional protein (DBP) deficiency resemble each other.¹⁻⁵ They cannot always be distinguished without laboratory tests. The clinical syndromes comprising the ZSDs, i.e., Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD), were described before their biochemical and molecular bases were understood.^{4,5}

This article is focused on the milder NALD-like variant of ZSDs, and ACOX1 and DBP deficiency. Most patients with these disorders present at or soon after birth, but reach some milestones before neurologic deterioration starts between 1 and more than 7 years.¹⁻⁵ MRI in

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Supplemental data at www.neurology.org

Supplemental Data



From the Department of Child Neurology (M.S.v.d.K., N.I.W.), VU University Medical Center, Amsterdam, the Netherlands; Department of Child Neurology (E.W.), Birmingham Children's Hospital, Birmingham, UK; Department of Medical Genetics (P.F.), Alberta Children's Hospital, Calgary, Canada; Department of Child Neurology (M.T.), Hacettepe Children's Hospital, Ankara, Turkey; and Departments of Paediatrics and Clinical Chemistry (R.J.A.W., H.R.W., S.F.), Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

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these disorders shows perisylvian polymicrogyria or no gyral abnormalities.⁶ The earliest changes related to demyelination are seen in the cerebellum and subsequently involve brainstem, posterior limb of the internal capsule, and posterior cerebral white matter.⁶

Biochemical abnormalities in ZSDs include increased plasma levels of very long-chain fatty acids (VLCFAs), phytanic and

pristanic acid, bile acid intermediates di- and trihydroxycholestanic acid (DHCA and THCA), and decreased erythrocytes levels of plasmalogens.^{4,5} The abnormalities in DBP deficiency are the same except for normal plasmalogen levels.¹ ACOX1 deficiency is characterized by the exclusive accumulation of VLCFAs.² Peroxisomal parameters can also be studied in fibroblasts, including investigation of number and size of peroxisomes by immunofluorescence microscopy.¹

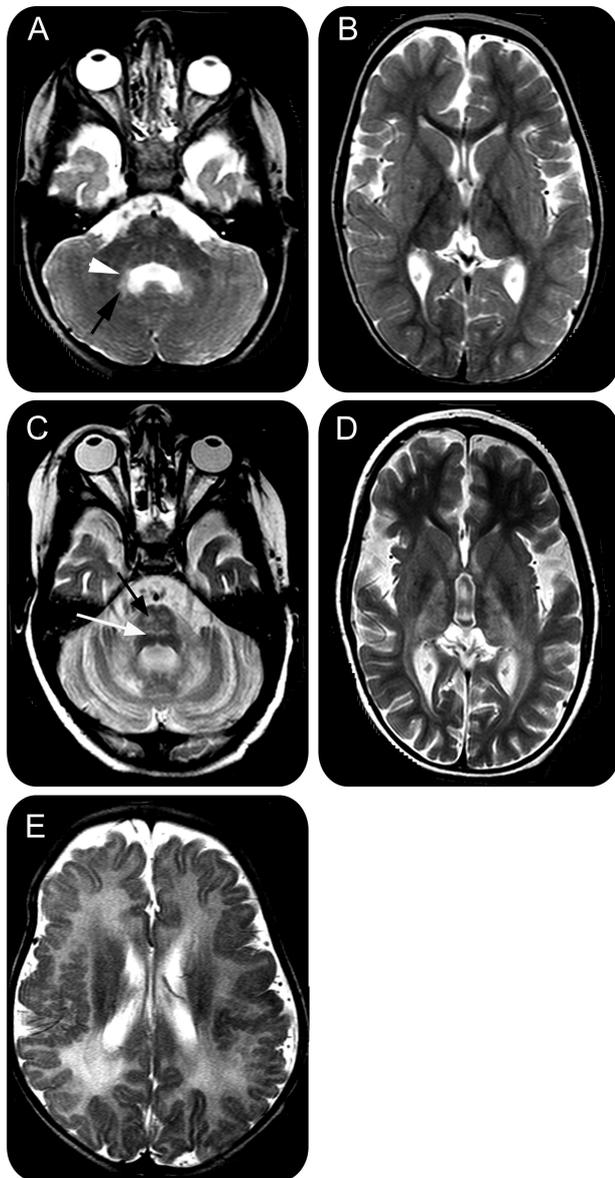
We observed patients with the clinical and MRI phenotype of an infantile onset peroxisomal disorder, but without convincing abnormalities of peroxisomal parameters in plasma. We decided to evaluate the accuracy of the MRI diagnosis with further peroxisomal testing.

