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Case Report

Primary adrenal insufficiency in two siblings with D-bifunctional protein deficiency

Cristel C. Chapel-Crespo[a,b,*], Ricardo Villalba[c], Raymond Wang[a,b], Monica Boyer[a,b], Richard Chang[a,b], Hans R. Waterham[d], Jose E. Abdenur[a,b]

[a] CHOC Children's, Division of Metabolic Disorders, Orange, CA, USA
[b] University of California, Irvine, Department of Pediatrics, Irvine, CA, USA
[c] Baystate Children's Specialty Center, Springfield, MA, USA
[d] Laboratory Genetic Metabolic Diseases, Academic Medical Center at the University of Amsterdam, Amsterdam, The Netherlands

1. Introduction

Peroxisomal D-bifunctional protein (DBP) deficiency is a rare single enzyme disorder of peroxisomal fatty acid beta-oxidation caused by biallelic pathogenic variants in the *HSD17B4*. DBP is composed of three domains, 2-enoyl-coenzyme-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and sterol carrier protein-2-like (3). Deficiency of either or both (hydratase and dehydrogenase) results in impaired catabolism of very long-chain fatty acids (VLCFA), di- and trihydroxycholestanoic acids (DHCA and THCA) and pristanic acid.

Classic clinical presentation is similar to Zellweger Syndrome with neonatal onset seizures, hypotonia, dysmorphic features and brain MRI abnormalities. However, due to increased availability of whole exome sequencing milder/atypical phenotypes presenting with juvenile onset adrenoleukodystrophy, sensorineural hearing loss, hypogonadism, cerebellar ataxia and/or peripheral neuropathy have been described [1–5].

Biochemical diagnosis of DBP deficiency is based on plasma accumulation of VLCFA, DHCA, THCA, and pristanic acid. However, milder cases have been reported with normal or atypical biochemical profiles [6]. Therefore, most cases, especially atypical forms, are currently being diagnosed by molecular testing. There is no current treatment for this disorder and management is supportive and aimed at screening for treatable complications.

Individuals with DPB deficiency are at risk of developing adrenal dysfunction due to the marked clinical overlap with peroxisomal biogenesis disorders, DBP homology with steroid converting enzyme and previously reported changes in the adrenal cortex of patients with DBP deficiency [7–12]. However, in the over 130 cases of DBP deficiency described in the literature there has been limited report to date of this disease manifestation.

Here we report the clinical, biochemical and molecular characteristics of two siblings with DBP deficiency and adrenal insufficiency. In addition, we provide information about long-term follow-up and treatment in one of the patients.

2. Methods

This study was granted a CHOC Children's IRB review exemption due its status as a single-family, anonymized chart review and minimal risk. Retrospective chart review of the patients was performed. VLCFA, plasmalogen levels, and fibroblast enzymatic studies were performed at the Kennedy Krieger Institute laboratories, Baltimore, MD using their proprietary methods. Molecular analysis of the *HSD17B4* gene was performed under research basis at the Laboratory Genetic Metabolic Disease, Academic Medical Center at the University of Amsterdam as previously published [12].

3. Case reports

3.1. Patient 1

Our first patient, male, is the first child of consanguineous Mexican parents (second cousins). He was born at 40–6/7 weeks gestation via emergency cesarean section for persistent fetal decelerations. APGAR scores were 2, 6 and 8 at 1, 5 and 10 min respectively. Resuscitation at delivery included tactile stimulation, suction, free flow oxygen, and bag and mask ventilation. Birth weight was 3.12 kg. After birth, patient was noted to have low tone and feed poorly. At around 24 h of age, he was noted to have focal seizure activity, was loaded with phenobarbital and transferred to our Institution for further evaluation and treatment. On physical exam, he was lethargic, had frontal bossing with large anterior fontanelle, was hypotonic with absent primitive reflexes including Moro, grasp, root and suck reflexes; deep tendon reflexes were + 2 and no ankle clonus was elicited. Initial laboratory tests showed elevation of liver enzymes AST (244 u/L [ref 22–58]) ALT (131 u/L [ref 11–39]) and normal electrolytes. He failed newborn hearing screen bilaterally. No structural brain abnormalities were noted on brain MRI. Patient remained in the neonatal intensive care unit (NICU) for 35 days and a g-tube was placed prior to discharge due to dysphagia and poor feeding.

* Corresponding author.
E-mail address: cristel.chapel.crespo@choc.org (C.C. Chapel-Crespo).

