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# Further Delineation of the Clinical and Pathologic Features of *HIKESHI*-Related Hypomyelinating Leukodystrophy

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## Abstract

**Background:** A recurrent homozygous missense variant, c.160G>C;p.(Val54Leu) in *HIKESHI*, was found to cause a hypomyelinating leukodystrophy with high frequency in the Ashkenazi Jewish population. We provide extended phenotypic classification of this disorder based on clinical history of a further seven affected individuals, assess carrier frequency in the Ashkenazi Jewish population, and provide a neuropathological study.

**Methods:** Clinical information, neuroimaging, and biosamples were collected. Brain autopsy was performed for one case.

**Results:** Individuals with *HIKESHI*-related disease share common clinical features: early axial hypotonia evolving to dystonia or with progressive spasticity, hyperreflexia and clonus, feeding difficulties with poor growth, and nystagmus. Severe morbidity or death during febrile illness

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Supplementary data

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occurred in five of the nine affected individuals. Magnetic resonance images of seven patients were analyzed and demonstrated diffuse hypomyelination and thin corpus callosum. Genotyping data of more than 125,000 Ashkenazi Jewish individuals revealed a carrier frequency of 1 in 216. Gross pathology examination in one case revealed abnormal white matter. Microscopically, there was a near-total absence of myelin with a relative preservation of axons. The cerebral white matter showed several reactive astrocytes and microglia.

**Conclusions:** We provide pathologic evidence for a primary disorder of the myelin in *HIKESHI*-related leukodystrophy. These findings are consistent with the hypomyelination seen in brain magnetic resonance imaging and with the clinical features of early-onset spastic/dystonic quadriplegia and nystagmus. The high carrier rate of the recurrent variant seen in the Ashkenazi Jewish population requires increased attention to screening and diagnosis of this condition, particularly in this population.

### Keywords

Hypomyelinating leukodystrophy; Whole genome sequencing; Hikeshi; Ashkenazi Jewish

## Introduction

Variants in *HIKESHI* (OMIM:614908, previously known at *C11ORF73*) were recently identified as the cause of a novel hypomyelinating leukodystrophy (OMIM:616881).[1,2] Leukodystrophies are genetic disorders affecting growth, development, or maintenance of the cellular and structural components of myelin in the central nervous system.[3] Myelination is a highly complex and tightly regulated process, rendering it highly vulnerable to damage during the synthesis or maintenance of developing myelin.[4] For many leukodystrophies, neuropathology is primarily characterized by the involvement of oligodendrocytes, astrocytes, and/or other non-neuronal cell types, although in many disorders the mechanism of disease remains unknown.[5-7]

The protein encoded by *HIKESHI*, Hikeshi, mediates the heat stress-induced nuclear import of heat shock protein (Hsp70) through a nuclear pore complex.[8,9] Upon nuclear translocation, HSP70 dissociates from Hikeshi and exerts its protective functions as a molecular chaperone. Hikeshi function is essential to cell viability in conditions of heat stress, although this appears to work in a cell-type-specific manner.[8,10]

Few cases of *HIKESHI*-related leukodystrophy have been previously reported.[1,2] In an effort to further understand the clinical spectrum and pathologic mechanisms underlying this newly found disorder, we report the neuropathology of an affected individual and provide clinical information on nine affected individuals, including seven new cases.

## Methods

Relevant clinical information, magnetic resonance imaging (MRI), DNA for sequencing, and autopsy samples were collected under Myelin Disorders Bioregistry Project at Children's Hospital of Philadelphia and the leukodystrophy center at Tel Aviv Sourasky Medical Center with approval from their respective institutional review boards. Clinical information and

neuroimaging of nine affected individuals was reviewed. MRIs were scored using an MRI scoring system previously described and validated.[11]

### Carrier frequency determination

Carrier screening for variants in *HIKESHI* in the Ashkenazi Jewish (AJ) population was performed as previously described in Rabin et al. from the Dor Yeshorim screening program[12] and are detailed in the Supplemental Methods.[13] In brief, written consent was provided and anonymous blood samples were collected and screened for single-gene disorders in the AJ population using a previously described high-throughput TaqMan allelic discrimination genotyping method on the Fluidigm platform.[14] Samples were classified by self-identification as AJ (n = 94,679), Sephardi Jewish (n = 16,372), Ashkenazi/Sephardi Jewish (n = 13,781), convert (n = 966), or unknown (n = 88). Samples from converts and unknown origins were excluded from frequency analysis. Ancestry by country was based on self-reported ancestry of four grandparents. Individuals with only one, two, three, or all four grandparents from the same country were designated according to country of origin. Individuals were excluded if (1) they listed two or more countries of origin, (2) listed the United States or Israel as country of origin, or (3) did not provide information regarding grandparental origin. Ancestry analysis was performed based on a dataset of 14,379 individuals.

### Tissue handling and preparation

Biospecimens of one of the affected individuals and age-matched control brain samples were collected at the University of Maryland Brain Bank. Signed informed consent from the families was obtained at the University of Maryland Brain Bank, a repository approved by the University of Maryland ethics board. Tissue blocks were obtained and sections were cut from 10% neutral buffered formalin-fixed paraffin-embedded specimens, stained with hematoxylin and eosin (H&E) and erichrome cyanine (EC), and immunostained for glial fibrillary acidic protein, neurofilament, CD45, CD68, aspartoacylase and proteolipid protein (PLP), oligodendrocyte transcription factor (OLIG2), and ionized calcium binding adaptor molecule 1 (Iba1).

