Chapter 7

Acute intermittent porphyria-related leukoencephalopathy

Sietske H. Kevelam, Rochus A. Neeleman, Quinten Waisfisz, Edith C.H. Friesema, Janneke G. Langendonk, and Marjo S. van der Knaap

Accepted for publication.
ABSTRACT

Objective
To identify the genetic etiology of a distinct leukoencephalopathy with autosomal recessive inheritance in a single family.

Methods
We analyzed available magnetic resonance images (MRIs) and retrospectively reviewed clinical information and laboratory investigations. We performed whole-exome sequencing (WES) to find the causal gene variants.

Results
We identified 3 family members with a similar MRI pattern characterized by symmetrical signal abnormalities in the periventricular and deep cerebral white matter, thalami, and central part of the pons. Cerebellar atrophy was noted in advanced disease stages. Clinical features were childhood-onset slowly progressive spastic paraparesis, cerebellar ataxia, peripheral neuropathy and in 2 patients optic atrophy as well as vertical gaze and convergence palsies and nystagmus. WES revealed compound heterozygous missense variants in the HMBS gene, both associated with the autosomal dominant disorder acute intermittent porphyria (AIP). Sanger sequencing of 6 healthy siblings confirmed the bi-allelic location of the variants and segregation with the disease. Patients had a slight and moderate increase in urinary and plasma porphobilinogen and 5’-aminolevulinic acid respectively and a 50-66 % decrease in hydroxymethylbilane synthase enzyme activity compared to normal.

Conclusions
Bi-allelic HMBS variants have been reported before as cause of severe encephalopathy with early childhood fatality in AIP. Our cases demonstrate childhood onset, but milder and slower disease progression in middle-aged patients. With this, a novel phenotype can be added to the disease spectrum associated with bi-allelic HMBS variants: a leukoencephalopathy with early onset, slowly progressive neurological symptomatology and long life expectancy.
INTRODUCTION

Acute intermittent porphyria (AIP) is characterized by attacks of severe abdominal pain with nausea, vomiting and hypertension and can be accompanied by psychiatric symptoms including anxiety, psychosis, and hallucinations.\textsuperscript{1,2} Attacks, which are caused by liver derived accumulation of 5’-aminolevulinic acid (ALA) and porphobilinogen (PBG), can be precipitated by drugs, alcohol, fasting, menarche, and stress.\textsuperscript{1,2} Without treatment acute porphyria attacks can progress to life-threatening hyponatremia, paralysis, epilepsy, coma, and death.\textsuperscript{1,2}

AIP is an autosomal dominant disorder caused by variants in the \textit{HMBS} gene, encoding hydroxymethylbilane synthase (HMBS, EC 2.5.1.61), also named porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.\textsuperscript{3} The carrier frequency in European countries is estimated at one in 75,000 people, but less than 10% of the carriers actually develop acute porphric attacks.\textsuperscript{1,4}

Reports of bi-allelic AIP are extremely rare and only 5 patients have been published so far.\textsuperscript{5-10} We report 3 siblings from a single family with a childhood-onset slowly progressive neurological disorder of unknown origin and a distinct leukoencephalopathy on MRI. Whole-exome sequencing (WES) revealed bi-allelic \textit{HMBS} variants segregating with the disease. We describe the phenotype, biochemical tests results, and genotype of all tested siblings, and we compare the patients’ phenotype with the published cases of bi-allelic \textit{HMBS} variants.

PATIENTS AND METHODS

Patients

Three siblings from 1 family shared a distinct pattern of MRI abnormalities. S.H.K. and M.S.v.d.K. evaluated the MRIs as previously described.\textsuperscript{11} We reviewed the clinical information and laboratory investigations retrospectively. With 6 healthy siblings and non-consanguineous parents, who never had signs or symptoms of neurological dysfunction, we considered the pedigree suggestive of an autosomal recessive disorder. We included all 6 healthy siblings for genetic segregation analysis, and 2 of them for further biochemical studies upon identification of the candidate gene. The parents were deceased and no DNA was available.
Standard protocols, registration and patient consents

The ethical standards committee approved our gene identification studies on patients with unclassified leukoencephalopathies at the VU University Medical Center in Amsterdam, the Netherlands. We received written consent from all individuals participating in this study.

