ARTICLE

Recurrent subacute post-viral onset of ataxia associated with a *PRF1* mutation

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Inflammation is an important contributor to pediatric and adult neurodegeneration. Understanding the genetic determinants of neuroinflammation provides valuable insight into disease mechanism. We characterize a disorder of recurrent immune-mediated neurodegeneration. We report two sisters who presented with neurodegeneration triggered by infections. The proband, a previously healthy girl, presented at 22.5 months with ataxia and dysarthria following mild gastroenteritis. MRI at onset showed a symmetric signal abnormality of the cerebellar and peritrigonal white matter. Following a progressive course of partial remissions and relapses, she died at 5 years of age. Her older sister had a similar course following varicella infection, she died within 13 months. Both sisters had unremarkable routine laboratory testing, with exception of a transient mild cytopenia in the proband 19 months after presentation. Exome sequencing identified a biallelic perforin1 mutation (*PRF1*; p.R225W) previously associated with familial hemophagocytic lymphohistiocytosis (FHL). In contrast to FHL, these girls did not have hematopathology or cytokine overproduction. However, 3 years after disease onset, the proband had markedly deficient interleukin-1 beta (IL-1 β) production. These observations extend the spectrum of disease associated with perforin mutations to immune-mediated neurodegeneration triggered by infection and possibly due to primary immunodeficiency.

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INTRODUCTION

Childhood neurodegeneration is a diagnostic challenge because it arises as a primary or secondary consequence of many genetic and acquired etiologies, particularly infectious causes.¹ Genetic bases include disorders of metabolism, myelination, vascular integrity and inflammation.^{2,3} Dysfunction of the innate immune system is the most common primary inflammatory cause of neurodegeneration.⁴ The aberrant release of cytokines by activated microglia and circulating macrophages in disorders of the innate immune system damage the myelin sheath directly, and also enhance blood-brain barrier permeability, allowing further recruitment of leukocytes to the CNS.

The myriad of inflammatory neurodegenerative disorders rarely have pathognomonic clinical or radiographic features, and are generally subclassified as affecting primarily the white matter, the gray matter, or both. Even when specific neuroradiological signs suggest a particular subset, diagnosis remains challenging and requires additional investigations. Consequently, many neurodegenerative disorders defy a precise diagnosis.

Pro- and anti-inflammatory cytokines can both cause and mitigate neurodegeneration.^{5,6} Interleukin-1 beta (IL-1 β) is a potent pro-inflammatory cytokine associated with the induction and maintenance of the innate immune response. It has been implicated in neurodegeneration as well as neuroprotection (reviewed by Fogal and Hewett⁷). IL-1 β is overexpressed within the central nervous system of patients with multiple sclerosis, in animal models of experimental autoimmune encephalomyelitis, and systemically in some patients with active hemophagocytic lymphohistiocytosis (HLH) with CNS involvement.^{8–10}

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder of immune dysregulation, resulting in hypercytokinemia associated with fever, hepatosplenomegaly, and cytopenia.^{10,11} Identified genetic causes of FHL include mutations in perforin1 (*PRF1*; FHL2),¹² *UNC13D* (FHL3),¹³ *STX11* (FHL4),¹³ and *STXBP2* (FHL5).¹⁴ The diagnostic criteria for HLH include fever, cytopenia (≥ 2 cell lineages), hypertriglyceridemia and/or hypofibrinogenemia, and hemophagocytosis in the bone marrow, spleen, or lymph nodes. Additional diagnostic criteria include

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decreased NK-cell activity, hyperferritinemia, and elevated secretion of soluble IL-2 receptor.¹⁵ Treatment includes chemo-immunotherapy and hematopoietic stem cell transplantation.

We present a family with two daughters who died of a previously undescribed neurodegenerative disorder triggered by infection. Both girls had biallelic mutations in *PRF1* without diagnostic features of FHL2.

MATERIALS AND METHODS

Patient data

Clinical data on each affected individual were obtained through retrospective chart review and interviews of the parents. Data on the probanda were also collected prospectively.

Standard protocol approvals, registrations, and patient consents

Family members gave informed consent/assent for protocol H09-01228 (University of British Columbia, Vancouver, Canada) and for protocol 76-HG-0238 (National Institutes of Health, Bethesda, MD, USA).

Exome sequencing and analysis

Exome sequencing was performed on the nuclear family (both affected sisters, their three unaffected siblings, and both parents – Figure 1) as previously described.¹⁶ We used NextGENe software version 2.2.2 (SoftGenetics, LLC, State College, PA, USA) to align sequences to the human reference genome (NCBI, Build 37 v2) and call variants. Each sample alignment was performed allowing one mismatched base and $\geq 85\%$ of the read matching the reference sequence. Variant calling required that the variant was observed in $\geq 20\%$ and ≥ 3 short reads, with a minimum of $5 \times$ coverage.