## Results

### Case reports

Clinical information is summarized in Supplemental Table S1. Nine affected individuals from six families are described.

Individuals 1 and 2 are twin brothers of AJ heritage. Intrauterine growth retardation was noted on ultrasound at 24 weeks of gestation, and emergency Caesarean section was performed at 34 weeks gestational age for maternal pre-eclampsia. Both patients received care in the neonatal intensive care unit and were discharged at 6.5 weeks and nine days, respectively. Both siblings had a history of nystagmus, progressive spasticity, poor growth, dystonia, and developmental delay. I-1 was slightly delayed in his developmental milestones, rolling at four months and sitting independently at nine months but had a regression and loss of skills at 13 months. I-1 died at age 12 years in the context of a febrile illness that had

originally been attributed to gastroenteritis. His brother, I-2, never achieved independent sitting or standing and was delayed in other milestones. He died at age five and a half years after being hospitalized due to altered mental status and complex focal status epilepticus, with presumed septic shock, leukocytosis, and evidence of multisystem organ failure with persistent lactic acidosis, elevated transaminases, elevated creatinine, and coagulopathy. There was no relevant family history. Skeletal muscle biopsy revealed a defect in mitochondrial respiratory chain subunit complex I in I-1, but no variants in complex I-related genes were found. Electrodiagnostic studies revealed no neuropathy or myopathy. Chromosomal microarray (CMA) was normal. Previous molecular studies for leukodystrophies were all negative, and the diagnosis was made by genome sequencing.[15]

I-3 is the younger of a sibling pair of AJ heritage and is currently aged 23 years. He has a history of microcephaly, developmental regression, spastic quadriparesis and dystonia, nystagmus, and optic atrophy. he is currently wheelchair bound and has significant spasticity and limited communication. His older brother, I-4, had a history of plagiocephaly with surgical correction, chronic nonprogressive spastic encephalopathy, developmental delay, and quadriplegia. He died at age 3.5 years due to complications of sepsis. An autopsy report was thought at the time to be consistent with a diagnosis of Pelizaeus-Merzbacher disease, although no molecular diagnosis was pursued, and no remaining tissue was available for analysis. There was no other relevant family history. Diagnostic testing performed for the younger sibling, I-3, included *PLP1* and *GJC2* sequencing and deletion/duplication analysis, and these were reported as negative. Testing sent for lysosomal enzymes showed lower enzyme activity, but was neither diagnostic of metachromatic leukodystrophy nor indicative of lysosomal storage disease. CMA was normal, and initial exome sequencing was negative. Later recognition of the phenotype led to targeted testing in I-3 and led to diagnosis.

I-5 is the only child to healthy nonconsanguineous parents of AJ origin born after an unremarkable pregnancy and delivery. Family history is negative for a neurodevelopmental disorder. She has a history of developmental delay, exotropia, nystagmus, hypotonia, hyperlaxity, and failure to thrive. She had multiple hospitalizations due to febrile illness and encephalopathy, notably at 9 months, and at 21 months, with mild lactic acidosis. Metabolic profile including lactate, organic and amino acids, very long chain fatty acids, and thiamine level was normal. Echocardiogram was normal. Muscle biopsy demonstrated slightly elevated fat but was otherwise normal. CMA and exome sequencing were initially normal. At age three years, she had fever and drowsiness. She was hospitalized with a suspected septic shock with elevated transaminases and lactate and died two hours after hospitalization. Reevaluation of exome sequencing was performed after the individual's death and revealed the diagnosis.

I-6 is the son of healthy nonconsanguineous parents of AJ origin. Family history is negative for a neurodevelopmental disorder. He has a history of global developmental delay with predominantly motor findings, hypotonia, dystonia and mild spasticity, nystagmus, and failure to thrive, with onset noted at age eight months. On examination at 4.5 years, he had made developmental gains mainly in speech and communication function yet delayed for his age. He is communicative and speaks with short sentences with a dysarthric speech. He can sit independently, creep, and stand with support. He has dystonic spastic quadriplegia,

titubation, and tremor. He has had no exceptional febrile illnesses and no hospitalizations. Metabolic profile was normal including serum amino acids, urine organic acids, blood pH, carnitine, acylcarnitine, very long chain fatty acids, lactate, and congenital disorders of glycosylation. Echocardiogram was normal. Genetic evaluation included a normal CMA and exome sequencing that revealed the diagnosis.