Whole-exome sequencing

We performed WES in 2 affected (patient 1 and 2) and 1 unaffected sibling. We extracted genomic DNA by standard methods. We enriched exonic targets with SeqCap EZ Human Exome Library v3.0 kit (Nimblegen). We performed sequencing with 100 bp paired-end reads on a Hiseq2000 (Illumina). For data analysis we used an in-house pipeline previously described.12 We performed variant filtering under the hypothesis of an autosomal recessive inheritance pattern assuming that the variant had a minor allele frequency of < 1% in the dbSNP137 (http://www.ncbi.nlm.nih.gov/projects/SNP), NHLBI Exome Sequencing Project (ESP6400 release) (http://evs.gs.washington.edu/EVS/), 1000genomes project (release February 2012), and GoNL database13 and was not present in-house control exomes. We excluded synonymous variants that were not located adjacent to the consensus splice site.

Segregation and validation of candidate variants

We performed Sanger sequencing in the 3 patients for confirmation of the 2 variants in HMBS (NM_000190.3) and in the 6 healthy siblings for segregation analysis. Primer sequences are available on request.

Measurement of ALA and PBG in urine and plasma

We collected urine and plasma samples for patients 1 and 2, and 2 healthy siblings following standard protocols during outpatient clinic visits in the National Porphyria Center in the Erasmus MC, Rotterdam, the Netherlands, after the outcome of the WES analysis. The samples were analyzed within 24 hours or stored at -80º Celsius for later examination. Methods for measuring ALA and PBG in urine and plasma have been published.14 In short, urinary ALA and PBG are separated by ion-exchange chromatography. At pH 6–6.5, ALA is a cation and PBG an anion. The urine is passed through an anion-exchanger on top of a cation exchanger, after which interfering substances are removed by washing the columns. ALA and PBG are then eluted separately. Reaction with Ehrlich's reagent gives a red condensation product that can be measured spectrophotometrically. In plasma, ALA is converted enzymatically to PBG by added ALA-dehydratase. PBG, present in plasma or formed from ALA by the addition of ALA-dehydratase, is subsequently converted to uroporphyrinogen I by the
addition of HMBS. Both ALA-dehydratase and HMBS are present in control red blood cell lysate, from which they are derived making use of their difference in sensitivity to heat denaturation. Following oxidation of the uroporphyrinogen to uroporphyrin using ultraviolet light, the latter is measured by fluorometric assay.

**Hydroxymethylbilane synthase in erythrocytes**

HMBS catalyzes the formation of hydroxymethylbilane from 4 molecules of PBG. The tetrapyrrroles are converted to uroporphyrinogen III by uroporphyrinogen cosynthase (an essential step in the synthesis of heme) or nonenzymatically cyclized to uroporphyrinogen I. We incubated erythrocyte lysate with PBG. We measured the formed porphyrins by specific fluorescence detection. We used a coproporphyrin standard as a reference.

**Literature search**

We performed a literature search to identify published cases with compound bi-allelic AIP. A PubMed search in November 2015 with the terms, “Porphyrias”[Mesh] AND compound heterozygous (33 hits), “Porphyrias”[Mesh] AND ‘homozygous’ (169 hits), “Porphyrias”[Mesh] AND ‘encephalopathy’ (32 hits) resulted in 234 articles. Available publications with descriptions of homozygous or compound heterozygous AIP patients were included. All papers mentioned in the OMIM database for the HMBS gene were checked for other cases with bi-allelic variants. Furthermore, the reference lists of the selected articles and review articles on acute porphyria, that resulted from our literature search were scanned with this objective. Case reports without definitive diagnosis, as defined by decreased enzyme activity or gene mutation, were excluded from the case series.

**RESULTS**

**Patients, laboratory findings and neurophysiological investigations**

Patient 1, the first child, was born after an uneventful pregnancy and delivery. He had a congenital deafness, for which he received a unilateral cochlear implant at age 53 years. Initial development was unremarkable. In his teenage years he developed a slowly progressive spastic paraparesis. At latest follow-up, 58 years old, he had a normal cognition, and his vision was normal with glasses. His neurological examination showed a spastic paraparesis and a distal peripheral neuropathy of his legs. He was able to walk using a walking aid. He was independent regarding activities of daily living (ADL).

