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Expanding the Clinical and Radiological Phenotype of Leukoencephalopathy due to bi-allelic *HMBS* mutations

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Abstract

Pathogenic heterozygous variants in *HMBS* encoding the enzyme hydroxymethylbilane synthase (HMBS), also known as porphobilinogen deaminase, cause acute intermittent porphyria (AIP). Bi-allelic variants in *HMBS* have been reported in a small number of children with severe progressive neurological disease and in three adult siblings with a more slowly progressive neurological disease and distinct leukoencephalopathy. We report three further adult individuals who share a distinct pattern of white matter abnormality on brain MRI in association with bi-allelic variants in *HMBS*, two individuals with homozygous variants and one with compound-heterozygous variants. We present their clinical and radiological features and compare these with the three adult siblings previously described with leukoencephalopathy and bi-allelic *HMBS* variants. All six affected individuals presented with slowly progressive spasticity, ataxia, peripheral neuropathy, with or without mild cognitive impairment and/or ocular disease with onset in childhood or adolescence. Their brain MRIs show mainly confluent signal abnormalities in the periventricular and deep white matter and bilateral thalami. This recognizable pattern of MRI abnormalities is seen in all six adults described here. Bi-allelic variants in *HMBS* cause a phenotype that is distinct from AIP. It is not known whether AIP treatments benefit individuals with *HMBS*-related leukoencephalopathy. One individual reported here had improved neurological function for 12 months following liver transplantation followed by decline and progression of disease.

Keywords

Acute intermittent porphyria; homozygous dominant acute intermittent porphyria; hydroxymethylbilane synthase; porphobilinogen deaminase

Author contributions

Conception and design of study: CAS, AK, CF, EJ, AR, JA, RJL, DJA MSvdK, MBD

Analysis and interpretation of data: CAS, AK, CF, EJ, MF, AR, TW, JA, RJL, VL, MB, PG, PJL, MSvdK, MBD

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Competing interest statement

The authors have no competing interests to declare

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Details of Ethics Committee approval

This study was approved by the Royal Children's Hospital Human Research Ethics Committee (Project Number: 28097).

Patient consent statement

All individuals provided written informed consent to their participation in this study and its publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Introduction

Hydroxymethylbilane synthase (HMBS; EC 2.5.1.61), also known as porphobilinogen deaminase (PBGD), is the third enzyme of the heme biosynthetic pathway and catalyzes the condensation of four molecules of porphobilinogen (PBG) to form hydroxymethylbilane. HMBS is encoded by the gene *HMBS* which is alternatively spliced to produce a housekeeping transcript of the gene containing exons 1 and 3-15, and an erythroid-specific transcript containing exons 2-15.(Grandchamp et al., 1989) Heterozygous pathogenic variants in *HMBS* cause acute intermittent porphyria (AIP) which is dominantly inherited with incomplete penetrance and presents with acute attacks often precipitated by dieting or fasting, hormonal changes and certain porphyrinogenic medications. (Puy et al., 2010) By contrast, bi-allelic pathogenic variants in *HMBS* are a rare cause of leukoencephalopathy presenting in childhood with progressive neurological disease of variable severity.(Beukeveld et al., 1990; Dixon et al., 2019; Hessels et al., 2004; Kevelam et al., 2016; Llewellyn et al., 1992; Solis et al., 2004) This form of the disease has been referred to throughout the literature as homozygous dominant AIP. (Beukeveld et al., 1990; Hessels et al., 2004; Yasuda et al., 2019) As the clinical presentation differs significantly to AIP and also compound heterozygous variants can be causative, we refer to this condition here as '*HMBS*-related leukoencephalopathy'. Of the rare individuals with bi-allelic pathogenic variants in *HMBS*, only three have survived to adulthood, (Kevelam et al., 2016) Dutch siblings in whom detailed radiological assessment identified a distinct pattern of white matter abnormalities on brain MRI. Here we present three additional adults with *HMBS*-related leukoencephalopathy who share this pattern of MRI abnormalities, confirming a recognizable MRI phenotype in associated with bi-allelic *HMBS* variants.

Methods

Recruitment

All three individuals were recruited from a clinical genetics service following referral for diagnostic assessment of leukoencephalopathy. All provided written consent to participate in a gene discovery research project investigating unclassified leukodystrophies approved by the Royal Children's Hospital Human Research Ethics Committee (Project Number: 28097).

Genetic testing

Individuals 1 and 2: exome sequencing of genomic DNA isolated from blood of both affected individuals was carried out at the Australian Genome Research Facility. Exons were captured using the SureSelect Human All Exon, V5+UTRs (Agilent) and sequencing, linkage and variant analysis was performed as previously described.(Smith et al., 2011) Individual 3 was tested for a familial *HMBS* pathogenic variant by an accredited New Zealand laboratory and then had analysis of the *HMBS* gene for a second pathogenic variant by the same laboratory. All coding regions and flanking intronic sequences of the *HMBS* gene were amplified by PCR and analyzed by automated fluorescent DNA sequencing. The resulting sequence was compared against the GRCh38 reference sequence REGION: (chr11:119,082,877-119,095,549). Variant nomenclature is based on the NCBI HMBS transcript (NM_000190.3) and protein reference sequence NP_001019553.1.