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Table 1
Initial D-BPD deficiency diagnostic testing. Both patients had elevated C26:1, C26:0, C26/C22, and C24/C22 ratios with normal plasmalogens. Fibroblast studies in patient 1 showed increased VLCFA levels, deficient phytanic and pristanic acid oxidation, with normal catalase solubility. Fibroblast studies were not performed in patient 2. Abnormal results are in bold; (H), value is above reference range; (L), value is below reference range.

	Patient 1	Patient 2	Control Mean ± 1 SD
Very Long Chain Fatty Acid			
C26:1 (ug/ml)	**1.41 (H)**	**1.44 (H)**	0.18 ± 0.09
C26:0 (ug/ml)	**2.25 (H)**	**1.88 (H)**	0.23 ± 0.09
C22:0 (ug/ml)	13.23	10.82	20.97 ± 6.27
C24:0 (ug/ml)	26.75	19.85	17.59 ± −5.36
C22:1 (ug/ml)	0.83	0.55	1.36 ± 0.79
C26/C22	**0.17 (H)**	**0.172 (H)**	0.01 ± 0.004
C24/C22	**2.022 (H)**	**1.835 (H)**	0.84 ± −0.1
Plasmalogen:Fatty Acid Ratio			
C16:0 DMA/C16:0	0.102	0.084	0.079–0.128
C18:0 DMA/C18:0	0.207	0.212	0.199–0.284
Pipecolic Acid			
Urine Pipecolic Acid (umol/g creatinine)	**261.1 (H)**		26.8 ± 15.2
Cultured skin fibroblasts studies			
C22:0 (ug/mg protein)	0.406		0.68 ± 0.26
C26:0 (ug/mg protein)	**0.645 (H)**		0.06 ± 0.01
C26:1 (ug/mg protein)	**0.379 (H)**		0.08 ± 0.02
C26:0/C22:0 (ug/mg protein)	**1.594 (H)**		0.10 ± 0.55
Catalase (%soluble)	30.6		57 ± 11.1
Peroxisomal substrate oxidation			
Pristanic Acid oxidation (% of mean control value)	**12.7 (L)**		100
Pristanic acid oxidation (pmol/48 h/mg protein)	**58.9 (L)**		463.8 ± 146.2
Phytanic Acid oxidation (pmol/48 h/mg protein)	**488.7 (L)**		1884 ± 275
Phytanic Acid oxidation (% of mean control value)	**25.9 (L)**		100

Diagnostic evaluation revealed elevated plasma VLCFA with normal plasmalogens. Fibroblast studies revealed elevated VLCFA content, reduced phytanic acid and pristanic acid and normal catalase solubility consistent with DBP deficiency (Table 1). Molecular testing subsequently reveled a homozygous pathogenic variant in *HSD17B4*, c.742C > T (p.Arg248Cys).

Over the ensuing six months, patient progressively deteriorated requiring tracheostomy placement due to recurrent aspiration pneumonias, obstructive sleep apnea and hypotonia leading to respiratory insufficiency. By 20 months of age he had refractory seizures, global developmental delays, severe scoliosis and neurogenic bladder requiring frequent catheterizations.

At age 2 years 9 months, routine labs showed hyponatremia (124 mEq/L [ref 135–145]) with normal potassium levels for which he was started on sodium chloride supplements (2.8 mEq of sodium/kg). Repeat labs showed improved sodium (131 mEq/L [ref 135–145]), normal potassium levels and low random cortisol (0.6 μg/dL [3.00–27.00]). Shortly thereafter he was hospitalized for pneumonia and was noted to have persistent hyponatremia (107–124 mEq/L [ref 135–145]) with poor response to IV sodium replacement, fluctuating potassium levels (3.8–6.3 mMol/L [ref 3.6–5.0]) and significant skin hyperpigmentation (lips, nipples, groin, genitals). Morning cortisol and aldosterone levels were low, renin and adrenocorticotropic hormone (ACTH) were elevated (Table 2). Based on these results, he was diagnosed with adrenal insufficiency and was started on hydrocortisone replacement with initial stress dosing of 100 mg/m^2/day. Hyponatremia improved within 48 h and he was subsequently discharged on hydrocortisone dose of 26.8 mg/m^2/day, fludrocortisone 0.05 mg tab daily, NaCl 60 mEq daily (4.3 mEq/kg/day). Hydrocortisone sodium succinate (Solu-Cortef® 100 mg/2 ml, 1 ml = 50 mg IM) was prescribed for acute illness.

Table 2
Adrenal insufficiency diagnostic testing. Patient 1 after presenting with hyponatremia at 2 y 9 m had inappropriately low cortisol and aldosterone with high ACTH and renin levels. Patient 2 was prospectively screened for adrenal insufficiency. Initial AM and random cortisol levels were normal. At 23 months she had a suboptimal response to cosyntropin stimulation test and at 25 months had elevated ACTH consistent with the diagnosis of adrenal insufficiency. Renin levels were normal. Abnormal results are in bold; (H), value is above reference range; (L), value is below reference range; (AM), morning.