I-7 is a male born to nonconsanguineous parents of AJ origin. Family history is negative for neurodevelopmental disorders. He came to medical attention in the neonatal period with early feeding difficulties. He has a history of developmental regression, predominantly in motor skills, torticollis, and failure to thrive. His early development until age one year was reportedly normal. He achieved independent sitting and cruising, but at this age he had a regression in motor skills and plateauing of development with later slow regaining of motor skills. During his first year of life, he had frequent febrile illnesses but no acute regression in association with these episodes. At age 13 months, nystagmus was noted on clinical examination and pale optic nerve. Brainstem auditory evoked potentials and behavioral hearing test demonstrated combined moderate conductive and sensorineural hearing impairment. On last examination at age 2.8 years he is communicative, uses few single words with dysarthric speech, and uses multiple gestures. He can creep and can sit without support and stand with support. He has dystonic spastic quadriplegia, titubation, and tremor. Blood and urine metabolic profile was normal. *HIKESHI*-related disorder was suspected, and targeted founder mutation analysis revealed the diagnosis.

Individuals I-8 and I-9 consist of two female individuals previously reported as Family B by Edvardson et al.[2] The elder sibling, I-8, is now aged eight years and four months. She had disease onset in the first year of life with microcephaly noted at six months, and at age one year she was reported to have feeding difficulties, global developmental delay, and lower limb spasticity. She had persistent developmental delays, predominantly in motor skills. She had a hospitalization at age two years due to febrile illness with decreased consciousness, acute heart failure with perimyocarditis, acute liver failure, and seizures. She required resuscitation and mechanical ventilation during that illness. She required gastrostomy insertion and is still partially gastrostomy depended for feeding. A thorough evaluation for infectious disease etiology revealed only an enterovirus-positive stool culture. After this hospitalization she regained most of her developmental skills but lost her ability to crawl. In the fourth year of life, she was hospitalized due to cardiogenic shock with multiorgan failure and focal seizures during febrile illness. Computed tomography on this admission demonstrated periventricular hypodensities with progression when compared with previous studies. Cerebrospinal fluid testing showed high protein with normal glucose and cells. She was treated with high-dose corticosteroids, clonazepam, and levetiracetam and recuperated but lost some right-hand function. She has had no further regressions and has made developmental gains, particularly in language skills. On examination at 6 years and 10 months, she was communicative and could speak in five-word sentences. She has severe progressive spasticity of the lower limbs and mild asymmetric spastic dystonia in upper limbs. She can sit with support, can roll over, but cannot crawl. She is mobile using an electric wheelchair.

I-9 had symptom onset at age five months with increased muscle tone and lower limb spasticity evident on examination. She achieved assisted walking but lost this skill in the context of increased spasticity. She has had no significant regression and was hospitalized once at the age of 3.5 years due to pertussis infection, with symptoms of coughing persisting over a three-month period. On examination at age 4.5 years, she had short stature. She had mild hypertonicity in the upper extremities and spasticity in lower limbs. She could sit independently and could crawl. She was communicative using single words and gestures. Her comprehension was better than expression. She uses augmentative and alternative communication. Diagnosis for I-8 and I-9 was made as reported previously in Edvardson et al.[2]

## MRI analysis

Neuroimaging was available for seven of nine individuals in this cohort. Age at MRI ranged from as early as 12 months through age six years.

In all individuals, the initial MRI was suggestive of diffuse hypomyelination. On T2 images there were prominent diffuse hyperintense signals (Fig 1). Abnormal signal extended through the brainstem (Fig 1, 1B-6B) and early myelinating structures such as the central tegmental tracts and posterior columns—medial longitudinal fasciculus in the pons that appear to normalize over time (Fig 1, 4B and 5B), internal capsule (Fig 1, 1C-6C), deep periventricular white matter (WM), and through to the supratentorial WM. Signal abnormalities in the corpus callosum were progressive over time, as was demonstrated on serial scans in I-5. In general, there was mild asymmetric thinning of the posterior corpus callosum (6/7, Fig 1, 1C-6C). The cerebellum was normal in nearly all individuals (6/7, Fig 1, 1A-6A, 6B). Follow-up scans, when available, were relatively unchanged.

Magnetic resonance spectroscopy was variable. In two cases (I-5 and I-8) there was reduced n-acetyl aspartate peak, suggesting neuronal loss. Decreased choline was noted in I-5, and both individuals had an elevated myoinositol peak. In two other individuals, magnetic resonance spectroscopy was unrevealing.

## Causal variants and carrier frequency

All individuals in this cohort, when testing was possible, had a homozygous missense variant, NM_016401.3:c.160G>C; p.(Val54Leu), in *HIKESHI*. This variant was previously identified as a founder variant in the AJ population in the initial report of this disease.[2] In the case of I-4, the variant could not be confirmed due to the unavailability of DNA; however, the p.(Val54Leu) variant was found in his affected sibling. In the ClinVar database (accessed December 17, 2019) and published literature, there are only three other reported single-nucleotide variants beyond the p.(Val54Leu) variant that has been identified in all cases presented herein and in the original description of this case.