Patient 2, the second child, was born after an uneventful pregnancy and delivery. Her
gross motor development was mildly delayed; she achieved walking at 2 years. During childhood she experienced a progressive spastic paraparesis, which caused loss of unsupported walking and wheelchair dependency at the age of 4 years. She had a gradually progressive loss of vision due to optic atrophy. In the course of decades she experienced a slowly progressive neurological deterioration with also mild cognitive impairment. The latest neurological examination at age 63 years revealed clinical evidence of a peripheral neuropathy in arms and legs. She had a severe cerebellar ataxia and apraxia. Ophthalmological examination demonstrated an almost complete loss of vision, a pendular nystagmus and vertical gaze and convergence palsies. She was completely ADL dependent and lived in a nursing home. She could participate in simple conversations.

Patient 3, the third child, was also born after an uneventful pregnancy and delivery. He achieved walking at 18 months, but was never able to run. From 4 years of age, he experienced progressive vision loss due to optic atrophy and from 12 years of age he developed progressive gait problems leading to wheelchair dependency at 35 years. He developed mild cognitive impairment. At latest follow-up, at 57 years, he had a spastic paraparesis, a distal peripheral neuropathy of legs and arms, cerebellar ataxia and also apraxia. He had an almost complete loss of vision, pendular nystagmus and vertical gaze palsy. He was partially ADL dependent and could participate in simple conversations.

After identification of the \textit{HMBS} gene variants, the 3 patients and the 6 healthy siblings were questioned for AIP-related symptoms; none were reported. Preceding WES analysis routine hematologic and biochemistry tests and extensive metabolic testing were performed in urine, blood, and cerebrospinal fluid (CSF) and were unrevealing. Patient 2 and 3 had once a mildly elevated CSF lactate. Mitochondrial DNA screening in blood and muscle revealed no pathogenic point variants or deletions. Measurement of oxidative phosphorylation enzyme activities in fresh muscle biopsy was normal. Nerve conduction studies of patient 2 (at age 55 years) and patient 3 (at age 38 years) showed normal sensory and motor conduction velocities. A reduced amplitude of compound muscle action potentials was seen in patient 2, suggestive of axonal neuropathy. Somatosensory-evoked potentials of the tibial and median nerve showed no cortical response in patient 3. Two successive sural nerve biopsies performed in patients 1 and 3 revealed progressive loss of both thick and thin nerve fibers.

\textbf{MRI abnormalities}

For patient 1, one MRI was available, performed at age 43 years; patients 2 and 3 had two available MRIs, obtained at age 51 and 59 years for patient 2, and at 36 and 57 years for patient 3. The most recent MRIs are shown in Figure 1. All 3 patients had extensive,
confluent, symmetrical signal abnormalities in the periventricular and deep cerebral white matter with relative sparing of the U-fibers (Figure 1, A-L and O). In patients 2 and 3 the abnormalities were most extensive. The thalami were affected, but the basal nuclei, internal capsule and corpus callosum were spared (Figure 1, B, F and J). All patients had mild widening of the lateral ventricles and subarachnoid spaces. Patients 2 and 3 had signal abnormalities in the central part of the pons. In both patients 2 and 3, the cerebellar atrophy was more severe on recent MRIs (Figure 1, E, I, N, P) than on earlier MRIs (not shown). At most mild cerebellar atrophy was observed in patient 1 (Figure 1, M). The white matter abnormalities progressed very slowly over time.

Figure 1. MRI characteristics.
Axial T2-weighted images of patient 1 (A-D, 43 years), patient 2 (E-H, 59 years), and patient 3 (I-L, 57 years); sagittal T1-weighted images of patient 1 (M), sagittal and axial T1-weighted images of patient 2 (N, O); sagittal fluid-attenuated inversion recovery (FLAIR) image of patient 3 (P). Confluent, symmetrical T2 signal abnormalities are seen in the pons (A and E) and periventricular and deep cerebral white matter (B-D, F-H and J-L). The thalami are symmetrically affected (B, F and J). The axial T1-weighted image of patient 2 (O) shows that the corpus callosum and U-fibers are relatively spared. Cerebellar atrophy is seen in patients 2 and 3 (N and P), but is at most mild in patient 1 (M). Diffuse mild cerebral atrophy, with widening of the ventricles and subarachnoid spaces is present in all cases (B-D, F-H and J-L).
Genetic analysis
After filtering of the variants obtained by WES a single candidate gene remained. The 2 patients carried two heterozygous missense variants in HMBS: c.500G>A (p.(Arg167Gln)), c.674G>A (p.(Arg225Gln)), located in exon 10 and exon 12 respectively. Both variants are known pathogenic variants associated with AIP. Segregation analysis of the variants confirmed the bi-allelic location of the variants and the presence of both variants in all 3 patients, while the 6 healthy siblings were heterozygous for one of the variants or carried 2 wild-type alleles. No other candidate genes were identified with WES using the filtering steps described.

