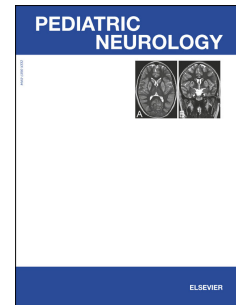


# Accepted Manuscript

D-bifunctional protein deficiency – a cause of neonatal onset seizures and hypotonia

João Nascimento, Céu Mota, Lúcia Lacerda, Sara Pacheco, Rui Chorão, Esmeralda Martins, Cristina Garrido



PII: S0887-8994(15)00037-5

DOI: [10.1016/j.pediatrneurol.2015.01.007](https://doi.org/10.1016/j.pediatrneurol.2015.01.007)

Reference: PNU 8573

To appear in: *Pediatric Neurology*

Received Date: 11 November 2014

Revised Date: 12 January 2015

Accepted Date: 17 January 2015

Please cite this article as: Nascimento J, Mota C, Lacerda L, Pacheco S, Chorão R, Martins E, Garrido C, D-bifunctional protein deficiency – a cause of neonatal onset seizures and hypotonia, *Pediatric Neurology* (2015), doi: 10.1016/j.pediatrneurol.2015.01.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Abstract**

Background: Peroxisomal disorders are classified in two major groups: (1) Peroxisome Biogenesis Disorders and (2) single Peroxisomal Enzyme/Transporter Deficiencies. D-bifunctional protein deficiency (DBP; OMIM #261515) included in this last group of rare diseases leads to an impaired peroxisomal beta-oxidation. D-bifunctional protein deficiencies are classified in four types based on the degree of activity of the 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase protein units.

Case report/Result: The authors present the first portuguese reported type II DBP deficiency patient, whose neonatal clinical picture is indistinguishable from a Zellweger spectrum disease. The clinical features and the neuroimaging findings of polymicrogyria raised suspicion of the diagnosis. After biochemical analysis, DBP deficiency was confirmed with the identification of p.Asn457Tyr (N457Y) mutation, present in homozygosity in *HSD17B4* gene. Parents were found to be carriers of the mutated allele, confirming the patient homozygosity status and allowing prenatal diagnosis to future pregnancies.

Conclusion: D-bifunctional protein deficiency is a rare and severe disease and final diagnosis can only be accomplished after *HSD17B4* gene sequencing. Treatment is generally of supportive nature, aimed at improving nutrition and growth, controlling the central nervous system symptoms and limiting the eventual progression of liver disease.

**Compliance with Ethics Guidelines and Acknowledgements section****Conflict of Interest:**

João Nascimento, Maria Céu Mota, Lúcia Lacerda, Sara Pacheco, Rui Chorão, Esmeralda Martins and Cristina Garrido declare that they have no conflicts of interest.

**Informed Consent:**

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from patient legal representatives to send the article. Informed consent was obtained from the patient parents to send the pictures.

**Author's contribution:**

**João Nascimento** - planned, conducted, and reported the work described in the article.

**Maria Céu Mota** – photo selection and reported the clinical work described in the article.

**Lúcia Lacerda** – planned, reported and revised biochemical and molecular work described in the article.

**Sara Pacheco** – reported the biochemical work described in the article.

**Rui Chorão** - The help of the neurophysiologist Rui Chorão was important to obtain and interpret the electroencephalograms. The authors want to include him as a co-author of the manuscript since he has chosen the EEGs and he wrote the Figure 3 legend. He also revised the clinical work described in the article.

**Esmeralda Martins** –reported and revised the clinical work described in the article. Photo selection.

**Cristina Garrido** - planned, conducted, and reported the clinical work described in the article. Photo selection.

**Corresponding author:** João Miguel Nascimento

Email address: [nascimentojoao10744@gmail.com](mailto:nascimentojoao10744@gmail.com)

## Introduction

Peroxisomal disorders are currently classified in two major groups: (1) Peroxisome Biogenesis Disorders and (2) single Peroxisomal Enzyme / transporter Deficiencies<sup>1-4</sup>. D-bifunctional protein deficiency (DBP; OMIM #261515) included in this last group of diseases leads to impaired peroxisomal beta-oxidation.