In addition, we screened 125,886 individuals from a different Jewish population for this variant in *HIKESHI*. We found 583 carriers identified from this group, determining an overall carrier rate of one in 216. In smaller ancestry groups, the carrier frequency is 0.59% or one in 168 in AJ individuals, 0.17% or one in 574 in mixed Ashkenazi/Sephardi Jewish individuals, and 0% in Sephardi Jewish individuals (Fig 2A). Data classified by country of

ancestral origin show high variation in number of samples per country, ranging from 39 (Belarus) to 7867 (Hungary). Analysis was limited to countries with an appreciable number of available samples. Frequencies derived for Poland and Galicia (0.99%) were compared with those for Hungarian self-classification (0.48%) (Fig 2B). The merging of Poland with Galicia was done on the historical and geographical common basis as in previous studies that studied Ashkenazi subgroups.[16]

### Neuropathology

Full neuropathological studies were performed on I-2. Prefixation brain weight was 1250 g (mean normal for age and gender: 1363.5 g [range 1291.8 to 1435.1 g]). Both gray matter (GM) and WM structures were demarcated on gross pathology, and there were no other significant gross pathologic findings. There was mild hydrocephalus and rounding of the lateral ventricle. The blood vessels and cranial nerves were unremarkable.

Microscopic examination of H&E-stained and EC stained sections revealed a near-total absence of myelin; there was no sparing of U-fibers (Fig 3A-D). The internal capsule was relatively preserved, but there was rarefaction of the deep gray nuclei. There were no definitive cystic lesions in the deep GM or WM (Fig 3A-D). PLP1 staining showed a decrease in myelin protein (Fig 3E), and neurofilament staining showed a relative preservation of axons in contrast to the total absence of myelin on EC stain (Fig 3D and F).

There was diffuse thinning and rarefaction of the cerebral WM with relative hypercellularity of the tissue with an increased reactivity of microglia (Fig 4A) and reactive astrocytes (Fig 4B) within both the GM and WM. Iba-1 staining showed morphological differences in microglia in GM that appear ramified and bushy, whereas the microglia in the WM have a round phagocytic appearance. H&E staining showed Alzheimer type II astrocytes (not shown). CD45 staining, expressed by microglia and peripheral leukocytes, shows no leukocytic infiltration in tissue from the affected individual compared with control samples (Fig 4C). There is perivascular accumulation of leukocytes on CD68 staining (Supplemental Figure S1)

There was a dramatic loss of Olig2 signal in postmortem samples of the affected individual compared with age-matched control tissue, with no labeling in the *HIKESHI*-affected individual (Fig 5A). Mature oligodendrocytes were visualized upon staining with an antibody against aspartoacylase in both the affected and controls, although the intensity of the staining is lower compared with control tissue (Fig 5B).

From the original autopsy report, it was noted that there were few axonal dilatations on cross-section of the medullary corticospinal tracts. The cerebellum showed multifocal loss of Purkinje cells with mild Bergmann gliosis, mild vacuolation of the WM, and gliosis of the dentate nucleus.

## Discussion

Defective Hikeshi results in a severe clinical presentation associated with significant morbidity and mortality. Individuals with *HIKESHI*-related disease have early hypotonia

and feeding difficulties with poor growth, dystonia, pyramidal signs, and lower limb spasticity that appears during the first year of life. There is severe motor delay with slow acquisition of basic skills followed by stagnation or regression in motor skills during early childhood and cognitive impairment. Nystagmus and visual impairment are also common features, frequently observed in hypomyelinating disorders. We also noted that several individuals had subtle dysmorphic features, although this was not formally assessed.

Of the 14 reported affected individuals, including an additional seven presented here in this study, eight have died from febrile illness within hours to days from presentation of fever, with complications due to sepsis and heart failure.[1,2] I-8, previously reported by Edvardson et al.,[2] had multiple hospitalizations: one due to acute perimyocarditis and a second with severe systemic inflammation secondary to cardiogenic shock. In the acute state, she received corticosteroids to which she responded favorably. In addition, based on the previously published cases, which demonstrated an overactive immune response due to accumulated HSP70, corticosteroids may play an important role in patient management in acute settings to prevent progression of comorbid features of systemic inflammation during febrile illness due to cytokine release. However, this preliminary finding will require prospective patient identification and further longitudinal study.

The causative reported variant, p.(Val54Leu), was previously published in a cohort of six patients, all of whom were homozygous for the variant.[2] In this work, the authors genotyped 1012 healthy individuals of AJ origin and identified five carriers, which allowed them to derive a carrier rate of 1:202 individuals. This same variant was found in each of the individuals in our cohort. Owing to the increasing numbers of families identified in our bioregistry with this condition, we found it important to assess the carrier status from available genotype information. We determined a carrier rate of one in 216 for the p.(Val54Leu) variant across more than 125,000 individuals of Jewish descent, but one in 168 for individuals of AJ descent without Sephardi designation. In addition, we searched the gnomAD browser (v2.1.1)[17] and found 28 carriers in a group of 10,370 individuals of AJ descent, estimating the variant's population frequency to be greater than one in 370 across another cohort. Thus, it is likely that the carrier rate is in the range of 1:168 to 1:370, dependent on different communities of AJ descent. This significant carrier rate suggests that previous cases may have gone unsolved, as is the case of a number of leukodystrophies, unless penetrance is more variable than currently recognized. Furthermore, over-representation of the *HIKESHI* p.(Val54Leu) variant in AJ individuals of Polish origin when compared with Hungarian follows a similar pattern to other Ashkenazi subgroups studied in the context of Bloom syndrome (OMIM:210900)[16,18] and suggests that some Ashkenazi subgroups may be at particular risk.