Results

Clinical features, laboratory findings and neurophysiological investigations

Individual 1, a 43-year-old female, is one of five children born to healthy first cousin Lebanese parents. She has one similarly affected brother, three unaffected siblings and no children. She was

born after an uneventful pregnancy and delivery. She was diagnosed with cataracts at four years of age and these were treated with removal and insertion of intraocular lens implants. She had normal motor development until age seven years when she had onset of slowly progressive ataxia. On assessment at age 36 years she had spastic/ataxic paraparesis, distal sensory impairment, mild dysarthria and normal cognitive function. Nerve conduction studies confirmed a mild generalized sensorimotor peripheral neuropathy with reduction in the sural and ulnar sensory nerve action potentials and mild prolongation of F-waves in the lower limbs. She was independent with activities of daily living (ADL), could walk unaided for short distances and used a motorized scooter for longer distances. Prior to whole exome sequencing (WES), extensive investigations for metabolic and genetic causes of leukodystrophy and ataxia had been unrevealing, including testing for spinocerebellar ataxia types 1, 2, 3, 6 and 7. After diagnosis of recessive *HMBS*-related leukoencephalopathy, specific enquiry regarding symptoms of AIP revealed that she experienced attacks of abdominal pain and occasional episodes of red urine lasting 1-2 days. Porphyrin studies had not been performed at the time of these episodes and therefore confirmation that these were symptoms of AIP is not available. However, the episodes ceased following the introduction of regular carbohydrate containing meals, avoidance of periods of fasting related to irregular mealtimes and religious practices, and ceasing the oral contraceptive which she had been prescribed for over 20 years. After identifying a potentially treatable condition, Individual 1 had regular neurological assessments to monitor her response to lifestyle measures and need for further treatment. Over a period of 16 months following diagnosis, her ataxia and dysarthria worsened. The International Cooperative Ataxia rating Scale - ICARS (Trouillas et al., 1997) and Scale for Assessment and Rating of Ataxia - SARA (Schmitz-Hubsch et al., 2006) clinical scales showed a worsening trend with the ICARS score progressing from 34 to 40 (out of 100) and SARA score

from 13.5 to 15 (out of 40) from November 2017 to March 2019. Because of the progression of her disease, she underwent liver transplantation in May 2019. Histological examination of the explanted liver showed lipid deposition consistent with Individual 1 being overweight. The immunosuppressive regime used was tacrolimus, mycophenolate mofetil and prednisolone. Prednisolone was started on day one and was weaned over eight weeks after which time it was discontinued. Sulfamethoxazole and trimethoprim were used for Pneumocystis prophylaxis. Post-transplant, there was clinically observed improvement in ataxia and dysarthria. This correlated with a decreased level of ALA (Table 2). Eight months post-transplant the ataxia rating scores reflected this improvement with ICARS score 20/100 (improved from 40/100 pre-transplant) and SARA 13/40 (Figure 1). 12 month post-transplant however, her neurological function began to decline. Reassessment at 17 months post-transplant demonstrated significant decline to pre-transplant state with ICARS score of 41/100 and SARA 18/40 (Figure 1). A cause for the decline was not identified, specifically there was no evidence of transplant rejection. Unfortunately, limited results from urine and plasma porphyrin studies are available for the study period and summarized in Table 2.

Individual 2, a brother of Individual 1, is a 54-year-old male with three healthy children. He was born after an uneventful pregnancy and delivery. He was diagnosed with cataracts in early childhood and these were treated with removal and insertion of intraocular lens implants. From late childhood he had slowly progressive ataxia, dysarthria and mild cognitive impairment. On assessment at 53 years he could walk independently for short distances and had an ataxic gait. He had predominantly truncal ataxia and mild peripheral ataxia. He had lower limb spasticity and evidence of a mild peripheral neuropathy. He required assistance with most ADL. At 54 years he

suffered an acute pontine infarction and has since been wheelchair dependent and nonverbal. He had no known metabolic risk factors for stroke.