METHODS This retrospective study received approval of the institutional review board with waiver of informed consent. We searched our database of approximately 3,000 patients with a leukoencephalopathy of unknown origin with the following inclusion criteria: 1) MRI features typical of the NALD variant of ZSDs, ACOX1 or DBP deficiency, as outlined in the introduction; and 2) normal or only mildly abnormal peroxisomal parameters in blood (nonfasting samples) as determined by different laboratories, including plasma VLCFAs, phytanic and pristanic acid, bile acids, and plasmalogens in erythrocytes. We collected clinical data and evaluated available MRIs of these patients.

Studies in cultured fibroblasts, performed at the Laboratory Genetic Metabolic Diseases, Amsterdam, included 1) determination of VLCFA profile; 2) measurement of enzymatic activity of dihydroxyacetonephosphate-acyltransferase (DHAPAT); 3) immunofluorescence microscopy analysis using antibodies against catalase, a peroxisomal matrix enzyme; and 4) measurement of peroxisomal α - and β -oxidation. When these studies revealed a ZSD, complementation testing was performed in fibroblasts to identify the associated gene,⁷ followed by molecular analysis of this gene. When these studies revealed a single enzyme deficiency, activity of the peroxisomal β -oxidation enzymes DBP¹ or ACOX1² was determined, followed by molecular analysis of *HSD17B4* for DBP deficiency and *ACOX1* for ACOX1 deficiency.

RESULTS Clinical features. Six patients fulfilled the above MRI and laboratory criteria. Clinical details are summarized in table e-1 on the *Neurology*[®] Web site at www.neurology.org. Patient 1 has been described before.⁸ Patients 2 and 6 are brothers. Four patients presented after birth with seizures. Early development was severely delayed in 3 patients: mildly delayed in 1, who achieved walking with support; and normal in 2, who achieved unsupported walking. Onset of deterioration in our patients was around 2 years. Patients became spastic and ataxic and lost all developmental milestones. Sensorineural

Figure 1 D-bifunctional protein deficiency



Axial T2-weighted images of patient 2 at 2 years, 4 months (A, B) and 4 years, 3 months (C, D), and his brother at 1 month (E). The first MRI shows mild signal abnormalities in the hilus of the dentate nucleus (black arrow in A) and superior cerebellar peduncles (white arrowhead in A). There are no evident supratentorial signal abnormalities (B). Follow-up MRI shows signal abnormalities in the hilus of the dentate nucleus, cerebellar white matter, superior and middle cerebellar peduncles, medial lemniscus (white arrow in C), pyramidal tracts (black arrow in C), parieto-occipital white matter, posterior limb of the internal capsule, and thalami. Early MRI in the brother shows bilateral polymicrogyria in the area of the Sylvian fissure (E).

deafness, decreased vision, and polyneuropathy occurred in some.

Initial laboratory results. Laboratory findings are summarized in table e-2. In 5 patients, initial testing of peroxisomal parameters in blood revealed no or little evidence of peroxisomal dysfunction. In particular, VLCFAs were either normal or mildly elevated with abnormal C24:0/C22:0 and C26:0/C22:0 ratios. In patient 5, mildly increased levels of VLCFAs, phytanic acid, and pristanic acid were measured, but initial biochemical studies in fibroblasts were normal.

MRI abnormalities. MRI findings are summarized in table e-3. Patients 2 and 6 underwent neonatal MRI. In patient 2, this MRI was not available but reported as normal. Patient 6 was the only one with perisylvian polymicrogyria (figure 1E). Five patients underwent MRI after the onset of neurologic deterioration between ages 2 and 3½ years. Delayed myelination and signal abnormalities in the hilus of the dentate nucleus and the superior cerebellar peduncles were seen in all (figures 1 and 2). Four patients had more

extensive brainstem, cerebellar, and cerebral white matter abnormalities, especially the parieto-occipital white matter and posterior part of the corpus callosum.

