Levodopa Response Reveals Sepiapterin Reductase Deficiency in a Female Heterozygote with Adrenoleukodystrophy

Ronald Thibert • Keith Hyland • Joe Chiles •
Steven Steinberg • Florian Eichler

Received: 14 March 2011 / Revised: 16 May 2011 / Accepted: 23 May 2011 / Published online: 16 September 2011
© SSIEM and Springer-Verlag Berlin Heidelberg 2012

Abstract We report a 4-year-old girl heterozygous for X-linked adrenoleukodystrophy (ALD) who displayed dopa-responsive motor symptoms and was subsequently diagnosed with sepiapterin reductase deficiency (*SPR*; OMIM 182125). Her father and paternal uncle had known ALD, and she was found to have elevated plasma very long chain fatty acids and a mutation in the *ABCD1* gene. She had language delay, severe hypotonia and abnormal eye movements and responded dramatically to a trial of levodopa (4 mg/kg per day). Two mutations within the gene for sepiapterin reductase were found and cultured skin fibroblasts demonstrated near zero activity of the enzyme. This case illustrates the importance of treatment trials that may give rise to biochemical clues to the underlying diagnosis, and the need to continue to search for diagnoses despite a misleading family history.

Introduction

For patients with suspected inherited disorders of metabolism the family history will often guide the initial laboratory evaluation but it can also introduce bias that delays recognition of symptom mismatch and subsequent diagnosis. Treatment trials that assess the levodopa response reveal important biochemical clues even though the patient may not display the classic symptom constellation.

Defects in monoamine biosynthesis have been associated with developmental delay, diurnal variation in symptom severity and dystonia that may improve with levodopa (Hyland 1999, 2008). Collectively, the disorders that respond to levodopa are termed dopa-responsive dystonias (DRD), the best known being GTP cyclohydrolase deficiency (Segawa's disease). In addition, defects in tyrosine hydroxylase, aromatic L-amino acid decarboxylase and sepiapterin reductase (SR) can also lead to DRD (Zorzi et al. 2002; Friedman et al. 2006; Thöny and Blau 2006; Abeling et al. 2006; Neville et al. 2005). No association with peroxisomal disorders has ever been described. We present a patient who is a female heterozygote for ALD but also carries bi-allelic alterations in the sepiapterin reductase (*SPR*) transcript and exhibits a syndrome of dopa-responsive neurological symptoms.

Communicated by: Georg Hoffmann.

Competing interests: None declared.

R. Thibert • F. Eichler (✉)

Department of Neurology, Massachusetts General Hospital,
55 Fruit Street, ACC 708, Boston, MA 02114, USA
e-mail: feichler@partners.org; rthibert@partners.org

K. Hyland • J. Chiles

Medical Neurogenetics, Atlanta, GA, USA
e-mail: khyland@mnglab.com; jchiles@mnglab.com

S. Steinberg

Kennedy Krieger Institute/Johns Hopkins, Baltimore, MD, USA
e-mail: steinbergs@kennedykrieger.org

Clinical Report

The female patient was the offspring of a gravida 2, para 2 mother. Pregnancy and delivery were uneventful. The patient was born at term via elective repeat cesarian section. She was initially evaluated at 7 months of age for axial hypotonia and eye movements which proved to be

nonepileptic on video-EEG. Her family history was significant for her father and paternal uncle having adrenomyeloneuropathy confirmed by elevated plasma very long chain fatty acids (VLCFA) and the *ABCD1* mutation c.692_694delGGGinsC.

At the time of first evaluation, she had not achieved sitting position and had poor head control. Tests of peroxisomal function, including plasma VLCFAs, phytanic acid, plasmalogens, and bile acid intermediates, along with other metabolic tests such as urine organic acids, serum amino acids, lactate, pyruvate, and biotinidase were performed. All test results were normal except for elevated plasma VLCFA, consistent with obligate carrier status. Magnetic resonance imaging (MRI) and spectroscopy (MRS) of her brain was normal as was a lumbar puncture, which was sent only for routine studies.

Over the next few years, she continued to show significant motor delay with low muscle tone and inability to sit independently. Interestingly, there were some elements of activated tone with diurnal variation. Further metabolic testing, including serum carnitine levels and acylcarnitines, and urine acylglycines, were normal. Karyotype with subtelomeric FISH was also normal.

On examination at 4 years of age, she had significant axial and appendicular hypotonia, dysarthria and preserved reflexes. Language was not well articulated due to reduced oral motor control. Her tone would become activated with excitement and continued to show a diurnal variation. A muscle, nerve, and skin biopsy was performed which was normal with no pathologic findings on electron microscopy. Nerve conduction studies performed prior to biopsy were also normal. Southern blot analysis was performed to rule out the possibility of a second *ABCD1* mutation, but revealed no large deletions.

Due to the fluctuation in her muscle tone and the diurnal nature of her symptoms, she was started on a trial of levodopa (4 mg/kg per day) at 4 years of age. Carbidopa was given together with levodopa at a ratio of 1:4. She showed a dramatic response to treatment with improved energy and tone and improved sleep. In the first weeks after initiation of treatment she developed dyskinesias. Four months after initiating treatment, the patient ambulated without assistance.