Although this recurrent variant deserves attention, another published individual with *HIKESHI* mutations was the affected child of a consanguineous Finnish couple and harbored a homozygous variant, c.11G>C p.(Cys4Ser).[1] Two other variants are listed in the ClinVar database as likely pathogenic: a c.31-1G>T variant that affects the acceptor splice site of exon 2 and a variant c.164A>G p.(Tyr55Cys) that affects the adjacent amino acid to the founder variant found in the AJ population. Thus, although the majority of cases identified are due to the p.(Val54Leu) variant, agnostic sequencing approaches may identify

further individuals with variants in this gene and broaden the phenotypic spectrum of this disorder. Furthermore, this disorder along with genetic disorders of mitochondrial origin, vanishing white matter disease, or several genotypes of Aicardi-Goutières syndrome should be considered in affected individuals with decompensation during acute febrile illness or in patients with prolonged recovery from these periods.

Variants in *HIKESHI*, encoding the Hikeshi protein, have been associated with a hypomyelinating leukodystrophy with diffuse signal abnormalities throughout the brain WM and thinning of the corpus callosum.[1,2] In previously reported cases, over time there was evidence of cystic change, which was not observed in the individuals presented here, although this may be limited by the age at which imaging studies were performed.[1,2] The pathological findings seen in our affected individual are consistent with a leukodystrophy characterized by WM pallor and near-total absence of myelin with a relative preservation of axons on neurofilament stains. We provide pathologic evidence that variants in *HIKESHI* cause a primary disorder of the myelin.

In conclusion, the finding of myelin involvement in this first histopathologic examination of a genetically confirmed case of *HIKESHI*-related pathology supports the radiologic classification of this disorder as a hypomyelinating leukodystrophy. The high carrier rate of the recurrent variant seen in the AJ population suggests that this rare condition remains underdiagnosed or that penetrance is unexpectedly variable. Increased attention to screening and diagnosis of this condition, particularly for individuals of AJ origin, will provide important information concerning pregnancy planning, prognosis, and potential therapeutics in the future of this recently recognized condition.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

1. Vasilescu C, Isohanni P, Palomaki M, Pihko H, Suomalainen A, Carroll CJ. Absence of Hikeshi, a nuclear transporter for heat-shock protein HSP70, causes infantile hypomyelinating leukoencephalopathy. Eur J Hum Genet. 2017;25:366–370. [PubMed: 28000699]
2. Edvardson S, Kose S, Jalas C, et al. Leukoencephalopathy and early death associated with an Ashkenazi-Jewish founder mutation in the Hikeshi gene. J Med Genet. 2016;53:132–137. [PubMed: 26545878]
3. Duncan ID, Radcliff AB. Inherited and acquired disorders of myelin: the underlying myelin pathology. Exp Neurol. 2016;283:452–475. [PubMed: 27068622]
4. Pouwels PJ, Vanderver A, Bernard G, et al. Hypomyelinating leukodystrophies: translational research progress and prospects. Ann Neurol. 2014;76:5–19. [PubMed: 24916848]

5. Kevelam SH, Steenweg ME, Srivastava S, et al. Update on leukodystrophies: a historical perspective and adapted definition. Neuropediatrics. 2016;47:349–354. [PubMed: 27564080]
6. van der Knaap MS, Bugiani M. Leukodystrophies: a proposed classification system based on pathological changes and pathogenetic mechanisms. Acta Neuropathol. 2017;134:351–382. [PubMed: 28638987]
7. Vanderver A, Prust M, Tonduti D, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. Mol Genet Metab. 2015;114:494–500. [PubMed: 25649058]
8. Kose S, Furuta M, Imamoto N. Hikeshi, a nuclear import carrier for Hsp70s, protects cells from heat shock-induced nuclear damage. Cell. 2012;149:578–589. [PubMed: 22541429]
9. Ogawa Y, Imamoto N. Nuclear transport adapts to varying heat stress in a multistep mechanism. J Cell Biol. 2018;217:2341–2352. [PubMed: 29748335]
10. Rahman KMZ, Mamada H, Takagi M, Kose S, Imamoto N. Hikeshi modulates the proteotoxic stress response in human cells: implication for the importance of the nuclear function of HSP70s. Genes Cells. 2017;22:968–976. [PubMed: 28980748]
11. van der Knaap MS, Breiter SN, Naidu S, Hart AA, Valk J. Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. Radiology. 1999;213:121–133. [PubMed: 10540652]
12. Ekstein J, Katzenstein H. The Dor Yeshorim story: community-based carrier screening for Tay-Sachs disease. Adv Genet. 2001;44:297–310. [PubMed: 11596991]
13. Rabin R, Hirsch Y, Johansson MM, et al. Study of carrier frequency of Warsaw breakage syndrome in the Ashkenazi Jewish population and presentation of two cases. Am J Med Genet A. 2019;179:2144–2151. [PubMed: 31287223]
14. Fedick A, Su J, Jalas C, et al. High-throughput carrier screening using TaqMan allelic discrimination. PLoS One. 2013;8:e59722. [PubMed: 23555759]
15. Helman G, Lajoie BR, Crawford J, et al. Genome sequencing in persistently unsolved white matter disorders. Ann Clin Transl Neurol. 2020;7:144–152. [PubMed: 31912665]
16. Risch N, Tang H, Katzenstein H, Ekstein J. Geographic distribution of disease mutations in the Ashkenazi Jewish population supports genetic drift over selection. Am J Hum Genet. 2003;72:812–822. [PubMed: 12612865]
17. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–443. [PubMed: 32461654]
18. Shahrabani-Gargir L, Shomrat R, Yaron Y, Orr-Urtreger A, Groden J, Legum C. High frequency of a common Bloom syndrome Ashkenazi mutation among Jews of Polish origin. Genet Test. 1998;2:293–296. [PubMed: 10464606]