Individual 3 is a 45-year old male with non-consanguineous parents of Dutch ancestry. His mother was investigated for porphyria cutanea tarda after presenting with a rash and she was found to have AIP. She had no personal or family history suggestive of AIP but her only biological son aged 40 (individual 3) had a history of "unexplained walking difficulties" which had been extensively investigated. He had normal childhood development and onset of an unsteady gait aged 13. On assessment at 22 years he had bilateral lower limb spasticity with normal strength and otherwise normal neurological function. His gait deteriorated over time with progressive leg weakness and peripheral numbness. Initial nerve conduction studies were normal but somatosensory and motor evoked potentials showed marked slowing of central conduction times. He required intensive care whilst living in Thailand aged 40 for management of an epileptic seizure after taking over-the-counter medication for a diarrheal illness. At latest follow up at 46 years, he had moderate spastic paraparesis and peripheral neuropathy. He had normal cranial nerve and upper limb examination. He had normal cognition and was able to walk unaided with ankle orthotics. He was independent with ADL, could drive and was working as a baker. Repeat nerve conduction studies demonstrated an axonopathy with reduced amplitude compound motor action potentials with normal conduction velocities and absent lower limb sensory nerve action potentials.

MRI

For individual 1, six brain MRIs were available, obtained at ages 34, 37, 40, 42, 43 and 45 years. Individual 2 had two MRIs available, obtained at ages 48 and 53 years. Individual 3 had two MRIs

available, obtained at ages 37 and 46. Images from these MRIs are shown in Figure 2. All three individuals had extensive, mainly confluent, symmetrical signal abnormalities in the periventricular and deep cerebral white matter with relative sparing of the U-fibres. All individuals had bilateral involvement of the thalami with sparing of the basal nuclei, internal capsule and corpus callosum. Individuals 1 and 3 had signal abnormalities in the central part of the pons. For Individual 1, this presented post-transplant Individual 2 had mild widening of the lateral ventricles and subarachnoid spaces suggestive of cerebral atrophy. Individuals 1 and 3, the repeat MRIs showed slow progression of the white matter abnormalities with patchy changes becoming confluent in some regions. Additional abnormalities noted were subcortical cavernomata and dilated perivascular spaces for individual 1 and a subcortical cyst in individual 3.

Genetic testing

Individuals 1 and 2 had WES which identified a novel homozygous missense variant in *HMBS* (NM_000190.3) c.251C>A, (p.Ala84Asp) in both. Segregation studies confirmed that both parents and two unaffected siblings were heterozygous for this variant. This substitution causes a moderate amino acid change in an evolutionarily conserved residue in the second alpha-helix of the HMBS protein. The substitution of an alanine with aspartate in this buried position could impair protein folding and stability due to its polar, acidic and hydrophilic properties. This variant has not been reported in association with AIP and is absent from the population database gnomAD. (Lek et al., 2016) To confirm pathogenicity of the *HMBS* variant, both affected individuals were tested for HMBS enzyme activity in erythrocytes and measurement of the porphyrin precursor, porphobilinogen (PBG) in plasma and urine (where possible). The pathogenicity of this variant was evidenced by the significant reduction in HMBS activity in erythrocytes in these individuals

to 13% and 18% of normal mean respectively (Table 2). An unaffected sibling who is heterozygous for this variant had erythrocyte HMBS activity that was 50% of normal mean. No additional variants of clinical significance or potential clinical significance were identified from the WES analysis.

Individual 3 was tested for a familial variant in *HMBS* (NM_000190.3) c.500G>A (p.Arg167Gln) after his mother was incidentally found to have latent AIP. After detection of this heterozygous pathogenic variant, he had biochemical studies which confirmed an elevated quantitative urine porphobilinogen (PBG) of 3.7 umol/mol creatinine (normal <1.5). Porphyria was considered as the cause of his problems but after discussion with world experts it was concluded the underlying aetiology was probably unrelated. He was initially lost to follow up after residing overseas but was reevaluated after returning to New Zealand in 2016 and the report by Kevelam et al.(Kevelam et al., 2016) was published. Sequencing of the *HMBS* gene was requested to look for a second variant and *HMBS* (NM_000190.3) c.674G>A (p.Arg225Gln) was identified. This variant was classified by the laboratory as likely pathogenic in accordance with ACMG guidelines as indicated by in-silico predictions, population frequency and published case reports.(Floderus, Shoolingin-Jordan, & Harper, 2002; von Brasch et al., 2004) Segregation studies of the second variant showed that it was not maternally-inherited but was most likely acquired from the individual's father (of Dutch heritage) who was not available for testing. Bi-allelic inheritance was in addition assumed likely given the genotype and phenotype match with individuals in the Dutch family previously reported by Kevelam at al. (Kevelam et al., 2016)

Biochemical analysis

The results of urine 5-aminolevulinic acid (ALA) and porphobilinogen (PBG) concentrations and erythrocyte HMBS activity are presented for each individual in Table 2 and compared to those of the similarly affected adults previously reported by Kevelam et al.(Kevelam et al., 2016) Urine PBG/creatinine is elevated in all affected individuals who were tested, more significantly in those with the homozygous variant Ala84Asp compared to those with the compound heterozygous variants Arg167Gln/Arg225Gln. The reduction in HMBS enzyme activity in erythrocytes varies from 13-67% of normal mean in those who were tested. For Individual 1, red cell porphyrin was measured post-transplant and found to be elevated, a finding that is usually associated with cutaneous porphyrias rather than AIP and the explanation in this case is unknown.