Peroxisomes catalyze the beta-oxidation of a different set of fatty acids and this process involves the participation of several enzymes, including two acyl-coenzyme A (CoA) oxidases, two multifunctional enzymes and two thiolases<sup>3,4</sup>.

Enoyl-CoA esters of very-long-chain fatty acids (VLCFAs) like hexacosanoic acid (C26:0), pristanic acid and the bile acid intermediates di- and trihydroxycholestanoic acid (DHCA and THCA) are all handled by D-bifunctional protein. This 79 kDa protein, also called multifunctional protein 2, catalyzes the second (hydration) and third (dehydrogenation) steps of peroxisomal beta-oxidation. This protein also contains a sterol-carrier protein-2-like domain<sup>2,3</sup>.

D-bifunctional protein deficiencies are classified into three types: type I - deficiency of 2-enoyl-CoA hydratase unit and 3-hydroxyacyl-CoA dehydrogenase unit, type II - isolated hydratase deficiency and type III - isolated dehydrogenase deficiency<sup>2-4</sup>. Recently, *McMillan et al* (2012) proposed a type IV phenotype, based on the presence of a missense mutation in each of enzyme domains resulting in markedly reduced but detectable hydratase and dehydrogenase activity of DBP<sup>5</sup>.

A large cohort study, showed that type III was the most represented deficiency (45%), followed by type II (28%) and type I (27%)<sup>4</sup>. All three types are inherited in an autosomal recessive manner due to mutations in the gene (*HSD17B4*), mapped to chromosome 5q23.1<sup>3,4</sup>. Although prognosis is usually poor, with a mean age of death of 17.6 months on type III, 10.7 on type II and 6.9 months on type I, there are some reported patients which survive until the second decade of life<sup>4</sup>.

The value of this article is two-fold. Firstly, remind the wide spectrum and etiology of peroxisomal disorders and secondly to explain step by step the diagnostic evaluation of this rare disease. Obtaining a specific mutation diagnosis does not help with patient treatment but is crucial for family counseling and future prenatal diagnosis.

## Case report

The patient, a male born after 35 weeks of gestation by vaginal delivery, was the first child of a non-consanguineous couple. Family history was unremarkable. There was no reported information regarding exposure to known teratogens during pregnancy. Routine prenatal ultrasound screening was normal and the majority of congenital infections were excluded after serological prenatal testing.

Newborn needed resuscitation intervention after birth, with positive airway pressure due to apgar scores of 5 and 6 at 1 and 5 minute, respectively. Despite apgar score increase to 8 at 10 minute, the newborn was sent to the neonatal intensive care unit (NICU) due to severe hypotonia. Birth weight was 2080g (P10 Fenton chart), length 47.5cm (P50-90 Fenton chart) and occipital-frontal circumference was 31cm (P10-50 Fenton chart).

The first physical examination, after arrival in the NICU, revealed craniofacial dysmorphism, characterized by a high forehead, flat nasal bridge, microretrognathia, short neck and short toes (Figure 1). Severe generalized hypotonia, absence of spontaneous movements, absence of primitive reflexes and inconstant gaze were present since first day of life.

During the time in NICU, he was asymptomatic for two days. At 3 days of life, subtle clonic movements of the left arm and leg were noticed, but the amplitude integrated electroencephalogram (aEEG) monitoring was insensitive and did not detect any abnormality. Coincidental with these seizure episodes he developed respiratory distress and needed nasal continuous airway pressure for 6 days.

Cerebral ultrasound at 4th day of life was normal, but magnetic resonance imaging (MRI) at day 10th revealed cortical mantle malformation in both cerebral hemispheres and a cortical dysplastic area with polymicrogyria, more pronounced in the perisylvian region (Figure 2).