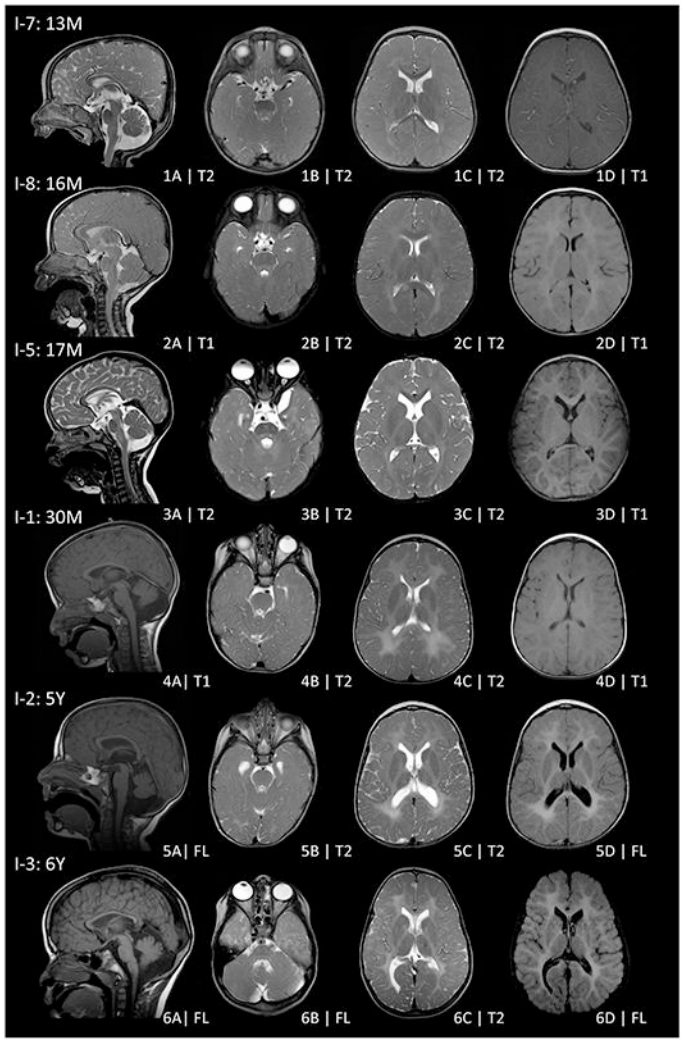


**FIGURE 1.**

Magnetic resonance imaging (MRI) of *HIKESHI*-affected individuals. Overview of MRI features in individuals with the p.(Val54Leu) variant in HIKESHI. (1A-6A) In general, there was mild asymmetric thinning of the posterior corpus callosum and normal cerebellum. (1B) MRI at age 13 months in one individual raised suspicion of a hypomyelinating disease with T2 hyperintensity in early myelinating structures such as the central tegmental tracts and posterior columns—medial longitudinal fasciculus in the pons. (4B and 5B) Over time, myelination in these tracts appears to normalize. (1C-6C) The signal abnormalities are diffuse and confluent, with T2 hyperintensity affecting the frontal white matter (WM) and extending posteriorly through the internal capsule (particularly the posterior limb), and into the deep periventricular WM and also superiorly through the supratentorial WM and inferiorly into the brainstem, also seen on T1 and fluid-attenuated inversion recovery (FLAIR) imaging (1D-6D). (3C) The corpus callosum was mildly spared in I-5. (5C and 6C) Over time, scans remain relatively unchanged; however, in some individuals there is increased T2 hyperintensity in the affected WM. At age 30 months, the abnormal signal was diffuse and confluent. M, months; Y, years; FL, FLAIR.