Discussion

HMBS-related leukoencephalopathy caused by homozygous and compound heterozygous pathogenic variants in *HMBS* can present with slowly progressive neurological disease and survival into adulthood. This phenotype has been recognized in six adult individuals, three presented here and three previously-reported. (Kevelam et al., 2016) These six individuals share a common phenotype characterized by childhood-onset disease with slow progression of lower limb spasticity, peripheral neuropathy, with or without ataxia, mild cognitive impairment and cataracts. Brain MRIs show all six individuals have extensive CNS white matter disease with a recognizable pattern of signal abnormality in the periventricular and deep white matter and bilateral thalami. Four of the six individuals have abnormal signal in the central pons. One individual had no signal abnormality in the pons at the time of his diagnosis of leukoencephalopathy at 48 years but had a pontine infarction at age 54 years. Four individuals have a degree of cerebral and/or cerebellar atrophy. Two individuals (siblings) have a novel, homozygous, likely pathogenic variant in *HMBS*:

Ala84Asp *HMBS* and the other four individuals from two families (likely distantly related) have compound heterozygote variants in HMBS: Arg167Gln/Arg225Gln (Table 1). *HMBS* dysfunction in these individuals is evidenced by increased levels of porphyrin precursors in urine and reduced HMBS activity in erythrocytes (Table 2). Although the clinical features and severity of disease is similar for all six individuals, their HMBS enzyme activity levels range from 13 – 67 % of normal, indicating that this biomarker may not be an accurate measure of variant pathogenicity in CNS disease. There is no autopsy tissue available, so we cannot be sure of the underlying CNS pathology and the reason for the different patterns of selective vulnerability is unknown.

The low HMBS enzyme activity detected in Individuals 1 and 2 (13% and 18% of the normal mean) provides support for pathogenicity of their novel homozygous *HMBS* variant Ala84Asp. For individual 3, a measurement of enzyme activity was not available; however, he shares the same phenotype and genotype with the Dutch family reported by Kevelam et al. who had enzyme activity levels ranging from 55-67% of normal mean, in association with compound heterozygous variants Arg167Gln and Arg225Gln. The pathogenicity of the variant Arg167Gln is well-established from clinical reports and functional studies, (Chen et al., 2016; Delfau et al., 1990; Llewellyn et al., 1992) however there are conflicting results from functional studies regarding the pathogenicity of the variant Arg225Gln. (Chen et al., 2016; Lenglet et al., 2018) Expression studies of the Arg225Gln variant in *E.coli* to determine HMBS activity demonstrated 102% of wild type activity in one study (Chen et al., 2016) and 65% in another (Lenglet et al., 2018). These *in vitro* results are inconclusive and furthermore do not exclude the possibility that Arg225Gln *in vivo* may be unstable. As *HMBS*-associated leukoencephalopathy requires both alleles to be affected with no single allele cases ever reported, a second variant *in trans* was expected in our patient.

Incorporating evidence from previously published case reports and segregation studies, (Floderus et al., 2002; Kevelam et al., 2016; von Brasch et al., 2004) the low population frequency of 0.02% with zero homozygotes as well as predominantly damaging *in silico* predictions, the Arg225Gln variant was classified as likely pathogenic with respect to an autosomal recessive leukoencephalopathy disorder in accordance with ACMG guidelines (Richards et al., 2015).

A likely pathogenic role of the Arg225Gln variant in recessive leukoencephalopathy does not necessarily confirm its pathogenicity in autosomal dominant AIP. For example, a recent report describing a severe phenotype associated with a homozygous *CASR* splice variant (Capozza et al., 2018) found that the heterozygous parents of the proband were biochemically and phenotypically normal. The reason was that the observed splice variant does not ablate normal splicing 100%. It was found that sufficient normal transcript was produced from the affected allele that likely explains the normal parental phenotype. A related example can be seen in the adult-onset polycystic kidney disease associated with heterozygous variants in *PKD1*; selected heterozygous missense variants in this gene can be tolerated without evidence of disease but, when coupled with a pathogenic variant in trans, cause a severe form of the disease with onset in early childhood. (Al-Hamed et al., 2019) Likewise, partial loss of HMBS activity observed with Arg225Gln may be sufficient for a clinical phenotype when in trans with a second pathogenic variant but only low penetrance AIP in the heterozygous state (Floderus et al., 2002; Kevelam et al., 2016). Furthermore, while (Floderus et al., 2002; Kevelam et al., 2016) report 55-67% of wild type HMBS activity in compound heterozygous Arg167Gln/Arg225Gln patients, the functional level of HMBS activity produced from each individual allele was not assessed, hence further studies are needed to accurately determine *in vivo* functional acivity of Arg225Gln. While the Arg225Gln variant