Biochemical analysis
Detailed results of the biochemical studies are outlined in Table 1. Patients had moderately increased values of ALA and PBG in plasma and urine, and HMBS enzyme activity was decreased within the reference range for heterozygous carriers of AIP. The healthy siblings, both carriers of the c.674G>A mutation, had normal urine ALA and PBG values, near-normal plasma ALA and PBG values and HMBS enzyme activity.

Table 1. Biochemical analysis

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Sib 1</th>
<th>Sib 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA/creatinine umol/mmol (N &lt; 4.6)</td>
<td>2.4</td>
<td>2.6</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>PBG/creatinine umol/mmol (N &lt; 1.3)</td>
<td>3.2</td>
<td>3.6</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA nmol/l (N &lt; 74)</td>
<td>72</td>
<td>158</td>
<td>98</td>
<td>77</td>
</tr>
<tr>
<td>PBG nmol/L (N &lt; 12)</td>
<td>293</td>
<td>176</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>HMBS enzyme activity in % of normal mean in erythrocytes</td>
<td>55</td>
<td>67</td>
<td>105</td>
<td>83</td>
</tr>
<tr>
<td><strong>HMBS variants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.500G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.674G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.500G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.674G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALA = 5'-aminolevulinic acid; PBG = porphobilinogen; N = normal reference range; HMBS = hydroxymethylbilane synthase

Literature search bi-allelic HMBS variants
Five patients with 2 pathogenic bi-allelic HMBS gene variants have been reported previously. All cases, including our patients, are summarized in Table 2. A patient published in 1977 by Gregor et al., who presented with a severe neurological disease and increased porphyrin precursors in urine presumably had bi-allelic HMBS variants, but no genotype was described and this patient was not included. Three of the 5 proven bi-allelic HMBS patients presented with an infantile-onset chronic, progressive neurological disease, two others had an onset during early childhood. They all
showed signs of psychomotor retardation and at least 4 died at an early age.⁵,⁸,⁹ All had an increased excretion of urinary ALA and PBG, up to a 100 fold increase relative to upper limits of the normal range, which was reflected in abnormally colored urine in all cases except for the patients reported by Llewellyn et al.⁸ All had a severely decreased erythrocyte HMBS enzyme activity (1%-17% of normal).⁵-¹⁰ Despite these findings, symptoms associated with acute porphyric attacks were not reported in any of the patients. Neurological features included a peripheral neuropathy (mixed axonal and demyelinating), cerebellar ataxia, reduced tendon reflexes and positive bilateral Babinski signs.⁵-⁹,¹⁷ MRI findings of 1 patient showed extensive signal abnormalities of the periventricular and deep white matter, sparing the thalami and pons.⁹

**DISCUSSION**

To our surprise, WES revealed bi-allelic variants in *HMBS*, known to be associated with autosomal dominant AIP, in 3 siblings with a leukoencephalopathy. All patients had a similar MRI pattern characterized by extensive symmetrical signal abnormalities in the periventricular and deep white matter, the thalami and the central part of the pons, with slow progression and late cerebellar atrophy. The clinical phenotype of the siblings, presently between 58 and 64 years old, was characterized by a childhood-onset very slowly progressive spastic paraparesis, cerebellar ataxia, peripheral neuropathy, and in 2 patients near-total vision loss due to optic atrophy. In the literature 5 other patients with bi-allelic *HMBS* variants have been reported. These patients also had neurological signs, but the onset of the disease was considerably earlier, the course more severe and the outcome less favorable (see table 2). One patient was reported to have white matter abnormalities, but without further details; the specific MRI pattern observed in our patients was not reported.⁹ Strikingly, similar to the 5 previously reported children with bi-allelic *HMBS* variants, our 3 affected siblings did not experience acute porphyric attacks.