At day 20th, he developed seizures characterized by initial crying with conjugate eye deviation to the right and myoclonus of the left eyelid, followed by chewing episodes with sialorrhea that lasted 1 minute. By that time, the electroencephalogram displayed multifocal epileptiform discharges with a burst-suppression pattern, which was improved after phenobarbital treatment (Figure 3).

By the end of the second month, there was aggravation with the development of clusters of epileptic seizures, characterized by clonic movements of the limbs with right head shifting. Phenytoin and levetiracetam were added at this time.

During the first two months of live, periodic respiration was observed along with several apnea episodes. Continuous positive airway pressure (CPAP) was initiated. After swallowing therapy, bottle-feeding was achieved, by the end of second month.

At 3 months, he restarted seizures, characterized by clonic movements of the left limbs and valproic acid was introduced. However one week later, he started infantile spasms and vigabatrin was added. Two weeks later, focal seizures were registered and carbamazepine treatment was associated.

At 11 months of age, in spite of being on combined treatment of valproate, carbamazepine and vigabatrin, parents reported a few daily brief focal seizures without cyanosis. On physical examination, the patient presented severe hypotonia without head control and poor spontaneous movements. He had horizontal nystagmus and an inconstant fixation and tracking of visual stimulus. Ophthalmological investigation did not detect any structural abnormalities, but the patient was unable to follow a moving object.

The newborn hearing screening revealed deafness confirmed later on, at 5 months, by auditory evoked potentials.

Prenatal laboratory studies including TORCH (toxoplasmosis, syphilis, rubella, cytomegalovirus, herpes simplex) serology, blood sugar, transaminases thyroid hormones and cortisol were normal. Karyotype was 46 XY and the fluorescence in situ hybridization (FISH) test for chromosome 15 and 22 was normal. Neonatal screening for inborn errors of metabolism was performed. Blood lactate:pyruvate ratio, blood ammonia and plasma/urine amino acids levels were below the highest age-related reference value.

A peroxisomal disorder or a congenital disorder of glycosylation was suspected due to the central hypotonia with craniofacial dysmorphism, epilepsy and the previously normal laboratory results. Most frequent congenital disorders of glycosylation were excluded by serum transferrin isoelectric focusing pattern that was found to be normal. The presence of polymicrogyria in the MRI was strongly suggestive of a peroxisomal disorder, and around 1 month of age very long chain fatty acids (VLCFA) plasma levels were found to be significantly increased (C26:0 of 4.22 $\mu$ g/ml, with reference range of 0.16-0.57) (Table 1). This finding was confirmed on cultured skin fibroblasts (C26:0 of 1.01 $\mu$ g/mg of protein, with reference range of 0.03-0.18) (Table 1).

Erythrocyte plasmalogen levels were found to be normal and the activity of dihydroxyacetone-phosphate acyltransferase (DHAP-AT), a peroxisomal enzyme catalyzing the first step in ether-phospholipid biosynthesis, was also found to be normal on fibroblasts (Table 1). This last result allowed excluding a peroxisomal biogenesis disorder (Zellweger spectrum disorders or any type of rhizomelic chondrodysplasia punctata). Given these results, the patient was classified as having an enzymatic defect in peroxisomal  $\beta$ -oxidation. Although plasmatic bile acid intermediates (dihydroxy- and trihydroxycholestanoic acids) were within normal range, pristanic acid was increased (1.2 $\mu$ g/ml with reference range of 0-0.9) (Table 1).

This led the authors to consider a  $\beta$ -oxidation defect due to a D-bifunctional protein deficiency and initiate *HSD17B4* gene molecular analysis by *Sanger* sequence.

Diagnosis of a D-bifunctional protein deficiency was confirmed by the identification of a previously described deleterious mutation (*van Grunsven et al. 1999*), a c.1369A>T transversion, resulting in a p.Asn457Tyr (N457Y) substitution (gene/locus MIM number 601860.0004 allelic variant), present in apparent homozygosity<sup>7</sup>.