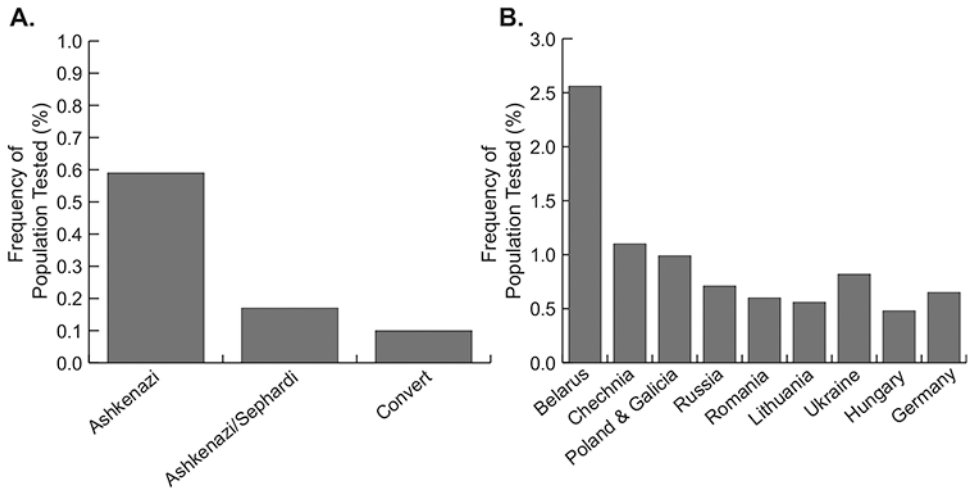


**FIGURE 2.**

*HIKESHI* carrier frequency among Ashkenazi Jewish and by country of origin. (A) The observed frequency for the c.160G>C p.(Val54Leu) is 0.59% or one in 168 in Ashkenazi Jewish individuals, 0.17% or one in 574 in mixed Ashkenazi/Sephardi Jewish individuals, and is absent in Sephardi Jewish individuals. (B) Data classified by country of ancestral origin show high variation in number of samples per country, ranging from 39 (Belarus) to 7867 (Hungary). We therefore limited the statistical test to include frequencies for Poland and Galicia 0.99% versus Hungary 0.48%. Merging of Poland with Galicia was done on the historical and geographical common basis as in previous studies that studied Ashkenazi subgroups.[16]

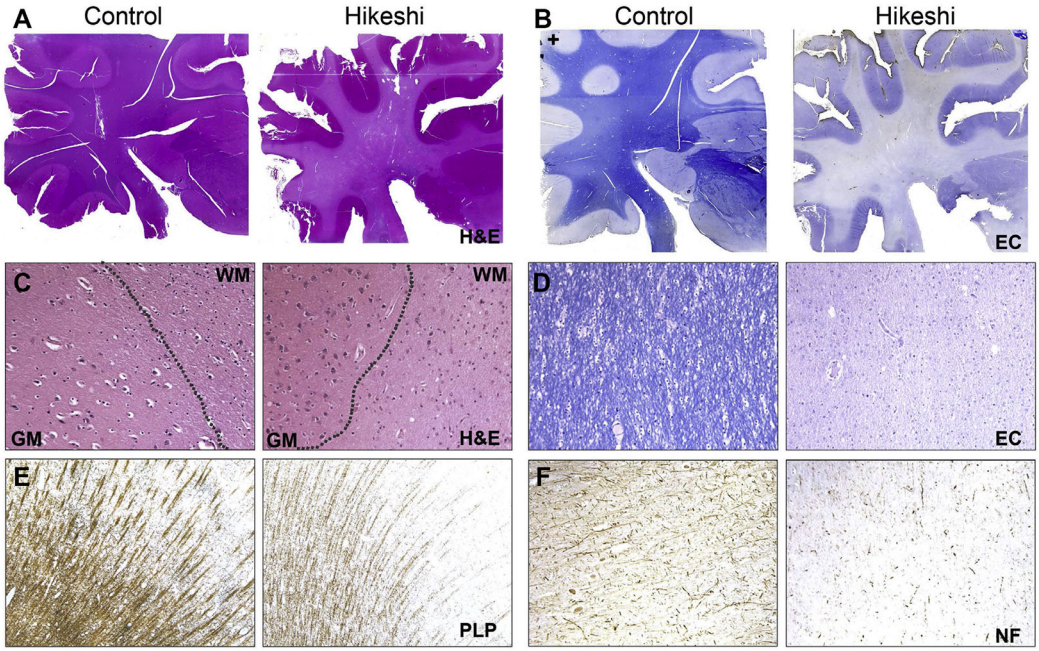


**FIGURE 3.**

Pathological findings in postmortem brain tissue of a *HIKESHI*-affected individual. (A) Hematoxylin and eosin (H&E) staining of coronal sections of control and *HIKESHI*-affected brain samples show diffuse myelin pallor in the *HIKESHI*-affected individual compared with the control sample. (B) Similarly, staining with erichrome cyanine (EC), which stains myelin lipid, reveals a dramatic loss of myelin in *HIKESHI*-affected tissue compared with control samples. (C) Examination at higher resolution shows hypercellularity due to reactive changes within the gray matter (GM) and white matter (WM) in the *HIKESHI*-affected tissue as well as pallor of myelin. (D) Higher resolution of EC-stained sections reveals a complete lack of myelin in the Hikeshi brain. (E) Further staining against the major myelin protein, proteolipid protein (PLP), shows a decrease in cortical myelin. (F) Neurofilament (NF) staining demonstrates a decrease in axon density in the *HIKESHI*-affected individual compared with the control sample with relative preservation of axons when compared with the total absence of myelin staining.

