BRIEF COMMUNICATION

Genetic variation in genes of inborn errors of immunity in children with unexplained encephalitis

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Pediatric encephalitis has significant morbidity and mortality, yet 50% of cases are unexplained. Host genetics plays a role in encephalitis' development; however, the contributing variants are poorly understood. One child with anti-NMDA receptor encephalitis and ten with unexplained encephalitis underwent whole genome sequencing to identify rare candidate variants in genes known to cause monogenic immunologic and neurologic disorders, and polymorphisms associated with increased disease risk. Using the professional Human Genetic Mutation Database (Qiagen), we divided the candidate variants into three categories: monogenic deleterious or potentially deleterious variants (1) in a disease-consistent inheritance pattern; (2) in carrier states; and (3) disease-related polymorphisms. Six patients (55%) had a deleterious or potentially deleterious variant in a disease-consistent inheritance pattern, five (45%) were heterozygous carriers for an autosomal recessive condition, and six (55%) carried a disease-related polymorphism. Finally, seven (64%) had more than one variant, suggesting possible polygenetic risk. Among variants identified were those implicated in atypical hemolytic uremic syndrome, common variable immunodeficiency, hemophagocytic lymphohistiocytosis, and systemic lupus erythematosus. This preliminary study shows genetic variation related to inborn errors of immunity in acute pediatric encephalitis. Future research is needed to determine if these variants play a functional role in the development of unexplained encephalitis.

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BACKGROUND

Pediatric encephalitis is inflammation of the brain parenchyma that is often complicated by significant morbidity and mortality. Encephalitis is accompanied by global dysfunction and encephalopathy, and inflammation manifesting as fever, seizures, cerebral spinal fluid (CSF) pleocytosis, and neuroradiologic abnormalities. A specific etiology is identified in only half of cases, with viral and autoimmune being most common [1]. In the remainder, encephalitis is often unexplained [1, 2]. While genetic variation is cited as contributing to the development of unexplained encephalitis, few specific loci have been implicated and their prevalence unknown [3].

While typically considered rare, neuroinflammatory disorders are impacted by genetics. For example, hemophagocytic lymphohistiocytosis (HLH) is a monogenic disorder characterized by a dysregulated hyperinflammatory cytotoxic NK and T cell response, where CNS involvement is a poor prognostic indicator [4]. In complex genetic disorders such as systemic lupus erythematosus (SLE), specific loci are associated with CNS involvement [5]. Additionally, TLR3 mutations have been associated with severe herpes simplex encephalitis [6]. Subsequently, we hypothesize that children with unexplained encephalitis may carry variants associated with neuroimmunologic conditions predisposing to CNS inflammation.

METHODS

The study was approved by the University of Pittsburgh's Institutional Review Board (#20010099). Written informed consent was obtained from one or more parents/guardians for minors, and from participants over 18 years of age. For minor participants, child assent was garnered when able. Patients admitted to the Children's Hospital of Pittsburgh Intensive Care Unit between June 2018–November 2021 with a diagnosis of acute encephalitis were eligible if they displayed altered consciousness, cognition, personality, or behavior for >24 h and had ≥2 of the following: (1) fever; (2) CSF WBC > 4 cells/ul; (3) neuroradiologic evidence of inflammation; (4) seizures not attributable to known diagnoses; (5) focal neurologic signs; and (6) encephalopathy on EEG. Enrollees were a limited convenience sample of eligible participants. Ten participants had unexplained encephalitis and one subject with anti-NMDA receptor encephalitis was included.

Genetic sequencing

Whole genome sequencing (WGS) was performed on DNA extracted from whole blood at the University of Pittsburgh Institute for Precision Medicine on Illumina's NovaSeq 6000 with a mean coverage of 43.7x. FASTQ files were aligned to *homo sapiens* reference sequence GRCh38. Resultant VCF files were analyzed in the Fabric Genomics Opal 5.2.2 software [7] to identify missense, nonsense or frameshift mutations. Variants were filtered for coverage >10, PHRED score >30.

We limited candidate variants to a 515 genes list, combining monogenic inborn errors of immunity classified by the International Union of Immunologic Societies [8] and a validated sequencing panel for pediatric

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neuroinflammation [9]. Next, candidates were restricted to rare variants with a minor allele frequency <5% (MAF < 0.05) in the ExAC [10] database. All rare variants were evaluated in Qiagen's Professional Human Genetic Mutation Database (HGMD), which classifies variants' pathogenicity based on peer-reviewed reports of the variant in human disease [11]. These reports were manually reviewed for inheritance pattern and presence of the corresponding neurologic/inflammatory phenotype consistent with Online Mendelian Inheritance in Man [12]. Thus, only variants classified as deleterious or potentially deleterious with respect to the particular phenotype of interest were included.