Following generation of a variant list fitting autosomal recessive inheritance by NextGENe Viewer's Variant Comparison Tool, we ranked the variants using

VAR-MD as described.¹⁷ To accept variants for ranking, we required a variant to meet the following criteria in at least four of the seven exomes: a cutoff for genotype confidence score of ≥ 16 , or for variants that had scores between 7 and 16, a genotype confidence score of at least one-fourth the coverage value.

Cell stimulation and measurement of cytokines

Peripheral blood mononuclear cells (PBMCs) were purified over a gradient of Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ, USA). PBMCs were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine.

For evaluation of inflammasome activation, extracellular IL-1 β was detected in culture supernatants after stimulation of PBMCs with lipopolysaccharide (LPS) in the presence or absence of exogenous ATP.¹⁸ An LPS challenge was performed (in triplicate) on patient IV-5, unaffected siblings, parents, and seven healthy controls. Briefly, 2×10^5 PBMCs/well were added to a 96-well plate and treated in triplicate with 10 ng/ml *E. coli* Ultra-Pure LPS (InvivoGen, San Diego, CA, USA) for 4 h with or without addition of 5 mM ATP for the last hour of incubation. The IL-1 β concentrations were measured using an ELISA Ready-SET-Go (eBioscience, San Diego, CA, USA). Statistical analysis was performed using SPSS v.19 (IBM, North Castle, NY, USA), applying independent sample *t*-tests.

A Luminex-based cytokine/chemokine assessment was performed on patient and healthy adult control whole blood.¹⁹ Briefly, 2.2 μ l of a $10 \times$ TLR ligand for stimulation of whole blood (final working concentrations are 10 ng/ml LPS, 1 μ g/ml PAM, 100 μ g/ml pIC, 10 μ g/ml PGN, and 10 μ M R848) was added to a well of a 96-well culture plate. Assays were performed in duplicate. Two hundred microliters of a 1:1 whole blood:RPMI 1640 (Invitrogen, Carlsbad, CA, USA) mixture was then added to each well. After a 24-hour incubation, cytokine levels (IL-1 β , IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IP-10, MIP-1 α , MIP-1 β , TNF- α , IFN- α 2, and IFN- γ) were measured in the supernatants using a Luminex immunobead-based 12-plex assay (Luminex running the Masterplex software, MiraiBio, Alameda, CA, USA).

RESULTS

Clinical features

The proband and her sister (Table 1) were daughters of a consanguineous couple of Lebanese origin. The parents were first cousins but had additional loops of consanguinity such that the estimated coefficient of inbreeding for the proband was 0.13 (Supplementary data), the equivalent of the offspring of half-sibs.

Patient IV-5. The proband (IV-5, Figure 1) was born at term after an unremarkable pregnancy and delivery. She had normal early growth and development and good health aside from upper respiratory and middle ear infections. Beginning at 18 months, her weight gain declined, and at 22.5 months, a week after a bout of gastroenteritis with an eczematous rash, she developed ataxia, abnormal eye movements and dysarthria as well as brain white matter abnormalities (see below; Figure 2). She had elevated CSF protein concentration (0.56 g/l; ref.: 0.10–0.35 g/l). Bone marrow biopsy showed iron depletion. Additional testing was otherwise unremarkable (Table 1 and Supplementary Table 1).

Soon after partial resolution of the ataxia and dysarthria, she developed progressive disease. At age 32.5 months, her brain MRI documented further white matter degeneration, and a skeletal muscle biopsy showed mild variation in fiber size and type II fiber atrophy on light microscopy; fiber and mitochondrial ultrastructure as well as mitochondrial respiratory chain activity were normal. Also, her CSF had unremarkable chemistry and cytology, and by PCR, no detectable nucleic acid indicative of infection with Cytomegalovirus, Herpes virus type 6, Varicella-Zoster, Epstein-Barr virus, Adenovirus, West Nile virus, Enterovirus, Herpes simplex viruses 1 and 2, and toxoplasmosis (Table 1). By age 42 months, she developed mild

Figure 1 Familial pedigree and photographs of the affected girls. (a) The pedigree shows two loops of consanguinity; however, molecular studies and further family history analysis confirmed additional loops. The affected girls are noted by shaded shapes and the proband by an arrow. (b) Photograph of the elder affected girl. (c) Photograph of the proband.