Parents of the patient here reported were found to be carriers of the mutated allele, confirming the patient homozygosity status and allowing prenatal diagnosis in future pregnancies.

Current treatment is of supportive nature, aimed at improving nutrition and growth, controlling the central nervous system symptoms and avoiding liver disease progression by giving oral bile acids.

## Discussion

D-bifunctional protein deficiency is a rare, but severe peroxisomal disorder, and most affected children die in the first 2 years of life. The clinical picture, indistinguishable from that of a peroxisome biogenesis defect like Zellweger syndrome, is characterized by neonatal hypotonia, seizures presenting in the first two months of life, psychomotor delay, failure to thrive, neuronal migration defects and/or demyelination<sup>4,6</sup>.

The majority of these patients, as the patient here reported, display facial dysmorphic features along with hearing or visual impairment. Suspicion of a peroxisomal disorder was raised both by the clinical features and by neuroimaging findings<sup>9-10</sup>, namely cortical dysplastic area with polymicrogyria. This is a relatively common malformation of cortical development, characterized by multiple small and partly fused gyri with abnormal cortical lamination. The different forms of polymicrogyria encompass a wide range of clinical, aetiological (prenatal infections, cerebral vascular insults, maternal drug ingestion, loss of a twin *in utero*, genetically determined syndromes) and histological findings. Advances in imaging studies have improved the diagnosis and classification of this condition and region specific polymicrogyria syndromes have been identified<sup>10</sup>.

Patient MRI presented a combination of multiple gyri and white matter abnormalities especially over the frontal and lateral aspects of the brain, particularly in the area of the sylvian fissure. No inflammatory reaction was observed in the areas of disturbed myelination.

These findings are present in other D-bifunctional protein deficiency reported cases and resemble those of Zellweger syndrome<sup>9-10</sup>. Recent data indicate that this overlap with Zellweger syndrome may not be due to deficiencies of substrate degradation by the D-bifunctional protein enzyme, but rather to other secondary peroxisomal deficiencies that occur as a consequence of the DBP defect<sup>6,7</sup>.

Despite most D-bifunctional protein deficiency patients have plasma accumulation of VLCFA, pristanic acid and bile acid intermediates, there have been some reports identifying patients without plasma abnormalities<sup>6</sup>. Therefore, a final diagnosis can only be established after performing molecular analysis of the *HSD17B4* gene.

The bile acid abnormalities in patients with a peroxisomal disorder have been shown to contribute to the liver disease associated with these disorders and also have been hypothesized to have an effect on the developing nervous system<sup>11,12</sup>.

Unconjugated C<sub>27</sub>-bile acid intermediates (DHCA and THCA) are especially cytotoxic and they can be reduced by treatment with C<sub>24</sub>-bile acids, which downregulate bile acid biosynthesis via activation of the nuclear receptor farnesoid X receptor (FXR), resulting in reduced transcriptional activation of cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), which is the rate-limiting enzyme in bile acid biosynthesis. Bile acid therapy will also increase bile flow by replenishing the decreased levels of C<sub>24</sub>-bile acids and it will increase the intraluminal bile acid concentration, thereby facilitating the absorption of fats and fat soluble vitamins. Bile acid therapy can alleviate intrahepatic cholestasis and may also improve some aspects of central nervous system development. However, the therapy can exacerbate the degree of hepatic steatosis and worsen the already severe mitochondrial and cellular damage in the liver<sup>12</sup>.

Studies have shown that peroxisome-deficient hepatocytes are particularly sensitive to bile acid toxicity, indicating that bile acid therapy will be more beneficial in patients with a relatively mild peroxisome biogenesis disorder (neonatal adrenoleukodystrophy or infantile Refsum disease) or patients with a single enzyme defect (enzyme  $\alpha$ -methylacyl-CoA racemase, D-bifunctional protein or sterol carrier protein X deficiency)<sup>12</sup>.