Candidate variants were classified into three groups. In group 1, variants were limited to those reported as deleterious (DM) or potentially deleterious (DM?) in the HGMD professional database [11] and found in a disease-consistent inheritance pattern (one variant for autosomal dominant (AD) or X-linked (XL) disorders in males, and two variants for autosomal recessive (AR) disorders). The DM? designation indicates an uncertain linkage of variant and phenotype, and represents a potential rather than definitive association. Group 2 included heterozygous DM or DM? AR variants, and variants of unknown significance (VUS) if another DM or DM? AR variant was identified at the same locus. Finally, group 3 variants were disease risk polymorphisms in HGMD.

RESULTS

Ten of 11 subjects were previously healthy prior to admission and one had a history of epilepsy (Table 1). Participants were between 10 months to 18 years of age. Clinical diagnoses included anti-NMDA receptor, parainfectious, limbic, and acute necrotizing encephalitis, and febrile illness-related epilepsy syndrome. CSF WBC was elevated in 8 of 11 (73%) participants. In total, 64% had seizures and 100% showed EEG slowing consistent with encephalopathy. MRI findings consistent with CNS inflammation were found in 91% of participants. Notably, 3 of 11 (27%) had poor neurologic outcome, with Pediatric Cerebral Performance Score ≥ 4 (severe disability, coma/vegetative state or death) at between 4 months to 8 years of follow-up.

In total, six patients (55%) had a DM or DM? variant with an AD inheritance (Table 2, Group 1). These included atypical hemolytic uremic syndrome (aHUS): *CFI* p.Pro64Leu, *CD46* p.Ala353Val, and *CFHR5* p.Gly145Glu; common variable immunodeficiency (CVID): *TCF3* p.Lys101Arg; congenital neutropenia: *ELANE* p.Pro257Leu; aplastic anemia: *TERT* p.His412Tyr; autoimmune lymphoproliferative syndrome (ALPS): *CASP10* p.Pro501Leu; and familial Mediterranean fever: *CARD14* p.Q422K. Five patients (45%) were compound or synergistic heterozygotes for an AR condition (Table 2, Group 2). These included CVID: *LRBA* p.Met467Val, *SKIV2L* p.Arg324Trp, and *PSMB9* p.Arg173Cys; HLH: *UNC13D* p.Arg928Cys and p.Met795Thr (VUS); Primary immunodeficiency: *IL21R* p.Gly345Ser and p.Leu329Val (VUS); Cerebrotendinous xanthomatosis: *CYP27A1* p.Pro384Leu; and Neuronal ceroid lipofuscinosis: *CLN6* p.Gly259Ser. Six subjects (55%) carried a risk polymorphism (Group 3), including two related to SLE: *DNASE*1 p.Arg2Ser and p.Gly127Arg. Other risk polymorphisms included Crohn's disease: *NOD2* p.Leu248Arg; *FCN3* deficiency: *FCN3* p.Leu117SerfsTer65; Increased IL-6, TNF-α, Ig levels: *CD40* p.Pro227Ala; *MASP2* deficiency: *MASP2* p.Asp120Gly; Reduced apoptotic function: *CASP10* p.Tyr446Cys; and leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation: *DARS2* p.Gly338Gln.

DISCUSSION

In this pediatric encephalitis study, DM and DM? immunologic variants affected 64% of participants, possibly suggesting a genetic contribution to CNS inflammation. Several study participants had variants that impact adaptive immunity, suggesting either an autoimmune or increased susceptibility mechanism. Our data also suggest complex genetic risk, with 64% carrying multiple variants at diverse immunologic loci. However, It must be emphasized that currently, the link between immunogenetic variation and pediatric encephalitis is only associative. Further efforts to establish causality will require additional research at the population and variant level. For some variants, these follow up studies will likely demonstrate that variants are incidental findings. Others may represent true causal relationships, emphasizing the need for genome wide sequencing to categorize the full landscape of genetic risk.

For example, sequencing of Patient 6—who has anti-NMDA receptor encephalitis, caused by autoantibodies to the glutamate receptor—demonstrated risk variants for SLE, another autoantibody-mediated disease. Patient 6 also had a DM? complement variant. Classic activation of the complement innate immune pathway can be triggered by autoantibodies [13]. In CNS SLE, terminal complement complexes are elevated in CSF [14] and murine models suggest that blood brain barrier dysfunction can be inhibited by complement antagonists [15]. Alternatively, complement variants may impair immune complex clearance, a mechanism previously implicated in a case of refractory anti-NMDA receptor encephalitis with genetic complement abnormalities [16]. However, while anti-NMDA antibodies activate complement in vitro, CNS complement deposition has not been demonstrated in vivo [17, 18]. These direct relationships lend biologic plausibility to our findings in other cases of unexplained encephalitis with less well-characterized molecular pathology.