Endocrine Testing	Patient 1	Patient 2	Reference Range
ACTH	**940 (H)**	**327 (H)**	16–48 pg/mL
Cortisol, AM	**0.2 (L)**	10	5–27 μg/dL
Cortisol, Random	**0.6 (L)**	15.6	3–27 μg/dL
Cortisol, post-Cosyntropin	N/A	**14.1 (L)**	> 18 μg/dL
Renin	**31 (H)**	3.6	1.7–11 ng/mL/h
Aldosterone	**< 1 (L)**	N/A	7–93 ng/dL

Long term follow-up of his adrenal insufficiency was difficult, due to frequent intercurrent illnesses and hospital admissions requiring multiple changes in medications. His hydrocortisone maintenance dose was titrated to 16 mg/m^2/day while ACTH remained mildly elevated (80 pg/mL) or even normalized at times (17 pg/mL). At age 3 years 10 months, while receiving his baseline cortisol treatment, he presented to the emergency room for tracheostomy site bleeding that was thought to be secondary to a granuloma excision 2 weeks prior. A tracheal culture revealed *Pseudomonas aeruginosa* for which he was treated at home with ciprofloxacin. Shortly thereafter patient had a respiratory arrest and was taken to an outside hospital emergency room where he was pronounced dead. Family declined autopsy.

3.2. Patient 2

The younger sister of patient 1, was born at term 39 4/7 weeks via repeat cesarean section. Prenatal history was unremarkable and prenatal testing for DBP was declined. Her APGAR scores were 7 and 7 at 1 and 5 min respectively (poor tone, nasal flaring and subcostal retractions). Birth weight was 3.3 kg (40.5 percentile). After birth she was noted to feed poorly, and due to family history, was immediately transferred to our institution for evaluation of possible DBP deficiency. Upon arrival she was noted to have relative macrocephaly, frontal bossing with normal fontanelle size and low nasal bridge. She had a 2/6 systolic murmur, a palpable liver edge and hypotonia with ineffective grasp reflex and decreased deep tendon reflexes. Initial laboratory tests revealed normal electrolytes and liver enzyme levels. An echocardiogram showed a patent ductus arteriosus. At 24 h of life the patient developed seizure activity and an EEG was abnormal, showing moderate non-specific generalized abnormalities and prominent multifocal spikes and sharp waves, compatible with a diffuse or multifocal encephalopathy. Although no actual electrographic seizures were detected, she was started on phenobarbital. A brain MRI was unremarkable. Patient remained in the NICU for a total of 14 d and a gastrostomy-tube was placed prior to discharge. Evaluation for DBP deficiency was notable for elevations of C26:0, C26:1, and C24/C22 and C26/C22 ratios, with normal plasmalogen levels (Table 1). Molecular testing revealed a homozygous pathogenic variant in *HSD17B4*, c.742C > T (p.Arg248Cys), as previously seen in her brother.

At 7 months of age, she was diagnosed with infantile spasms, which progressed to complex partial seizures after treatment with ACTH. Clinical course remained stable during the following year.

Because of her brother's history of primary adrenal insufficiency, the patient was periodically monitored for adrenal insufficiency and was prophylactically treated with stress doses of hydrocortisone when ill (Solu-Cortef® 100 mg/2 ml, 25 mg IM). At 23 months of age she had a suboptimal response to cosyntropin stimulation test (250 mCg) but was

not started on hydrocortisone replacement at the time, because morning cortisol levels remained normal (Table 2). Subsequently, at 25 months of age she had elevated ACTH (Table 2), suggesting progressive deterioration of the adrenal function, for which she was started on routine hydrocortisone replacement at 14 mg/m2/day. Around this time, she was found to have a neurogenic bladder requiring intermittent catheterization, profound sensorineural hearing loss and severe sensory and mild axonal motor peripheral neuropathies. Patient had several admissions for respiratory distress/pneumonia and at 36 months, after a prolonged admission, she became ventilator dependent requiring tracheostomy placement.

During the following years, she continued being admitted frequently for intercurrent illness and refractory seizures requiring stress doses of hydrocortisone 45–60 mg/m^2 per day.

At 5 years of age, monitoring for mineralocorticoid deficiency revealed an elevated renin level of 8.7 ng/mL/h for which she was started of fludrocortisone at 0.05 mg/day.