Table 1 Summary of key laboratory investigations

		Age (months)				
<i>Patient IV-5</i>		22.5	23.5	32.5	44–44.5	45 59 60–61
<i>Time after onset (months)</i>		Onset	1	10	21.5–22	22.5 36.5 37.5–38.5
Diagnostic cutoff for		Test result (reference range)				
Analyte and units	HLH ^{1,5}					
Ferritin µg/l	> 500	7 (6–30)	113 (103–135)	—	79 → 96 (105–135)	27 (12–30)
Hemoglobin g/l	< 90	114 (103–135)	1.72 (1.5–8.5)	—	0.96 → 1.53 (1.5–8.5)	127 (105–135)
Neutrophils × 10 ⁹ /l	< 1.0	2.05 (1.5–8.5)	253 (200–550)	—	77 → 108 (200–550)	3.34 (1.5–8.5)
Platelets × 10 ⁹ /l	< 100	219 (200–550)	—	—	2.75	187 (200–490)
Fibrinogen g/l	< 1.5	—	23 (5–45)	—	703 → 321 (5–45)	27 (< 36)
ALT U/l	Elevated	46 (20–60)	54 (20–60)	—	740 → 117 (20–60)	35 (< 36)
AST U/l	Elevated	Total 74 (56–75)	—	—	Total 64 (59–78)	77
Serum protein g/l	Low	0.67 (0.30–1.41)	—	—	—	—
Triglycerides mmol/l	> 3.0	3.96 (1.15–4.70)	—	—	—	—
Cholesterol mmol/l	—	1.70	—	—	—	—
HDL mmol/l	Low	0.56 (0.10–0.35)	0.28 (0.10–0.35)	—	—	—
CSF protein g/l	Elevated	12 (0–20)	12 (0–20)	4 (0–20)	—	—
CSF-nucleated cell × 10 ⁶ /l	Elevated	Negative ^a	—	Negative	—	—
CSF microbiology	—	Normocellular for age. No abnormal cells. Iron deficiency (Bilateral iliac crests)	—	—	Normocellular for age. Mild dyserythropoiesis consistent with iron deficiency (Right iliac crest)	—
Bone marrow aspirate and biopsy	—	—	—	—	—	—
<i>Patient IV-3</i>		75.5	82.5	83	88	
<i>Time after onset (months)</i>		Onset	6.5	7	12	
Diagnostic cutoff for		Test result (reference range)				
Analyte and units	HLH ^{1,5}					
Hemoglobin g/l	< 90	—	133 (118–146)	121 (118–146)	—	132 (118–146)
Neutrophils × 10 ⁹ /l	< 1.0	—	1.49 (1.8–5.4)	1.16 (1.8–5.4)	—	3.1 (1.7–5.0)
Platelets × 10 ⁹ /l	< 100	—	254 (180–440)	230 (180–440)	—	406 (180–440)
Fibrinogen g/l	< 1.5	—	3.24 (1.68–4.40)	—	—	—
ALT U/l	Elevated	—	5 (10–25)	—	—	—
AST U/l	Elevated	—	21 (15–50)	—	—	—
protein g/l	Low	—	Albumin 43 (35–52)	—	—	—
HDL mmol/l	Low	—	—	—	—	—
CSF protein g/l	Elevated	—	0.30 (0.15–0.45)	—	—	—
CSF-nucleated cell × 10 ⁶ /l	Elevated	—	4 (< 1)	—	—	—

Abbreviation: HLH, hemophagocytic lymphohistiocytosis.

Values fulfilling diagnostic criteria for HLH are shown in **bold**.

PCR for Cytomegalovirus, Herpes virus type 6, Varicella-Zoster, Epstein-Barr virus, Adenovirus, West Nile virus, Enterovirus, Herpes simplex virus 1 and 2, and toxoplasmosis. It is performed on a cryopreserved sample.

^aPCR for Cytomegalovirus, Herpes virus type 6, Varicella-Zoster, CSF bacterial and viral culture.

Figure 2 Brain MRI of patients IV-5 and IV-3, comparing the initial and follow-up studies. Patient IV-5: axial and coronal T2 images (a–d, 22.5 months of age, 2 weeks after onset; e–h, 32.5 months of age, 10 months after onset) show progression of signal abnormality in the cerebellar white matter (fine arrows) with atrophy of the cerebellum and subsequent involvement of the cerebral white matter (thick arrows). Patient IV-3: axial T2 and axial post-contrast T1 images (i–l, 79 months of age, 3 months after onset; m–p, 88 months of age, 12 months after onset) show extensive and progressive signal abnormality involving the cerebellar (thin arrow) and cerebral (thick arrows) white matter, thalami and basal ganglia, with some lesions showing post-contrast enhancement (asterisks).

splenomegaly and lymphadenopathy without hepatomegaly or detectable acute viral infection. Two months later, she was admitted to hospital for vomiting, tachycardia, tachypnea and fever (39 °C); she had hypertrophic cardiomyopathy with mild diastolic dysfunction, mild pancytopenia, and elevated AST and ALT levels. Again her bone marrow biopsy was unremarkable except for iron depletion (Table 1). Her immune studies showed normal numbers and distribution of T, B, and NK cells as well as unremarkable responses to mitogens and normal complement activity (Supplementary Table 1). No viral or bacterial pathogens were identified. During her recovery, she manifested persistent neck rigidity suggestive of meningeal inflammation.