Results

Given her dramatic response to treatment, a lumbar puncture for diagnostic neurotransmitter metabolite and pterin analysis was considered but forfeited while she was on L-Dopa. We proceeded to sequence analysis of genes encoding enzymes responsible for the DRDs. No mutations were found in the GTP cyclohydrolase, tyrosine hydroxy-

lase or aromatic L-amino acid decarboxylase genes; however, two mutations were found within the sepiapterin reductase (*SPR*) gene. Both were in exon 2: one novel mutation (c.407 C>T; p.S136F) and one known pathogenic mutation (c.448 A>G; p.R150G) (Thöny and Blau 2006). The mother was heterozygous for the p.S136F mutation. The father was heterozygous for the p.R150G mutation. Measurement of SR activity in cultured skin fibroblasts showed a severe deficiency with activity being less than 1% of the control mean.

A year after commencing Sinemet, she had made further gains in motor function but only modest gains in expressive language skills. We increased levodopa (5 mg/kg per day) and began the serotonin precursor 5-hydroxytryptophan (5-HTP) (0.5 mg/kg per day). Within 4 months of these changes, she was communicating in full sentences despite continued articulation difficulties.

Discussion

This case illustrates the importance of persistence in the diagnostic evaluation of patients with a known genetic disorder whose symptoms fall outside the expected disease spectrum. Although female heterozygotes with X-ALD can show a spastic paraparesis in adulthood, severe symptoms in early childhood have not been reported in carriers (Moser et al. 2001, Maier et al. 2002, Heffungs et al. 1980). Furthermore, by testing of the healthy *ABCD1* allele we have reduced the chance of a second mutation on the opposing allele.

More importantly, our patient displayed prominent motor delay with subtle but reproducible diurnal variation. This raised the suspicion for an aberration in monoamine metabolism. The monoamine neurotransmitters include the catecholamines (dopamine, norepinephrine, and epinephrine) and serotonin. Figure 1 illustrates the key pathways involved in their synthesis and degradation. Deficiencies in the primary enzymes involved in monoamine synthesis and degradation have been described at the level of tyrosine hydroxylase, aromatic L-amino acid decarboxylase, monoamine oxidase, and dopamine beta-hydroxylase. Tetrahydrobiopterin is a necessary cofactor for both tryptophan hydroxylase and tyrosine hydroxylase. Therefore, enzymatic deficiencies that lead to reduced levels of tetrahydrobiopterin can interfere with the synthesis of the monoamine neurotransmitters. Deficiencies have been identified in each of the synthetic enzymes in the tetrahydrobiopterin pathway: guanosine triphosphate cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, and SR. The finding of biallelic alterations in the *SPR* gene and the near zero fibroblast SR enzyme activity demonstrates SR deficiency as the primary cause of our patient's symptoms.

Fig. 1 Pathway for synthesis and catabolism of dopamine and serotonin. Dopamine and serotonin are formed from tyrosine (Tyr) and tryptophan (Trp) respectively. The rate limiting enzymes are tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) and these enzymes require the cofactor tetrahydrobiopterin (BH4). In brain, BH4 is synthesized from GTP in a three step pathway with the final step being catalyzed by sepiapterin reductase (SR). SR deficiency leads to conversion of 6-pyruvoyltetrahydropterin (6PTP) to sepiap-

terin which is in turn converted to 7,8-dihydrobiopterin (BH2) (*dashed line*). SR deficiency causes decreased levels of BH4, and consequently low levels of dopamine, serotonin and their metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (HVA). *GTP* GTP cyclohydrolase; *NH₂TP* dihydroneopterin triphosphate; *PTPS* 6-pyruvoyltetrahydropterin synthase; *DHPR* dihydropteridine reductase; *5-HTP* 5-hydroxytryptophan; *AADC* aromatic L-amino acid decarboxylase; *MAO* monoamine oxidase; *COMT* catechol-O-methyltransferase

The *SPR* gene is composed of 3 exons on 2p13, and more than 19 different mutations have been identified in affected patients (Thöny and Blau 2006). Patients exhibit psychomotor retardation, inconsolable crying, neurological abnormalities (hypotonia, dystonic posturing, oculogyric crises, spasticity, tremor, ataxia, gait disorder, chorea, Parkinsonism, seizure-like movements), psychiatric symptoms (depressed affect, aggressive behavior, hypersomnolence), and occasional physical findings (microcephaly, growth deficiency) (Abeling et al. 2006, Neville et al. 2005). As with other dopa-responsive disorders, symptoms are characteristically worse in the evenings, so-called diurnal variation. The disease is hard to diagnose due to lack of peripheral markers and brain MRI is normal in most cases. CSF reveals low HVA and 5-HIAA and high levels of biopterin and dihydrobiopterin with the presence of sepiapterin (Zorzi et al. 2002). As in our case the diagnosis can be also be established by mutation analysis and confirmed by documenting low SR activity in skin fibroblast cultures.