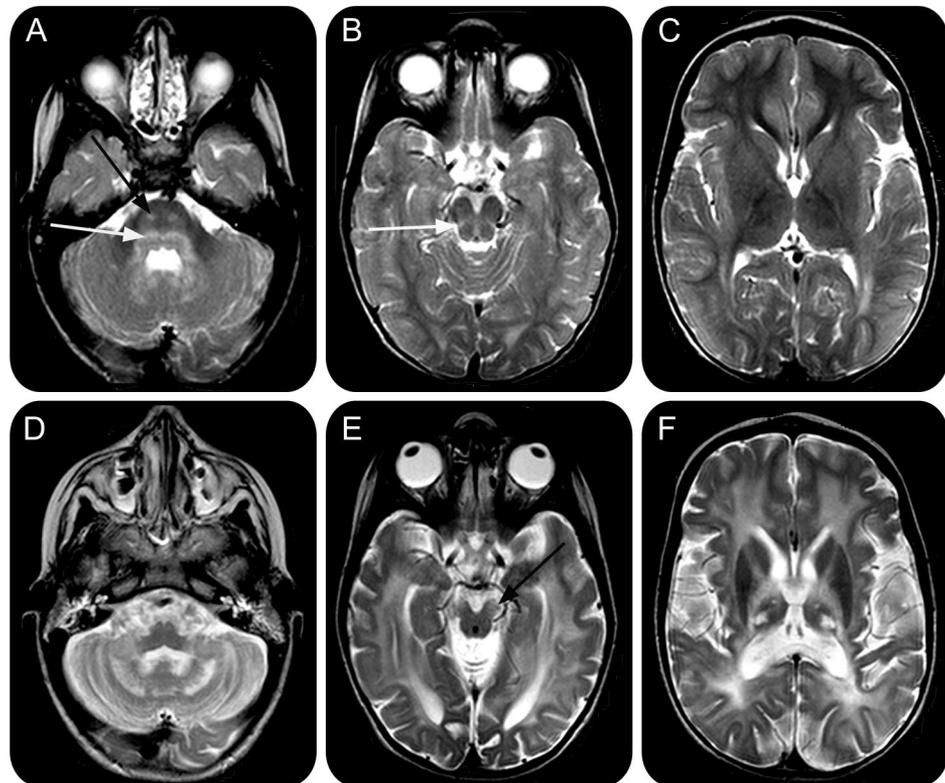
Four patients had follow-up MRIs 1–2 years later (figures 1 and 2). These showed diffuse abnormalities of the brainstem, cerebellar and cerebral white matter. The thalamus became affected in all patients. Two patients received contrast; no enhancement was found.

These MRI findings indicated that the patients most likely had a peroxisomal disorder and instigated further studies.

Peroxisomal testing in fibroblasts. Although also in fibroblasts some parameters, like VLCFAs, were normal or only mildly abnormal, immunofluorescence microscopy analysis showed aberrant peroxisomal staining in all cell lines (table e-2).

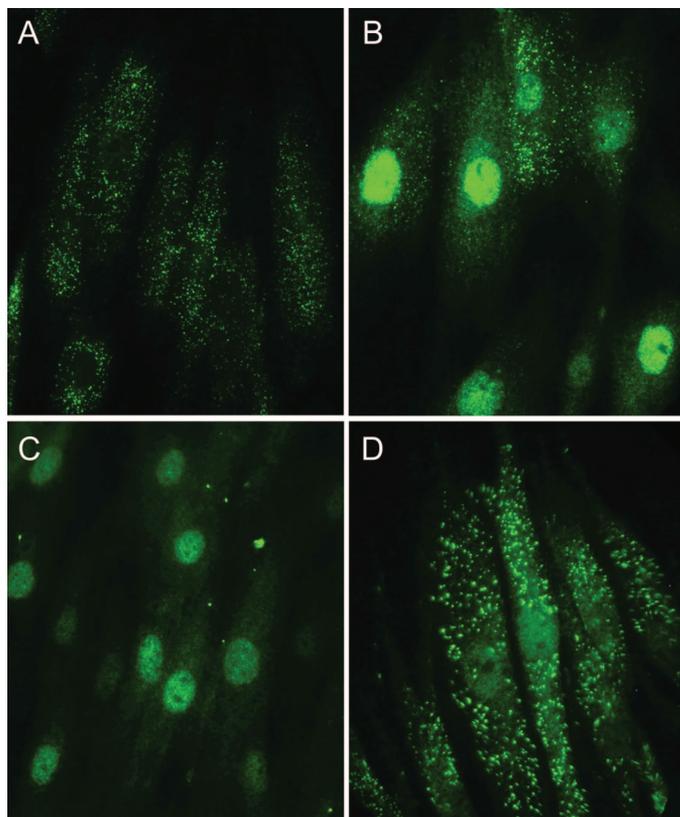
In cells from patients 3, 4, and 5, a mosaic pattern was seen with cells with peroxisomal staining, cells with a reduced number of peroxisomes, and cells without peroxisomal staining (figure 3B). When cultured at 40°C, cells lost all peroxisomal staining (fig-