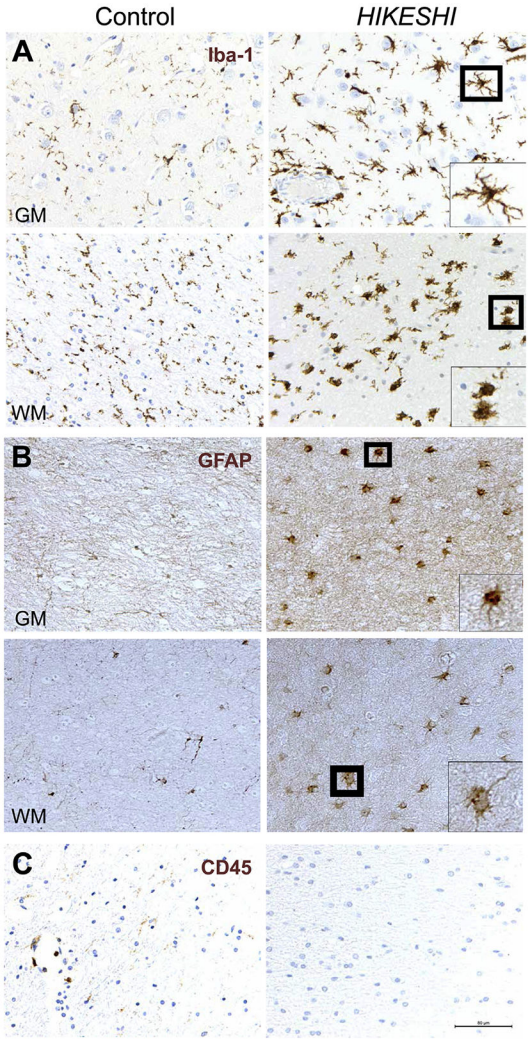


**FIGURE 4.**

Astrogliosis and microgliosis in white and gray matter of Hikeshi brain sample. (A) Iba-1 labeling in white matter (WM) and gray matter (GM) in postmortem Hikeshi brain shows reactive microgliosis compared with age-matched control tissue. The microglia in GM appear ramified and bushy, whereas the microglia in the WM have a round phagocytic appearance. (B) Similarly, glial fibrillary acidic protein (GFAP) stain shows reactive gliosis in both WM and GM of *HIKESHI*-affected tissue compared with control. (C) Examination of CD45, a marker for peripheral leukocytes, shows no infiltration of leukocytes in the affected individual.

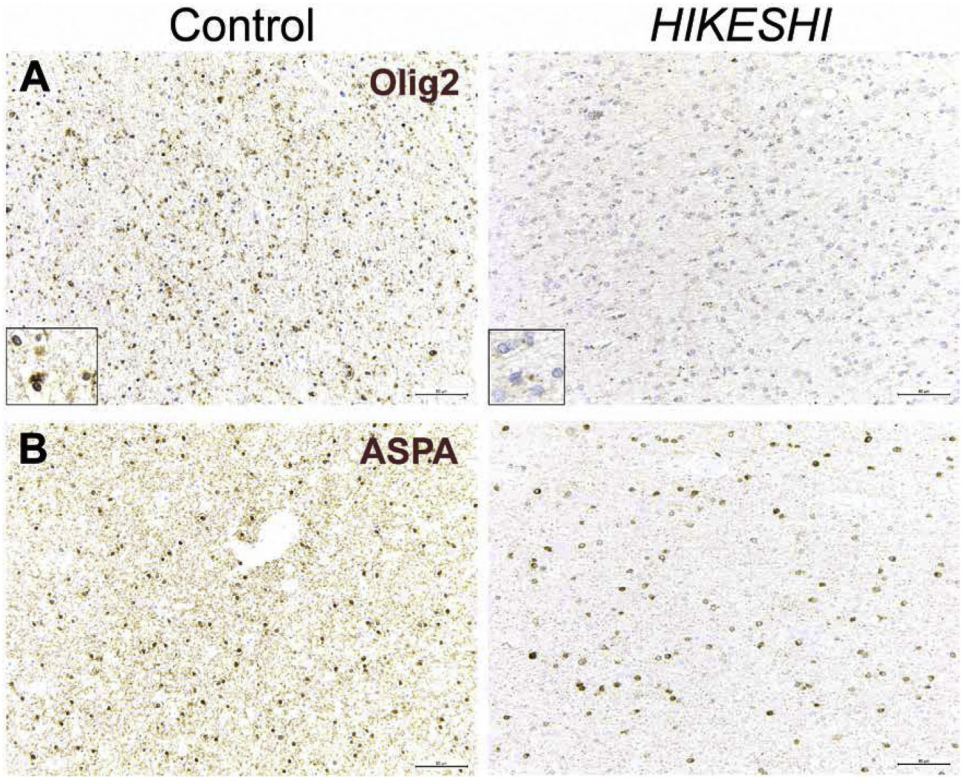


**FIGURE 5.**

Oligodendrocyte loss and degeneration occurs in postmortem Hikeshi brain. (A) Olig2 stain labels cells of the oligodendrocyte lineage, and a dramatic loss of this transcription factor is seen in the postmortem *HIKESHI*-affected brain tissue compared with age-matched control tissue. The inset shows co-localized olig2 (brown) and nuclear stain (blue), with no labeling in the *HIKESHI* brain. (B) The mature oligodendrocytes were visualized with staining for aspartoacylase (ASPA), and whereas *HIKESHI*-affected tissue stains with ASPA, the intensity of the staining is lower compared with the control sample.