Both variants, found in our patients, have been reported in heterozygous patients with a deleterious effect on enzyme function and occurrence of acute porphyric attacks. *In vitro* studies in *Escherichia coli* showed that the c.500G>A variant in homozygosity is associated with a residual HMBS enzyme activity of less than 2%,⁹ and X-ray crystallization studies indicated disturbances of pyrrole polymerization.¹⁹ The c.674G>A variant was previously reported in 3 family members from Sweden, one of whom was symptomatic. This woman had intermittent periods of abdominal pain and pain in shoulders and limbs. Urinary ALA and PBG ranged from normal to mildly increased.¹⁶ The authors argue
that this variant would lead to severe disruption of the enzyme structure and activity due to its importance for the conformation of the enzyme.\textsuperscript{16} In light of this information we found the biochemical results in our patients remarkable. Both urinary and plasma ALA and PBG levels were slightly to moderately increased with an 55-67\% erythrocyte HMBS enzyme activity of normal, values similar to heterozygous carriers of \textit{HMBS} gene variants outside episodes of AIP.

Previously reported patients with bi-allelic \textit{HMBS} variants had HMBS enzyme activity levels of 1-17\% of normal, with an excessive excretion of porphyrin precursors.\textsuperscript{5-10} This would implicate that our family has a new disease phenotype, caused by bi-allelic \textit{HMBS} variants, with milder neurological features than the other bi-allelic cases and biochemical results in the range of heterozygous carriers. The latter would indicate that either the reduced enzyme activity with elevated ALA and PBG levels does not cause chronic leukoencephalopathy in our patients or that the enzyme activity in erythrocytes is not comparable with brain activity.

During attacks, heterozygous carriers of \textit{HMBS} variants frequently experience neurological manifestations, most commonly motor neuropathy, but seizures, pyramidal signs, cerebellar dysfunction, transient blindness, and decreased consciousness have also been reported.\textsuperscript{4,20} Clinical presentation can mimic the Guillain-Barré syndrome.\textsuperscript{21} Chronic axonal neuropathy is seen both in heterozygous carriers of \textit{HMBS} with active AIP and in individuals with latent AIP.\textsuperscript{22,23}

Several hypotheses have been proposed to explain the acute and chronic neurological dysfunction in AIP.\textsuperscript{24} First, it has been suggested that high levels of ALA are directly neurotoxic because of the structural similarity of ALA with gamma-aminobutyric acid (GABA), a potent inhibitor of neuronal synaptic activity. The neurotoxicity of ALA has been hypothesized to be due to direct interaction with GABA-receptors or by inhibiting glutamate uptake.\textsuperscript{25,26} However, mice partially deficient for HMBS (25-30\% rest activity) develop a chronic, progressive motor neuropathy in the presence of normal or only slightly elevated plasma and urinary levels of ALA, and no acute attacks.\textsuperscript{27} This suggests that at least for the chronic neurological symptoms alternative pathomechanisms would play a role.\textsuperscript{27} Another hypothesis concerns the effect of a long-term relative shortage of heme in neurons. Heme is an important molecule involved in a variety of biological processes such as oxygen, electron transport and cytochrome p450 system. If patients with AIP have a relative chronic heme deficiency because of the disruption in the heme biosynthesis pathway, this might disrupt axonal transport leading to axonal degeneration.\textsuperscript{27} This explanation would be in line with the evidence that acute porphyric attacks can be precipitated by drugs increasing Cyp450, in this way depleting the hepatic heme pool, as heme is a substrate for Cyp450. Via a negative feedback loop
### Table 2. Overview patients with bi-allelic *HMBS* variants