Shortly after this hospitalization, she developed seizures. Over the ensuing 17 months, she gradually became blind, lost cognitive and

most motor skills, and developed dysphagia. Throughout this period, she manifested neck rigidity following each of many infections but had no more recorded fevers until the week before her death when her temperature was 38.5 °C. She died at 61 months of age with upper respiratory infection and cardiac failure secondary to left ventricular hypertrophy with outlet obstruction and mitral regurgitation. The family declined an autopsy.

Patient IV-3. The proband's sister (IV-3; Figure 1) was the product of an uneventful pregnancy. She had mild developmental delay and hearing loss. At age 75.5 months, concurrent with varicella infection, she had a seizure in the context of fever, deterioration of mental status and was diagnosed with varicella meningitis. CT brain imaging

showed dilated ventricles and cerebellar swelling. Following 2 weeks of treatment with acyclovir and steroids, she regained baseline neurological function. Subsequently, however, she became ataxic and was unable to walk. Three months after initial presentation her brain MRI showed diffuse white matter changes (Figure 2). She gradually lost additional motor skills and strength; eight months after presentation the nerve conduction velocities of her sensory and motor evoked responses were at the lower limits of normal in the right upper and lower limbs (Right Tibial Motor at ankle: latency 3.22 ms, conduction 40.97 m/s). Her CSF showed normal protein, glucose and amino acid levels. CSF neurotransmitters were normal except for an elevated neopterin level; her skin fibroblasts had normal 6-pyruvoyltetrahydropterin synthase activity, excluding 6-pyruvoyltetrahydropterin synthase deficiency as the cause. All other testing was unremarkable (Table 1 and Supplementary Table 1). At age 88.5 months, her brain MRI showed progression of the white matter changes (see below; Figure 2). She died at 91 months of age, 1 year before the birth of her younger sister, the proband. No autopsy was performed.

Brain imaging

Patient IV-5. Two weeks after the onset of ataxia, a CT scan showed abnormal hyperintense areas in the cerebellar white matter. A subsequent brain MRI (Figures 2a–d) showed symmetrical areas of T2 hyperintense signal abnormality in the cerebellar white matter and the cerebral white matter of the frontal lobe, as well as in a peritrigonal distribution.

Ten months later a repeat brain MRI (Figure 2e–h) showed worsening of the cerebellar changes with atrophy and involvement of the gray matter. In addition, new areas of signal abnormality distributed along an anterior to posterior gradient were seen in the periventricular cerebral white matter.

Patient IV-3. Three months after her first seizure, a brain MRI (Figure 2i–l) showed cerebellar volume loss, diffuse symmetrical T1 hypointense and T2 hyperintense signal abnormalities in the cerebellar white matter, mild symmetrical ventriculomegaly and signal abnormalities in the periventricular white matter, thalami, basal ganglia, internal capsule, and midbrain. Some of these lesions enhanced after contrast, suggesting disruption of the blood-brain barrier.

Nine months later, a brain CT confirmed the prior findings, and a repeat brain MRI identified worsening of the T1 and T2 signal abnormalities involving the cerebellar white matter and extending into the gray matter, and greater involvement of the cerebral white matter, thalami, basal ganglia, internal and external capsules (Figure 2m–p), and brainstem. Many lesions in the subcortical white matter, basal ganglia, cerebellar peduncles and brainstem enhanced with contrast.

Defective production of IL-1 β

In the context of her normal B- and T-cell studies, the absence of fever with apparent infection and neurodegeneration triggered by minor infections, we suspected that the proband had a defect in pyrogen generation mediated by innate immunity. To assess this, we tested if her monocytes could produce the pyrogen IL-1 β in response to an LPS challenge in the presence of ATP (inflammasome activation assay).¹⁸ At 59 months (37 months after onset), the proband's PBMCs failed to produce IL-1 β , in contrast to healthy controls, the asymptomatic siblings, and the parents (Figure 3). Further testing using the Luminex cytokine assay confirmed the minimal IL-1 β production and revealed a broad decrease in inflammatory cytokines,

Figure 3 Inflammasome activation assay. Concentration of IL-1 β excreted by PBMCs measured by ELISA. The cells were treated with 10 ng/ml LPS, with or without treatment with ATP. All experiments were performed in triplicate. 'Controls' represents the mean of seven biological replicates in healthy controls; error bars indicate the standard error of the mean. Proband IL-1 β after stimulation with LPS and ATP is significantly decreased in comparison to other healthy controls, and to all five unaffected family members ($P < 0.05$). All heterozygous family members showed no significant difference from controls. Control experiments with media only, or media with ATP, yielded negligible concentration of IL-1 β in all samples (data not shown). An asterisk indicates a statistically significant difference between the patient and controls (independent samples t -test, $P \leq 0.05$).

following stimulation with a panel of innate immune activators (Supplementary Figure 1). The proband died before these abnormalities could be investigated further.