appears to have low penetrance in the heterozygous state, its true effect may not be accurately reflected in erythrocyte enzyme activity levels. Although *HMBS* is ubiquitously expressed, it is possible that the expression levels vary between the hematopoietic, hepatic and nervous systems and as such the relative effect of Arg225Gln may vary dependent on cell type. Discordance between clinical symptoms and measured enzyme activity in *HMBS*-related disease may occur due to genetic and/or environmental modifiers. Comparing the prevalence of AIP in the general population and the frequency of likely pathogenic variants identified in large public datasets, the penetrance of pathogenic heterozygous *HMBS* variants is estimated to be ~1% in the general population.(Chen et al., 2016) By contrast, the penetrance of pathogenic *HMBS* variants within families with AIP is close to 30%, suggesting a role for genetic and/or environmental modifiers of the AIP phenotype.(Lenglet et al., 2018) Just as there is variable penetrance of AIP in association with different heterozygous *HMBS* variants, the severity of neurological disease associated with bi-allelic variants may also vary with genotype.(Beukeveld et al., 1990; Llewellyn et al., 1992; Solis et al., 2004) This is also demonstrated in the mouse models generated by Yasuda et al. that found the mice homozygous for *Hmbs* variant Arg173Gln died in utero, mice homozygous for *Hmbs* variant Arg167Gln mice had early onset neurological disease and those with *Hmbs* compound heterozygote variants Arg173Gln/Arg167Gln mice were abnormal and died early.(Yasuda et al., 2019)

The cause for chronic central nervous system (CNS) disease in association with bi-allelic *HMBS* variants is not clear. Evidence from a recent study of bi-allelic *Hmbs* mutation knock-in mice indicates that the pathogenesis of this disease may be distinct from heterozygous AIP, including response to porphyrinogenic triggers and AIP treatments.(Yasuda et al., 2019) AIP results from

haploinsufficiency of HMBS, the third enzyme in the heme biosynthesis pathway. Upregulation of hepatic 5-aminolevulinate synthase I (ALAS1), the first enzyme in the heme biosynthesis pathway, in the setting of HMBS deficiency leads to an accumulation of the porphyrin precursors 5-aminolevulinic acid (ALA) and porphobilinogen (PBG). The neurological symptoms of AIP are thought to be due to a neurotoxic function of ALA, which has structural homology with gamma-aminobutyric acid (GABA), a potent modulator of neuronal synaptic activity, thereby inhibiting GABA receptors.(Brennan & Cantrill, 1979; Brennan, Cantrill, & Kramer, 1980) However, ALA does not readily cross the blood-brain barrier (Hu, Shen, Keep, & Smith, 2007; Terr & Weiner, 1983; Yasuda et al., 2019) and therefore, may accumulate within the CNS and not be liver-derived. Elevated ALA in CNS tissue was detected in the bi-allelic *Hmbs* mutation knock-in mouse model along with delayed myelination and decreased myelin volume.(Yasuda et al., 2019) For the individuals discussed in this report, levels of porphyrin precursor in the cerebrospinal fluid are not known. Their MRIs show evidence of dysmyelination rather than lack of myelin production. As the white matter abnormalities in these individuals are slowly progressive, they might be related to low-grade toxicity.

An alternative hypothesis for chronic neurological impairment in *HMBS*-related disease is a chronic deficiency in neuronal heme that interrupts axonal transport and causes axonal degeneration. (Lindberg et al., 1999) This may be relevant also to the polyneuropathy and progressive neurological disease associated with recessive *ALAD* mutations (Doss porphyria). (Doss et al., 2004; Souza et al., 2021) Mice with HMBS deficiency and normal ALA levels were shown to develop progressive neurological dysfunction, indicating the disease process was not mediated by elevated ALA.(Lindberg et al., 1999) White matter signal abnormalities on brain MRI

can be seen in both symptomatic and pre-symptomatic individuals with AIP who may have normal ALA levels,(Bylesjo, Brekke, Prytz, Skjeflo, & Salvesen, 2004) though these white matter abnormalities are not as extensive as the leukoencephalopathy seen in these individuals with bi-allelic *HMBS* variants. CNS hypomyelination has been reported in very rare individuals with homozygous variegate porphyria caused by bi-allelic mutations in the protoporphyrinogen oxidase gene (*PPOX*), whereas neurological manifestations of variegate porphyria due heterozygous mutations are the same as in AIP. The mechanism of disease in that condition is thought to be a toxic effect of accumulated metabolites on myelin production.(Pinder et al., 2013) The ubiquitous isoform of *HMBS* is a housekeeping gene and for unclear reasons, housekeeping genes are frequently implicated in recessive disorders of CNS white matter.(van der Knaap & Bugiani, 2017) Further study is required to understand the pathological mechanism for *HMBS*-related CNS disease and any genotype-phenotype correlations.