Our patient also showed several unique features for SRD. Dystonia and oculogyric crises, in contrast to most previously reported cases, were absent. Table 1 summarizes and contrasts the core clinical features of SRD and X-ALD. As the core features of SR deficiency are nonspecific and dystonia may not always be present, it is crucial to be cognizant of subtle signs that may suggest monoamine dysfunction. Although abnormal muscle tone and motor development improved dramatically with L-dopa/carbidopa,

language skills appeared to benefit most following 5-HTP administration.

Further investigation is needed to define genotype/phenotype relationships, optimize pharmacologic therapy, understand pathogenesis of tone fluctuation and clarify the role of individual monoamines in language disturbance. SRD and other disorders of monoamine metabolism should be considered in all cases of abnormal eye movements in the setting of significant motor delay. Diurnal fluctuation is often difficult to discern in cases of infantile onset as well as severe affected individuals, as in our patient. Therapies should be directed at not only dopamine deficiency, but also other biochemical deficiencies present in this disorder.

Acknowledgment We want to thank for support by NIH grant K08NS52550 (F.E.).

Synopsis

Levodopa response reveals sepiapterin reductase deficiency in a female heterozygote with adrenoleukodystrophy.

Details of the Contributions of Individual Authors

Thibert and Eichler performed the clinical evaluation of the patient and wrote up the report. Hyland and Chiles performed the sepiapterine reductase gene sequencing and enzyme assay.

Table 1 Signs and symptoms in patients with sepiapterin reductase deficiency (SRD) versus X-linked adrenoleukodystrophy (X-ALD)

	This case	SR	X-ALD	
		Patients	Hemizygotes	Heterozygotes
Age at diagnosis	4 years	0.5–26 years	4–65 years	8–65 years
Age at first symptom	6 months	6 months	4 years	8 years
Sex	F	6 F/7 M	M	F
<i>Clinical symptoms</i>				
Motor delay	+++	+++	–	–
Motor regression	–	–	++	+
Speech delay	++	++	–	–
Speech regression	–	–	++	–
Diurnal fluctuation	+	++	–	–
Abnormal eye movements +	++	–	–	–
Tremor	–	–	+	+
Pain	–	–	+	++
Dystonia	–	++	++	–
Axial hypotonia	+++	++	+	–
Limb hypertonia	–	+	++	+
Weakness	+	+	+	+

– absent, + uncommon, ++ common, +++ very common

The frequencies are derived from published reports (Friedman et al. 2006; Moser et al. 2001; Maier et al. 2002)

Steinberg performed the lipid assay and gene sequencing for adrenoleukodystrophy and contributed to write-up.

Name of One Author Who Serves as Guarantor

Florian Eichler accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

A Competing Interest Statement

Dr. Keith Hyland is co-owner of Medical Neurogenetics, a company that provides genetic and metabolite testing for sepiapterin reductase deficiency. Mr. Joe Chiles is an employee of Medical Neurogenetics, a company that provides molecular and metabolite testing for sepiapterin reductase deficiency. None of the other authors report disclosures.

The authors confirm independence from any potential sponsors; the content of the article has not been influenced by potential sponsors.

Details of Ethics Approval

Consent was obtained from the parents to disclose the video material.

References

- Abeling NG, Duran M, Bakker HD et al (2006) Sepiapterin reductase deficiency an autosomal recessive DOPA-responsive dystonia. *Mol Genet Metab* 89:116–120
- Friedman J, Hyland K, Blau N, MacCollin M (2006) Dopa-responsive hypersomnia and mixed movement disorder due to sepiapterin reductase deficiency. *Neurology* 67:2032–2035
- Heffungs W, Hameister H, Ropers HH (1980) Addison disease and cerebral sclerosis in an apparently heterozygous girl: evidence for inactivation of the adrenoleukodystrophy locus. *Clin Genet* 18:184–188
- Hyland K (1999) Presentation, diagnosis, and treatment of the disorders of monoamine neurotransmitter metabolism. *Semin Perinatol* 23:194–203
- Hyland K (2008) Clinical utility of monoamine neurotransmitter metabolite analysis in cerebrospinal fluid. *Clin Chem* 54:633–641
- Maier EM, Kammerer S, Muntau AC, Wichers M, Braun A, Rocher AA (2002) Symptoms in carriers of adrenoleukodystrophy relate to skewed X inactivation. *Ann Neurol* 52:683–688
- Moser HW, Smith KD, Watkins P, Powers J, Moser AB. X-linked adrenoleukodystrophy, in *The metabolic and molecular bases of inherited disease*, C. Scriver, et al., Editors. 2001, McGraw Hill: New York. 3257–3301.
- Neville BG, Parascandolo R, Farrugia R, Felice A (2005) Sepiapterin reductase deficiency: a congenital dopa-responsive motor and cognitive disorder. *Brain* 128:2291–2296
- Thöny B, Blau N (2006) Mutations in the BH4-metabolizing genes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, carbinolamine-4a-dehydratase, and dihydropteridine reductase. *Hum Mutat* 27(9):870–878
- Zorzi G, Redweik U, Trippe H, Penzien JM, Thöny B, Blau N (2002) Detection of sepiapterin in CSF of patients with sepiapterin reductase deficiency. *Mol Genet Metab* 75:174–177