Exome sequencing identifies a homozygous pathogenic *PRF1* mutation

To determine the molecular etiology for this unusual innate immune response, we performed exome sequencing on the DNA of the affected girls and immediate family members (parents and unaffected siblings; Figure 1). Analysis for homozygous variants unique to the affected siblings identified a mutation (NM_005041.4: c.673C>T, p.R225W) in exon 3 of the *PRF1* gene (Supplementary Figure 2). Both parents and all of the unaffected siblings were heterozygous for the same variant. The p.R225W mutation is in the transmembrane region of perforin and has been associated with FHL2.^{11,12,20,21} In an animal cell model, p.R225W causes decreased cytotoxicity and impaired trafficking of secretory granules.²²

DISCUSSION

We present two sisters with fatal neurodegeneration showing similar patterns of white matter cerebellar degeneration. We also found that the proband had a defective innate immune response, and that both she and her affected sister had pathogenic biallelic mutations in *PRF1* as a possible cause of their disease.

The *PRF1* mutation identified in our patients has been associated with FHL2,¹¹ but the sisters did not manifest diagnostic features of FHL2.¹⁵ Specifically, they had minimal or no fevers, no hypertriglyceridemia, no hypofibrinogenemia, and no hyperferritinemia. The youngest had only mild transient cytopenia. Because both sisters died before the *PRF1* mutation was identified, they were not tested for

Table 2 Comparison of neurological features of patients reported with PRF1 mutations for whom MRI images or detailed description was provided

Reference	PRF1 mutation	Age		Infectious trigger	Features onset	Systemic features of		MRI
		onset/ death				HLH	Clinical Dx at onset	
Present case IV-5	p.R225W	22.5 m/ 61 m		Unidentified GI virus at onset	Ataxia, Dysarthria, Nystagmus	Inconsistent ^a	Metabolic neurodegeneration	Progressive symmetrical cerebellar white matter signal abnormality with cerebellar atrophy. Less involvement of the cerebellar white matter
Present case IV-3	p.R225W	5 y and 8 m/ 7 y and 7 m		Varicella at onset	Seizure, Ataxia	No	Encephalomyelitis	Progressive symmetrical signal abnormality of the cerebellar and cerebral white matter, cortex, gray matter structures and brain stem, with multiple enhancing lesions.
Chiapparini <i>et al</i> ²⁶	p.R225W	13 y/ alive 18 m post-HSCT		Not reported	Headache, Gait imbalance	Yes	Inflammatory immune-mediated disorder	Symmetrical cerebellar and cerebellar gray and white matter signal abnormality, worse in the cerebellum, with multiple enhancing lesions.
ME van Egmond <i>et al</i> ³⁶	p.G149S + p.E253K	11 m/ alive 18 m post-HSCT		Not reported	Regression in motor and language skills	Yes	Suspected acute disseminating encephalomyelitis (ADEM).	Signal abnormalities in the cortex, cerebellar and cerebellar white matter, with multiple enhancing perivascular foci.
Astigarraaga <i>et al</i> ⁴⁴	p.L215I + p.A262D	4 y/ alive 16 m post-HSCT		varicella	Ataxia, nystagmus, dysmetria	Yes	Cerebellitis	Signal abnormalities in the periventricular and cerebellar white matter.
Moshous <i>et al</i> ⁴⁴	p.E6X + p.A91Val/ p.R119W	3 y and 6 m/ alive 5 m post-HSCT		URTl at exacerbation 3 months after onset	Headaches, ataxia, visual disturbances	Yes	CNS vasculitis	Asymmetrical cerebellar and cerebellar gray and white matter signal abnormality, with multiple supra and infratentorial lesions with peripheral post-contrast enhancement and central necrosis.
Feldman <i>et al</i> ⁴³	p.P459L	5 m/44 m		Upper respiratory infection at onset	Poor head control, tremor, abnormal tone	Inconsistent ^a	Chronic lymphocytic meningitis	Signal abnormalities in the periventricular, deep supratentorial and cerebellar white matter, corpus callosum and brainstem with some enhancing lesions.
Beaty <i>et al</i> ²⁵	p.R356W + T450W ^b	16 y/18 y		Not reported	Unilateral upper extremity weakness and sensory deficits	Inconsistent ^a	Multiple sclerosis	Patchy areas of focal demyelination

Abbreviations: GI, gastrointestinal; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; m, months; PRF1, perforin 1 mutation; URTl, upper respiratory tract infection; y, years.