Understanding the pathogenesis of *HMBS*-related CNS disease is essential for the clinical management of affected individuals. Current treatment for AIP is aimed at downregulating hepatic heme synthesis by administration of Haem and by avoiding medications and lifestyle behaviours which trigger haem synthesis. A phase 1 trial of givosiran, an RNA interference therapy targeting hepatic aminolevulinic acid synthase 1 (*Alas1*), resulted in near normalization of neurotoxic intermediates ALA and PBG and severity of AIP symptoms in the treatment group compared with placebo.(Sardh et al., 2019) A phase III study ENVISION was recently published(Balwani et al., 2020). It involved 94 patients with acute hepatic porphyrias of whom 89 had AIP and showed over 70% reduction in frequency of symptoms. FDA granted approval for use of this drug on 20 November 2019. Resolution of disease with liver transplantation has been reported for a number

of individuals with heterozygous AIP. (Dowman et al., 2012; Soonawalla et al., 2004; Yasuda et al., 2015) In the bi-allelic *Hmbs* mutation knock-in mouse model, hepatic *Alas1* mRNA levels were normal (Yasuda et al., 2019) suggesting givosiran may be ineffective for bi-allelic *HMBS*-related disease. This was the case for a child with bi-allelic *HMBS*-related disease and severe developmental delay, for whom there was little, if any evidence of benefit from liver transplantation.(Dixon et al., 2019) Likewise for a child with Doss porphyria, liver transplantation did not halt the progression of his neurological disease. (Doss et al., 2004) For Individual 1 in this study, liver transplantation led to a significant but transient improvement in neurological function. The emergence of novel therapies for AIP emphasizes the importance of early diagnosis and improved understanding *HMBS*-related leukoencephalopathy as a rare and potentially treatable cause for neurodegeneration.

Conclusions

Leukoencephalopathy due to bi-allelic *HMBS* variants is a rare but likely under-recognized cause for chronic progressive neurological disease with or without ophthalmological abnormalities. Erythrocyte HMBS enzyme activity levels in affected individuals may not correlate with CNS disease severity or *HMBS* variant pathogenicity. For Individuals 1 and 2, the diagnosis of *HMBS*-related leukoencephalopathy demonstrates the power of exome sequencing in revealing unexpected diagnoses in otherwise non-specific progressive ataxia presentations and we recommend *HMBS* be included in gene panels for leukoencephalopathy, spastic paraplegia and ataxia. MRI pattern recognition can guide genomic testing and assist with *HMBS* variant interpretation.The utility of AIP treatments in slowing the course of this progressive neurological disease is unknown and requires further study.

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Figure legends

Figure 1: Ataxia rating scale scores for Individual 1

Ataxia rating scale scores for Individual 1 on six occasions between November 2017 and October 2020. The last assessment pre-liver transplantation was three months prior to the transplant and the first assessment following liver transplantation was six-week post-transplant. SARA: Scale for the Assessment and Rating of Ataxia; ICARS: International Cooperative Ataxia Rating Scale.

Figure 2: Brain MRI abnormalities

Representative images are shown for all individuals. All images are axial T2-weighted. The images 1-3 are taken from individuals 1, 2 and 3 at ages 34 years, 48 years and 46 years respectively. The images 4-6 are taken from individuals previously reported by Kevelam et al.(Kevelam et al., 2016) at ages, 37 years, 51 years and 35 years respectively and included for comparison. All six individuals had extensive, mainly confluent, symmetrical signal abnormalities in the periventricular and deep cerebral white matter with relative sparing of the U-fibres. In individuals 5 and 6 the abnormalities were most extensive. All individuals had bilateral involvement of the thalami with sparing of the basal nuclei, internal capsule and corpus callosum. Individuals 3, 5 and 6 had signal abnormalities in the central pons. Mild widening of the lateral ventricles and subarachnoid spaces was present for individuals 2, 4, 5 and 6 suggestive of cerebral atrophy. In addition, individuals 4, 5 and 6 had mild cerebellar atrophy. For individuals 5 and 6, the cerebellar atrophy was more severe on recent MRIs, suggesting progression. All those for whom multiple MRIs were available, showed slow progression of the white matter abnormalities with patchy changes becoming confluent in some regions. Additional

abnormalities noted were subcortical cavernomata and dilated perivascular spaces for individual 1 and a subcortical cyst in individual 3.