As an alternative to oral bile acid therapy, treatment with an artificial FXR ligand should be considered. These artificial FXR ligands are not harmful for the hepatocyte but they will downregulate the synthesis of the toxic C<sub>27</sub>-bile acid intermediates<sup>12</sup>.

Although D-bifunctional protein deficiency clinical presentation is considered severe (*Ferdinandusse et al, 2006*), type II and III are less severe than type I, once mutations in those types are associated to residual, rather than null enzyme activity<sup>4</sup>.

On the basis of genotype analysis, patient here reported was classified as type II DBP deficiency due to homozygosity for p.Asn457Tyr (N457Y) mutation in *HSD17B4* gene, which leads to a disturbance in folding domain of hydratase unit. This mutation, according to the 110 patients' report of *Ferdinandusse et al (2006)* was described as the most frequent within type II DBP deficiency patients, as well as the second most frequent DBP deficiency causing mutation<sup>7</sup>. The missense mutation N457Y had an allele frequency of 11% and was found in 13 patients. This mutation had



already been reported as causing an isolated defect of the enoyl-CoA hydratase domain of the D-bifunctional protein. It was firstly reported in patients born of consanguineous parents, presenting abnormalities of peroxisomal beta-oxidation with elevated very long chain fatty acids and branched chain fatty acids, but normal levels of bile acid intermediates<sup>7</sup>. Expression studies of p.N457Y mutation in *Saccharomyces cerevisiae* confirmed that is a disease-causing mutation<sup>8</sup>. Moreover, in this study, patient's fibroblast immunoblot analysis showed that D-bifunctional full-length protein levels were severely reduced and enoyl-CoA hydratase domain was undetectable within the peroxisome.

DBP deficiency is a rare disease with an estimated prevalence of 1:100 000 and a complete final diagnosis can only be accomplished after *HSD17B4* gene sequencing<sup>7</sup>.

Two other Portuguese patients have a DBP biochemical and molecular diagnosis, although they may be classified as type I e III based on their genotype.

Family pedigree performed during genetic counseling of this family revealed a common related background, as the grandparents were from the same small village. Probable ancestor consanguinity could explain why the same rare mutation was found in both parents.

**Figure legends**

**Figure 1** – Newborn with severe hypotonia and facial dysmorphism.

**Figure 2** – MRI at day 10th revealed cortical mantle malformation and polymicrogyria (★), more pronounced in the perisylvian region.

**Figure 3** – The electroencephalograms on the left side (20<sup>th</sup> day of life) displayed multifocal epileptiform activity with sharp and slow waves or spikes followed by long periods of suppression of electric activity. The electroencephalograms on the right side (30<sup>th</sup> day of life, under therapy with phenobarbital) showed scarce focal right or left central epileptiform activity and disappearance of suppression-burst pattern.