The p.R225W mutation is highlighted in **bold**.

^aMild systemic features that did not fulfill diagnostic criteria for FHL, subsequently interpreted as possibly related to PRF1 in the context of the genetic diagnosis.

^bPredicted protein alteration according to the report of 1066C>T and 1349C>T mutations in the coding sequence; gene accession number not provided.

reduced NK-cell activity or elevated soluble IL-2 receptor levels. Also, two bone marrow biopsies in the proband did not demonstrate increased hemophagocytosis found in 82% of FHL2 patients.²³

Neurodegeneration has been reported as a possible feature of FHL2 and sporadic HLH,^{24–38} with a frequency of 37–69%.^{23,29,39,40} A third of patients have neurological symptoms at diagnosis,⁴⁰ and 36% of those with *PRF1* mutations have some CNS involvement.²³ Although there is an association of variations in *PRF1* with susceptibility to multiple sclerosis,⁴¹ very few patients with HLH have had neurological symptoms as the sole presenting feature, and consistent with the expected ascertainment and reporting bias,^{29,30,32,39} all developed the diagnostic features of FHL2–HLH, with the exception of one individual who was diagnosed by brain histopathology but without identification of a mutation.^{25,26,29,31–33,36,42} The sisters reported herein are therefore the first individuals reported with neurodegeneration, biallelic pathogenic *PRF1* mutations and no diagnostic features suggestive of FHL–HLH (Table 2).

Potential explanations for this lack of features include the nature of the triggering pathogen, pathogen–host interaction, the immunological landscape defined by prior infectious exposures, genetic background or another primary cause of neurodegeneration. By history, however, the sisters did not have common triggering pathogens or common environmental exposures. Thus, this precludes ready acceptance of the first three possible explanations. Notwithstanding the limitations of exome sequencing,¹⁶ extensive evaluation of all exome variants in the siblings failed to identify an alternative primary causal mutation for neurodegeneration or candidate modifier variant even though the high degree of consanguinity suggests that the affected children may both have inherited such modifier variants for the phenotype associated with the *PRF1* mutation (Supplementary Table 2 to 4). A genome-wide analysis of a larger cohort of patients harboring *PRF1* mutations, presenting with and without neurodegeneration might, however, identify such modifiers.

In contrast to the febrile cytokine storms observed in FHL2, the proband stopped developing fevers with infection during the course of her disease. Consistent with this, her PBMCs did not produce IL-1 β or other pro-inflammatory cytokines overexpressed in FHL2.¹⁰ This observation, consistent with a defect in the innate immune response, suggests that the link between *PRF1* mutations and cytokine overexpression may be indirect or complex. The discrepant cytokine phenotype between FHL2 patients and our patients can be explained by genetic modifiers or by a difference in the timing of cytokine testing. Studies of cytokine expression in patients with classical FHL have been performed early in the disease course, most typically before treatment,¹⁰ whereas our proband was tested long after onset of symptoms. Determining whether this cytokine profile is a distinguishing feature of the *PRF1*-related neurodegenerative disorder manifest by these girls, or whether loss of cytokine production or cytokine depletion is a feature common to the later stages of FHL2 will require testing of additional FHL2 patients.

The brain MRI findings in HLH are nonspecific. They include multiple focal or diffuse areas of signal abnormality within the cerebral and cerebellar white and gray matter.^{28,29,36} The lesions can have a nodular appearance and enhance after contrast, a finding suggestive of perivascular involvement.^{29,30} The involvement of the cerebellum with development of cerebellar atrophy is a prominent feature observed in the majority of HLH patients. Cerebellar gray matter involvement usually appears later in the disease, and thus is not constant. Involvement of the cerebral gray matter is less common. Even when the patient cohort is limited to those with perforin mutations, the MRI findings are also not consistent (Table 2),

although cerebellar disease predominates.^{24–26,36,43,44} In the absence of systemic findings of HLH, therefore, one is unable to make a clinical diagnosis of *PRF1*-related neurodegeneration, and molecular testing is required.