Figure 2 Zellweger spectrum disorder



Axial T2-weighted images of patient 3 at 3 years, 4 months (A–C) and 5 years, 4 months (D–F). The first MRI shows signal abnormalities in the hilus of the dentate nucleus, superior cerebellar peduncles, medial lemniscus (white arrows in A and B), and pyramidal tracts (black arrow in A). There are beginning signal abnormalities in the occipital white matter (C). Follow-up MRI shows signal abnormalities in the hilus of the dentate nucleus and cerebellar white matter (D), pyramidal tracts (black arrow in E), temporal (E), parieto-occipital (F), and frontal (F) white matter, splenium of the corpus callosum (F), posterior limb of the internal capsule (F), and thalami (F).

Figure 3 Immunofluorescence microscopy



Immunofluorescence microscopy analyses using antibodies raised against catalase in a control cell line cultured at 37°C (A), in cells from a patient with Zellweger spectrum disorder cultured at 37°C revealing a mosaic pattern with cells with peroxisomal staining and cells without peroxisomal staining (B), in cells from the same patient but cultured at 40°C revealing the absence of peroxisomal staining (C), and in cells from a D-bifunctional protein-deficient patient revealing a reduced number of enlarged peroxisomes (D).

ure 3C), indicative of ZSD.⁹ Complementation testing revealed defects of PEX13 for patients 3 and 4, and PEX6 for patient 5. Molecular studies in patient 4 revealed a homozygous mutation in *PEX13* and in patient 5 a homozygous mutation in *PEX6* (table e-2). In patient 3, no mutations were identified in the coding region of *PEX13*.

Immunofluorescence microscopy of cells from patients 1 and 6 revealed a reduced number of enlarged peroxisomes, indicative of a single peroxisomal β -oxidation defect (figure 3D). Enzyme analysis revealed deficient activity of the dehydrogenase component of DBP in patient 1, and deficient activity of both the hydratase and dehydrogenase components of DBP in patients 2 and 6. In all 3 patients mutations were found in *HSD17B4* (table e-2).

DISCUSSION The clinical picture of the present patients was suggestive of the NALD variant of ZSDs or single peroxisomal β -oxidation defects. Limited peroxisomal testing performed in different laboratories revealed no convincing abnormalities. In all patients MRI showed typical abnormalities associated

with ZSDs or single peroxisomal β -oxidation defects, which prompted further testing in fibroblasts. A peroxisomal defect was confirmed in all. A similar case has been published recently.¹⁰ These observations show that if MRI indicates a ZSD or single peroxisomal β -oxidation defect, negative initial peroxisomal tests do not rule out such disease, and that further peroxisomal studies in fibroblasts and molecular analyses to identify the gene defect are warranted.

Additionally, our study provides information on the sequence of demyelination in the NALD variant of ZSD and single peroxisomal β -oxidation defects. It starts in the hilus of the dentate nucleus and superior cerebellar peduncles, the outflow tracts of the dentate nucleus. Subsequently, the cerebellar white matter and brainstem are affected, especially the pyramidal tracts, medial lemniscus, and superior, middle, and inferior cerebellar peduncles. Next, the parieto-occipital white matter with relative sparing of the U-fibers, the splenium of the corpus callosum, and the posterior limb of the internal capsule are affected. The demyelination is progressive and finally involves all cerebral hemispheric white matter. The thalamus is typically affected as well. No contrast enhancement occurs.

AUTHOR CONTRIBUTIONS

Dr. van der Knaap conceptualized the study; has access to all the data; takes responsibility for the data; participated in the design of the study; interpreted the MRIs; collected, analyzed, and interpreted the data; and participated in drafting the manuscript. Dr. Wassmer, Dr. Wolf, Dr. Ferreira, and Dr. Topçu helped collect patient data and revised the manuscript for intellectual content. Dr. Wanders supervised the laboratory analyses and interpretation and revised the manuscript for intellectual content. Dr. Waterham performed the genetic analyses in the patients and revised the manuscript for intellectual content. Dr. Ferdinandusse participated in the design of the study, performed and interpreted the laboratory analyses, and participated in drafting the manuscript.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. **Go to Neurology.org for full disclosures.**

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