## References

- 1) Aubourg P, Wanders R. Peroxisomal disorders. *Handb Clin Neurol*. 2013; 113: 1593-609.
- 2) Moller G, van Grunsven EG, Wanders RJ, Adamski J. Molecular basis of D-bifunctional protein deficiency. *Mol Cell Endocrinol*. 2001; 171:61-70.
- 3) Wanders RJA, Waterham HR. Peroxisomal disorders: The single peroxisomal enzyme deficiencies. *Biochimica et Biophysica Acta*. 2006; 1763(12): 1707-1720.
- 4) Ferdinandusse S, Denis S, Mooyer PA, Dekker C, Duran M, et al. Clinical and biochemical spectrum of D-bifunctional protein deficiency. *Ann Neurol*. 2006; 59(1): 92-104.
- 5) McMillan HJ, Worthylake T, Schwartzentruber J, Gottlieb CC, Lawrence SE, et al. Specific combination of compound heterozygous mutations in 17 $\beta$ -hydroxysteroid dehydrogenase type 4 (HSD17B4) defines a new subtype of D-bifunctional protein deficiency. *Orphanet J Rare Dis*. 2012; 7:90
- 6) Grønborg S, Krätzner R, Spiegler J, Ferdinandusse S, Wanders RJ, et al. Typical cMRI pattern as diagnostic clue for D-bifunctional protein deficiency without apparent biochemical abnormalities in plasma. *Am J Med Genet A*. 2010; 152A(11):2845-9.
- 7) Ferdinandusse S, Ylianttila MS, Gloerich J, Koski K, Oostheim W, et al. Mutational Spectrum of D-Bifunctional Protein Deficiency and Structure-Based Genotype-Phenotype Analysis. *Am J Hum Genet*. 2006; 78(1):112-124
- 8) van Grunsven EG, Mooijer PA, Aubourg P, Wanders RJ. Enoyl-CoA hydratase deficiency: identification of a new type of D-bifunctional protein deficiency. *Hum Mol Genet*. 1999 8(8):1509-16.
- 9) van der Knaap MS, Wassmer E, Wolf NI, Ferreira P, Topcu M, et al. MRI as diagnostic tool in early-onset peroxisomal disorders. *Neurology*. 2012; 24; 78(17):1304-8.
- 10) Leventer RJ, Jansen A, Pilz DT, Stoodley N, Marini C, et al. Clinical and imaging heterogeneity of polymicrogyria: a study of 328 patients. *Brain*. 2010; 133:1415-1427.
- 11) Setchell KD, Bragetti P, Zimmer-Nechemias L, Daugherty C, Pelli MA, et al. Oral bile acid treatment and the patient with Zellweger syndrome. *Hepatology* 1992; 15(2):198-207
- 12) Ferdinandusse S, Denis S, Phyllis F, Wanders R. Bile acids: the role of peroxisomes. *J Lipid Res*. 2009; 50(11):2139-2147.

Diseases										
	ZSDs (ZS,NALD,IRD)	ACOX1 deficiency	D-BP deficiency	RCDP type 1	RCDP type 2	RCDP type 3	X-ALD	RD	SCPx	AMACRD
<b>Plasma</b>										
<b><math>\beta</math>-oxidation</b>										
Very-long chain fatty acids	↑	↑	↑ (↑)	N	N	N	↑	N	N	N
Di- and trihydroxycholestanoic acid	↑	N	N-↑ - (N)	N	N	N	N	N	↑	↑
Pristanic acid	N-↑	N	N-↑ (↑)	N	N	N	N	N	↑	↑
<b><math>\alpha</math>-oxidation</b>										
Phytanic acid	N-↑	N	N-↑ (N)	N-↑	N	N	N	↑	↑	N-↑
<b>Erythrocytes</b>										
<b>Etherphospholipid biosynthesis</b>										
Plasmalogens levels	↓	N	N	↓	↓	↓	N	N	N	N
<b>Fibroblasts</b>										
Enzyme		ACOX1	D-BP		DHAPAT	ADHAPS	ALDP	PhyH	SCPx	AMACR
DHAPAT activity	↓	N	N	↓	↓	↓	N	N	N	N
Mutated gene	PEX1,2,3,5,6,10, 12,13,14,16,19,26	ACOX1	HSD17B4	PEX7	GNPAT	AGPS	ABCD1	PAHX	SCP2	AMACR

**Table 1:** Differential diagnosis of peroxisomal disorders and patient data analysis

**Legend:** ACOX 1 - Peroxisomal acyl-coenzyme A oxidase 1; ADHAPS - Alkylidihydroxyacetone phosphate synthase; ALDP - Adrenoleukodystrophy protein; AMACRD - Alpha-methylacyl-CoA racemase; D-BP - D-bifunctional protein; DHAPAT - Dihydroxyacetonephosphate acyltransferase; IRD - Infantile refsum disease; NALD - Neonatal adrenoleukodystrophy; PhyH - Phytanoyl-CoA 2-hydroxylase; RCDP - Rhizomelic chondrodysplasia punctata; RD - Refsum disease; SCPx - sterol carrier protein X; ZD - Zellweger disease