In summary, we extend the neurodegenerative phenotype associated with biallelic mutations of *PRF1* and show that the neurodegenerative phenotype can occur in the absence of the hematological and immune signs of FHL2–HLH. We hypothesize that *PRF1*-related neurodegeneration is an under-recognized condition, and suggest that when suspicion of immune-mediated neurodegeneration arises, this diagnosis should be considered because it is potentially curable with hematopoietic stem cell transplantation.^{15,44}

WEB RESOURCES

<http://research.nhgri.nih.gov/software/Var-MD/>

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- Phelan J, Lowe L, Glasier C: Pediatric neurodegenerative white matter processes: leukodystrophies and beyond. *Pediatr Radiol* 2008; **38**: 729–749.
- Fernandes J, Saudubray J-M, Gvd Berghe, Walter JH (eds): *Inborn Metabolic Diseases: Diagnosis and Treatment*. Heidelberg, DEU, Germany: Springer, London, Ltd., 2006.
- Schlüter A, Espinosa L, Fourcade S *et al*: Functional genomic analysis unravels a metabolic-inflammatory interplay in adrenoleukodystrophy. *Hum Mol Genet* 2011; **21**: 1062–1077.
- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH: Mechanisms underlying inflammation in neurodegeneration. *Cell* 2010; **140**: 918–934.
- Amor S, Puentes F, Baker D, Van Der Valk P: Inflammation in neurodegenerative diseases. *Immunology* 2010; **129**: 154–169.
- Bélanger M, Allaman I, Magistretti PJ: Differential effects of pro- and anti-inflammatory cytokines alone or in combinations on the metabolic profile of astrocytes. *J Neurochem* 2011; **116**: 564–576.
- Fogal B, Hewett SJ: Interleukin-1 β : a bridge between inflammation and excitotoxicity? *J Neurochem* 2008; **106**: 1–23.
- Rossi S, Furlan R, De Chiara V *et al*: Interleukin-1 β causes synaptic hyperexcitability in multiple sclerosis. *Ann Neurol* 2012; **71**: 76–83.
- de Jong BA, Huizinga TWJ, Bollen ELEM *et al*: Production of IL-1 β and IL-1Ra as risk factors for susceptibility and progression of relapse-onset multiple sclerosis. *J Neuroimmunol* 2002; **126**: 172–179.
- Sumegi J, Barnes MG, Nestheide SV *et al*: Gene expression profiling of peripheral blood mononuclear cells from children with active hemophagocytic lymphohistiocytosis. *Blood* 2011; **117**: e151–e160.
- Stepp SE, Dufourcq-Lagelouse Rm, Deist FoL *et al*: Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* 1999; **286**: 1957–1959.
- Molleran Lee S, Villanueva J, Sumegi J *et al*: Characterisation of diverse *PRF1* mutations leading to decreased natural killer cell activity in North American families with haemophagocytic lymphohistiocytosis. *J Med Genet* 2004; **41**: 137–144.
- zur Stadt U, Schmidt S, Kasper B *et al*: Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet* 2005; **14**: 827–834.
- Cetica V, Santoro A, Gilmour KC *et al*: STXBP2 mutations in children with familial haemophagocytic lymphohistiocytosis type 5. *J Med Genet* 2010; **47**: 595–600.
- Henter J-I, Horne A, Aricó M *et al*: HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007; **48**: 124–131.
- Dias C, Sincan M, Cherukuri PF *et al*: An analysis of exome sequencing for diagnostic testing of the genes associated with muscle disease and spastic paraplegia. *Hum Mutat* 2012; **33**: 614–626.