Patient is currently 7 years old; she is encephalopathic and gastrostomy tube and ventilator dependent. Clinical course is otherwise unchanged. She has been maintained on hydrocortisone replacement at 11 mg/m^2/day with fluctuating ACTH levels and fludrocortisone at 0.05 mg/day with normal renin levels and stable blood pressures.

4. Discussion

Primary adrenal insufficiency has been associated with peroxisomal biogenesis disorders and X-linked adrenoleukodystrophy [9,10]. It has been postulated that adrenal insufficiency is also a complication of DBP deficiency, however it has never been described and prospectively ascertained in the literature.

Here we report two siblings with DBP deficiency and similar clinical course and highlight primary adrenal insufficiency as a manifestation of this disorder.

Many patients with DBP deficiency demonstrate the severe neonatal form, which is associated with early death, around 2 y of age [12], therefore primary adrenal insufficiency might have not manifested at the time of death or may have been overlooked. In 2006, Ferdinandusse et al. found postmortem adrenal cortex atrophy in 5 of 12 patients (42%) with DBP deficiency [13]. These findings are in agreement with Watkins' description of small adrenal glands with loss of all three zones of the cortex in a patient with DBP deficiency [11]. This finding may suggest that prior to death these patients may have had laboratory evidence of adrenal dysfunction, which is consistent with our findings.

It is possible that in patient 1, who presented with severe hyponatremia at 2 years of age, the diagnosis was made possible due to the life prolonging measures requested by the family. Interestingly, prospective monitoring of patient 2, led to the diagnosis of adrenal dysfunction at around the same age, suggesting that the onset of adrenal insufficiency in patients with DBP deficiency due to homozygous c.742C > T in *HSD17B4* is at approximately 2 years of life. This variant has been previously reported in a total of 6 patients (3 homozygous and 3 in compound heterozygous state) [12,14].

The c.742C > T (p.Arg248Cys) variant has been associated with DBP deficiency type III (affecting the dehydrogenase activity alone) due to defective dimerization of two C-domains, important for substrate binding [12,15]. While there is no clinical information about the 3 patients who were homozygous [12], two siblings with who were compound heterozygous for the c.742C > T and c.46G > A, (p.Gly16Ser) were described as having neonatal onset profound hypotonia and seizures; one of the siblings survived past age 5 y with resolution of seizures and ability to crawl [14]. The third patient with compound heterozygous variants (c.742C > T and c.745 T > G, p.Trp249Gly) was also reported to survive past age 5 y, however no further clinical information is available [12]. Based on the later and the previously reported residual enzyme activity, it has been postulated that the c.742C > T variant is associated with milder disease [15]. However, based on our experience this variant in the homozygous state is associated with severe neonatal disease; and primary adrenal insufficiency in the setting of prolonged survival secondary to life prolonging measures.

Due to its higher prevalence and earlier detection through newborn screening, adrenal insufficiency in X-ALD has been better characterized [16]. In contrast, the rarity and decreased survival of DBP-deficiency makes characterization of the adrenal insufficiency more difficult. Primary adrenal insufficiency in X-ALD develops in up to 86% of males and in less than 1% of female carriers [17]. In males, onset is typically between 3 and 10 y, however as seen in our Patient 2 biochemical evidence of adrenal insufficiency may be present prior to the development of symptoms [17]. As expected for an autosomal recessive condition, no difference in onset or severity related to sex was observed in our patients. No clear genotype-phenotype correlation regarding adrenal insufficiency has been found in X-ALD, and data available in DBP-deficiency is still insufficient to study if there is any correlation. Mild *HSD17B4* defects can also manifest as Perrault syndrome, a rare polygenic condition characterized by sensory neural hearing loss and premature ovarian failure. To our knowledge adrenal insufficiency has not been reported in this syndrome. However, in lieu of our findings screening for this complication should be considered in individuals with Perrault syndrome due to *HSD17B4* defects.

The pathophysiology of adrenal insufficiency in peroxisomal defects is poorly understood. Previous studies done in X-ALD and PBD patients suggest that the accumulation of VLCFA and cholesteryl-esters in adrenocortical cells decreases ACTH receptor responsiveness and cortisol release, resulting in adrenocortical atrophy [9,18,19]. Therefore, it is possible that the same mechanism applies to adrenal insufficiency in DBP deficiency and potentially in other peroxisomal defects with accumulation of VLCFA.

5. Conclusion

Patients with DBP deficiency are at risk of developing primary adrenal insufficiency and should therefore be prospectively screened and treated. Our findings constitute a clinically important expansion of the DBP deficiency phenotype and are particularly relevant as the addition of X-ALD to newborn screening panels may also identify new patients with this condition.

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