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Villeneuve et al., 1964</th>
<th>Llewellyn et al., 1992</th>
<th>Llewellyn et al., 1992</th>
<th>Solis et al., 2004</th>
<th>Hessels et al., 2004</th>
<th>This report (patient 1)</th>
<th>This report (patient 2)</th>
<th>This report (patient 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Caucasian</td>
<td>British</td>
<td>British</td>
<td>Spanish</td>
<td>Turkish</td>
<td>Caucasian</td>
<td>Caucasian</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>female</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>HMBS variants (c.DNA)</td>
<td>c.500G&gt;A</td>
<td>c.499C&gt;T</td>
<td>c.500G&gt;A</td>
<td>c.499C&gt;T</td>
<td>c.500G&gt;A</td>
<td>c.500G&gt;A</td>
<td>c.500G&gt;A</td>
<td>c.500G&gt;A</td>
</tr>
<tr>
<td>Age of onset</td>
<td>8 months</td>
<td>18 months</td>
<td>18 months -18 years</td>
<td>3 months</td>
<td>birth</td>
<td>during teens</td>
<td>4 years</td>
<td>4 years</td>
</tr>
<tr>
<td>Age last follow-up</td>
<td>8 years†</td>
<td>8 years†</td>
<td>10 years</td>
<td>3 years and 3 months†</td>
<td>7 years</td>
<td>58 years</td>
<td>63 years</td>
<td>57 years</td>
</tr>
<tr>
<td>Neurological signs</td>
<td>spasticity with contractures of arms and legs</td>
<td>ataxia, dysarthria, nystagmus</td>
<td>spasticity, ataxia, peripheral neuropathy (at age 8 years)</td>
<td>decreased muscle tone and reflexes, Babinski signs, peripheral neuropathy</td>
<td>developmental delay</td>
<td>spastic paraparesis legs and symmetrical axonal neuropathy</td>
<td>spastic paraparesis legs and symmetrical axonal neuropathy</td>
<td>spastic paraparesis legs and symmetrical axonal neuropathy</td>
</tr>
<tr>
<td>Ophthalmological signs</td>
<td>n.a.</td>
<td>bilateral cataract; optic nerve atrophy</td>
<td>normal</td>
<td>bilateral cataract</td>
<td>normal vision with classes</td>
<td>optic atrophy with vision loss (2/60 OD; 1/60 OS), fine peripheral cortical cataract, nystagmus</td>
<td>optic atrophy with vision loss (3/300), nystagmus</td>
<td></td>
</tr>
<tr>
<td>Acute porphyric attacks</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>No</td>
</tr>
<tr>
<td>Course and outcome, age of death</td>
<td>no progressive, death due to seizure (8 years)</td>
<td>no progressive, death due to chronic illness (8 years)</td>
<td>no progressive, severe debilitating</td>
<td>no stable, attended school for mentally handicapped children</td>
<td>no slowly progressive, walking with and ADL independent at age 58 years</td>
<td>no slowly progressive, wheelchair at age 63 years</td>
<td>ADL dependent at age 57 years.</td>
<td></td>
</tr>
<tr>
<td>MRI WM abnormalities</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>yes</td>
<td>n.a.</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Urine ALA levels</td>
<td>11-68 fold</td>
<td>n.a.</td>
<td>n.a.</td>
<td>14 fold</td>
<td>10 fold</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Urine PBG levels</td>
<td>9-45 fold</td>
<td>54 fold</td>
<td>3-11 fold</td>
<td>33 fold</td>
<td>128 fold</td>
<td>4 fold</td>
<td>2 fold</td>
<td>n.a.</td>
</tr>
<tr>
<td>HMBS enzyme activity (% of normal)</td>
<td>n.a.</td>
<td>14%</td>
<td>17%</td>
<td>1%</td>
<td>2-4%</td>
<td>55%</td>
<td>67%</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Abbreviations: ALA = 5-aminolevulinic acid; PBG = porphobilinogen; HMBS = hydroxymethylbilane synthase; n.a. = not available; AIP = acute intermittent porphyria; ADL = daily life quality; OD., = oculus dextrus; OS., = oculus sinister; WM = white matter; y = years; † = deceased
restoration of the hepatic heme pool occurs by increasing 5′ aminovulinate synthase enzyme production. With a decreased capacity to convert PBG to hydroxymethylbilane in patients with HMBS enzyme deficiency, accumulation of porphyrin precursors ALA and PBG occurs.1,2,4

In patient 3, however, the normal oxidative phosphorylation enzyme activities in muscle provide evidence against a chronic heme deficiency, as these enzymes are heme dependent. Also, heme deficiency cannot explain the complete resolution of acute and neurological symptoms in heterozygous AIP patients after liver transplantation, a curative treatment performed in very severe cases.28 Although the exact pathomechanism remains unsolved, the presented family adds new insight into the complex pathophysiology of acute porphyrias. Our family suggests that pain and neuropathy during attacks have a different pathomechanism than the progressive spastic paraparesis and leukoencephalopathy in AIP patients with bi-allelic variants.

As illustrated by this study, the unbiased approach of WES analysis may lead to unexpected and insightful results. In the case of our patients, the genetic findings also have direct implications for the ‘healthy’ siblings who are heterozygous carriers; they have an approximately 10% lifetime risk for AIP attacks, comparable to other heterozygous carriers. With this knowledge, measures can be taken to limit the risk of the potential life-threatening porphyric attacks. In addition, long-term complications of AIP like hypertension, chronic kidney disease, and hepatocellular carcinoma should be monitored in these patients and the family members who are carriers.29 Due to the increasing use of next-generation sequencing studies in patients with an unexplained neurological disorder we expect that more patients with bi-allelic HMBS variants will be identified.
REFERENCES