- 17 Sincan M, Simeonov D, Adams D *et al*: VAR-MD: A tool to analyze whole exome/genome variants in small human pedigrees with Mendelian inheritance. *Hum Mutat* 2012; **33**: 593–598.
- 18 Mehta VB, Hart J, Wewers MD: ATP-stimulated release of interleukin (IL)-1 β and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. *J Biol Chem* 2001; **276**: 3820–3826.
- 19 Kollmann TR, Crabtree J, Rein-Weston A *et al*: Neonatal innate TLR-mediated responses are distinct from those of adults. *J Immunol* 2009; **183**: 7150–7160.
- 20 Muralitharan S, Wali YA, Dennison D *et al*: Novel spectrum of perforin gene mutations in familial hemophagocytic lymphohistiocytosis in ethnic omani patients. *Am J Hematol* 2007; **82**: 1099–1102.
- 21 Clementi R, zur Stadt U, Savoldi G *et al*: Six novel mutations in the PRF1 gene in children with haemophagocytic lymphohistiocytosis. *J Med Genet* 2001; **38**: 643–646.
- 22 Voskoboinik I, Thia M-C, De Bono A *et al*: The functional basis for hemophagocytic lymphohistiocytosis in a patient with co-inherited missense mutations in the perforin (PFN1) gene. *J Exp Med* 2004; **200**: 811–816.
- 23 Trizzino A, Uz Stadt, Ueda I *et al*: Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutations. *J Med Genet* 2008; **45**: 15–21.
- 24 Astigarraga I, Prats JM, Navajas A, Fernández-Teijeiro A, Urberuaga A: Near fatal cerebellar swelling in familial hemophagocytic lymphohistiocytosis. *Pediatr Neurol* 2004; **30**: 361–364.
- 25 Beaty AD, Weller C, Levy B *et al*: A teenage boy with late onset hemophagocytic lymphohistiocytosis with predominant neurologic disease and perforin deficiency. *Pediatr Blood Cancer* 2008; **50**: 1070–1072.
- 26 Chiapparini L, Uziel G, Vallinoto C *et al*: Hemophagocytic lymphohistiocytosis with neurological presentation: MRI findings and a nearly miss diagnosis. *Neurol Sci* 2011; **32**: 473–477.
- 27 Chung TW: CNS Involvement in Hemophagocytic Lymphohistiocytosis: CT and MR Findings. *Korean J Radiol* 2007; **8**: 78–81.
- 28 Decaminada N, Cappellini M, Mortilla M *et al*: Familial hemophagocytic lymphohistiocytosis: clinical and neuroradiological findings and review of the literature. *Child's Nerv Syst* 2010; **26**: 121–127.
- 29 Goo H, Weon Y: A spectrum of neuroradiological findings in children with haemophagocytic lymphohistiocytosis. *Pediatr Radiol* 2007; **37**: 1110–1117.
- 30 Gurgey A, Aytac S, Balta G, Oguz KK, Gumruk F: Central nervous system involvement in Turkish children with primary hemophagocytic lymphohistiocytosis. *J Child Neurol* 2008; **23**: 1293–1299.
- 31 Haddad E, Sulis M-L, Jabado N, Blanche S, Fischer A, Tardieu M: Frequency and severity of central nervous system lesions in hemophagocytic lymphohistiocytosis. *Blood* 1997; **89**: 794–800.
- 32 Henter J-I, Nennesmo I: Neuropathologic findings and neurologic symptoms in twenty-three children with hemophagocytic lymphohistiocytosis. *J Pediatr* 1997; **130**: 358–365.
- 33 Puliyl MM, Rose W, Kumar S, Moses PD, Gibikote S: Prolonged neurologic course of familial hemophagocytic lymphohistiocytosis. *Pediatr Neurol* 2009; **41**: 207–210.
- 34 Rostasy K, Kolb R, Pohl D *et al*: CNS disease as the main manifestation of hemophagocytic lymphohistiocytosis in two children. *Neuropediatrics* 2004; **35**: 45–49.
- 35 Sieni E, Cetica V, Santoro A *et al*: Genotype-phenotype study of familial haemophagocytic lymphohistiocytosis type 3. *J Med Genet* 2011; **48**: 343–352.
- 36 van Egmond ME, Vermeulen RJ, Peeters-Scholte CMPCD *et al*: Familial hemophagocytic lymphohistiocytosis in a pediatric patient diagnosed by brain magnetic resonance imaging. *Neuropediatrics* 2011; **42**: 191–193.
- 37 Wada T, Nishiura K, Kuroda M *et al*: A case of acute encephalopathy with hemophagocytic lymphohistiocytosis and clonal T-cell expansion. *Brain Dev* 2011; **34**: 376–379.
- 38 Yang S, Zhang L, Jia C, Ma H, Henter J-I, Shen K: Frequency and development of CNS involvement in Chinese children with hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2010; **54**: 408–415.
- 39 Deiva K, Mahlaoui N, Beaudonnet F *et al*: CNS involvement at the onset of primary hemophagocytic lymphohistiocytosis. *Neurology* 2012; **78**: 1150–1156.
- 40 Horne A, Trottestam H, Aricò M *et al*: Frequency and spectrum of central nervous system involvement in 193 children with haemophagocytic lymphohistiocytosis. *Br J Haematol* 2008; **140**: 327–335.
- 41 Cappellano G, Orilieri E, Comi C *et al*: Variations of the perforin gene in patients with multiple sclerosis. *Genes Immun* 2008; **9**: 438–444.
- 42 Shinoda J, Murase S, Takenaka K, Sakai N: Isolated central nervous system hemophagocytic lymphohistiocytosis: case report. *Neurosurgery* 2005; **56**: 187.
- 43 Feldmann J, Ménasché G, Callebaut I *et al*: Severe and progressive encephalitis as a presenting manifestation of a novel missense perforin mutation and impaired cytolytic activity. *Blood* 2005; **105**: 2658–2663.
- 44 Moshous D, Feyen O, Lankisch P *et al*: Primary necrotizing lymphocytic central nervous system vasculitis due to perforin deficiency in a four-year-old girl. *Arthritis Rheum* 2007; **56**: 995–999.

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