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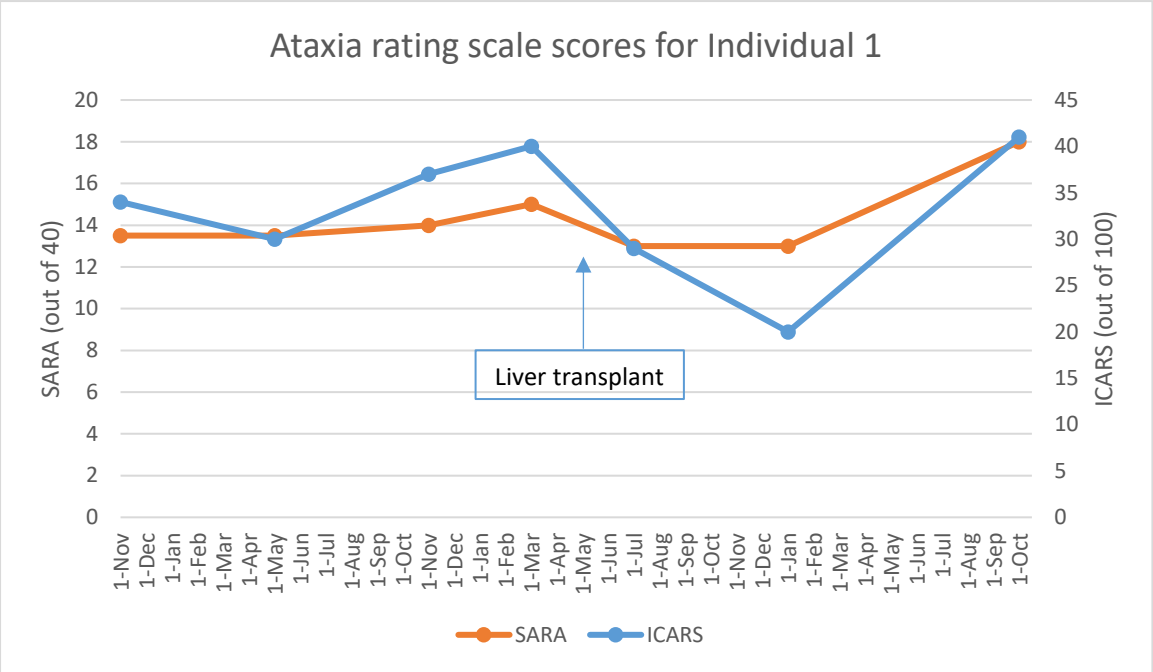
Figure 1: Ataxia rating scale scores for Individual 1



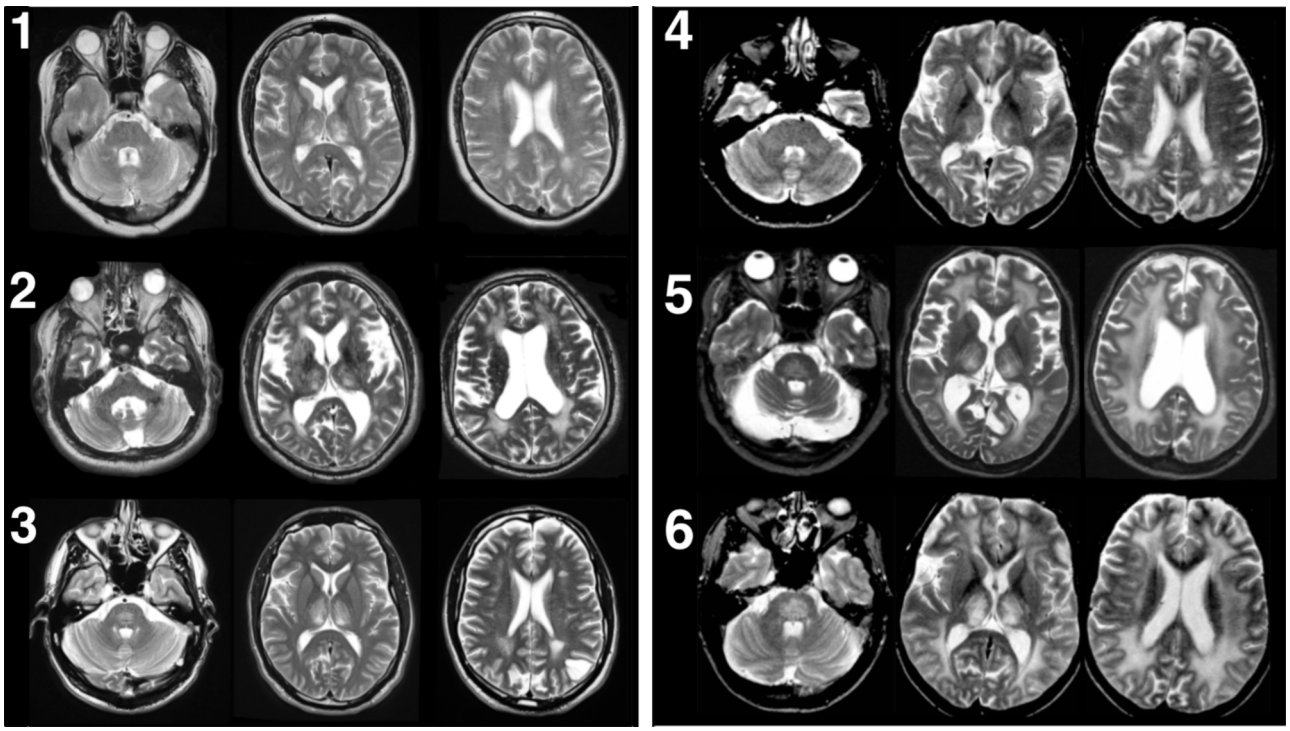


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Table 1: Clinical and neuroradiological features with comparison to three adult siblings previously-reported by Kevelam et al.(Kevelam et al., 2016)

	Family 1		Family 2	Kevelam et al.(Kevelam et al., 2016)		
	Individual 1	Individual 2	Individual 3	Patient 1	Patient 2	Patient 3
Ethnicity	Lebanese	Lebanese	Dutch	Dutch	Dutch	Dutch
Sex	Female	Male	Male	Male	Female	Male
HMBS variants	c.251C>A; p.Ala84Asp c.251C>A; p.Ala84Asp	c.251C>A; p.Ala84Asp c.251C>A; p.Ala84Asp	c.500G>A; p.Arg167Gln c.674G>A; p.Arg225Gln	c.500G>A; p.Arg167Gln c.674G>A; p.Arg225Gln	c.500G>A; p.Arg167Gln c.674G>A; p.Arg225Gln	c.500G>A; p.Arg167Gln c.674G>A; p.Arg225Gln
Age at onset	7 y	Late childhood	22 y	During teens	4 y	4 y
Age at last follow up	43 y	54 y	45 y	58 y	63 y	57 y
Spastic paraparesis	Yes	Yes	Yes	Yes	Yes	Yes
Peripheral neuropathy	Yes	Mild clinical features. NCS not done.	Yes	Symmetrical, axonal	Symmetrical, axonal	Symmetrical, axonal
Cerebellar ataxia	Yes	Yes	No	Yes	Yes	Yes
Apraxia	No	Unknown	No	No	Yes	Yes
Dysarthria	Yes	Yes	No	No	No	No
Cognitive impairment	No	Mild	No	No	Mild	Mild
Ophthalmologic signs	Childhood cataracts	Childhood cataracts	No	Normal vision with glasses	Optic atrophy, fine peripheral cortical cataract, nystagmus	Optic atrophy with vision loss, nystagmus
Acute porphyric attacks	Yes	No	No	No	No	No
Course of disease	Slowly progressive,	Slowly progressive,	Slowly progressive,	Slowly progressive,	Slowly progressive,	Slowly progressive,

	walking with walker and ADL independent at age 45 y	walking short distances prior to stroke at 54y	walking with ankle orthotics and ADL independent at age 46	walking with walker and ADL independent at age 58 y	wheelchair at age 4 y, ADL dependent at age 63 y	wheelchair at age 35 y, ADL dependent at age 57 y
MRI WM abnormalities	Symmetrical signal abnormality in periventricular and deep white matter	Symmetrical signal abnormality in periventricular and deep white matter	Symmetrical signal abnormality in periventricular and deep white matter	Symmetrical signal abnormality in periventricular and deep white matter	Symmetrical signal abnormality in periventricular and deep white matter	Symmetrical signal abnormality in periventricular and deep white matter
Bilateral signal abnormality in thalami	Yes	Yes	Yes	Yes	Yes	Yes
Signal abnormality in pons	Yes	No	Yes	No	Yes	Yes
Atrophy	No	Cerebral	No	Cerebral; mild cerebellar	Cerebral and cerebellar	Cerebral and cerebellar
Other	Subcortical cavernomata; Enlarged perivascular spaces		Subcortical cyst			

Table 2: Biochemical results where available with comparison to two adult siblings previously-reported by Kevelam et al.(Kevelam et al., 2016)

	Family 1						Family 2	Kevelam et al.(Kevelam et al., 2016)	
	Individual 1					Individual 2	Individual 3	Patient 1	Patient 2
	Pre-Tx	D8 post-Tx	3 mo post-Tx	7 mo post-Tx	15 mo post-Tx				
Urine PBG, umol/L (norm <10)	210				118				
Urine ALA/creatinine, umol/mmol (norm <3.8)	11.5	6.9					3.5	2.4	2.6
Urine PBG/creatinine, umol/mmol (norm <1.5)	11.6						3.7	3.2	3.6
Urine Total Porphyrin, nmol/L (norm <300)	4672				1347				
Urine Porph/Creat ratio (norm <35)	258.1				19.3				
HMBS enzyme activity in % of normal mean in erythrocytes (normal mean = 2.4 IU/L)	18			23		13		55	67
Total Porphyrin, umol/L rbc (norm <1.8)			17.3		18.7				

ALA=5-aminolevulinic acid; PBG=porphobilinogen; HMBS=hydroxymethylbilane synthase; Tx=transplant

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