Patient 2 also had variants in *CFI* and *CD46*, complement pathway downregulators. In aHUS, gain of function variants in complement activators, or loss of function variants in regulators lead to hyperactivation, endothelial damage, and organ injury—most commonly in the kidneys—but affecting the CNS in 25% of individuals [19, 20]. A 10 year old with recurrent hemorrhagic leukoencephalitis and *CFI* p.Pro64Leu, the same variant found in patient 2 in our study, had undetectable CFI, low C3, and low AP50 levels [21]. However, this individual also carried *CFI* p.Gln88Lys. *CFI* variants have also been described in sterile encephalitis, associated with C3 activation and terminal complement complex deposition on brain biopsy [22]. Together, this leads us to hypothesize that genetic risk for inappropriate humoral immune response and subsequent complement activation may contribute to unexplained encephalitis in a subset of children, however this will require future functional confirmation.

Patients 7 and 10 also showed shared genetic risk, both carrying *CASP10* variants, a gene which promotes lymphocyte apoptosis and, in ALPS, leads to uncontrolled proliferation and autoimmunity. We were unable to identify studies linking *CASP10* or ALPS with encephalitis. However, for both patient 7 and patient 10 and patient 2 and patient 6, shared genetic risk in this small cohort is raises a question of possible shared pathobiology that warrants further study.

Additionally, unique genetic findings were encountered. Patient 2 was compound heterozygous for an *UNC13D* DM? and a VUS variant, potentially consistent with HLH—an AR multisystem inflammatory disorder due to impaired cytotoxic killing. HLH has been hypothesized to manifest with isolated CNS involvement [4], as seen in our patient. In the literature, a 3 month old with complex genetics involving multiple variants in *PRF1, UNC13D, STXBP2* and *XIAP* had elevated CNS protein and neuroimaging findings consistent with acute necrotizing encephalitis [23], as observed in patient 2. Other unique findings include patient 5 who suffered devastating neurologic injury following nasopharyngeal adenoviral infection, who was compound heterozygous for an *IL21R* DM? and a VUS variant. Biallelic *IL21R* mutations cause immunodeficiency affecting both T and B cell compartments, with impaired immunoglobulin synthesis, T and NK cell dysfunction, and recurrent viral infections [24, 25].

Our study's main limitation is that identified variants cannot be equated with immunodeficiency, as literature-based stratification may misclassify pathogenicity. Further, genotype/phenotype correlations in the cohort may be atypical where CNS manifestations are primary, possibly due to variable penetrance,

Table 1. Clinical characteristics of study participants.

Patient	Age	Sex	Race	PMHx	Diagnosis	Fever	Seizure	AMS	Encephalopathy (EEG)	Focal neurologic signs	CSF WBC total: % differential (cells/μl)	CSF RBC (cells/μl)	CSF protein (mg/dl)	CSF Glu (mg/dl)	LP OP (cm H2O)	Other labs	Viral testing	MRI findings	PCPC score
1	6 years	M	Caucasian	–	Parainfectious	+	+	+	+	–	33: 10N, 70L, 20M	1	33	90	27	IgG: 858 mg/dl	–	T2 hyperintensities in the pons, midbrain, bilateral thalamic dorsomedial and pulvinar nuclei. Edema and scattered restricted diffusion in the bilateral frontal, temporal, parietal, and occipital lobes	2
2	12 years	F	Caucasian	–	Acute necrotizing encephalitis	+	+	+	+	–	39: 3N, 82L, 15M	6	34	97	36	–	–	Extensive diffusion restriction in frontal, temporal, and parietal lobes, and bilateral caudate and putamen with white matter sparing	1
3	13 years	M	Caucasian	–	Limbic encephalitis	–	+	+	+	–	6: 97L, 3M	0	21	65	19.8	–	–	T2 hyperintensities in the bilateral amygdala, hippocampi, insular cortices and gyrus recti	2
4	2 years	M	African American	–	Parainfectious	+	–	+	+	–	6: 12N, 39L, 49M	0	84	79	23	–	NP Adenovirus and Rhino/enterovirus +, CSF enterovirus PCR–	Normal	2
5	16 years	M	Caucasian	–	Parainfectious	+	–	+	+	–	25: 8N, 79L, 13M	108	65	56	19	–	NP Adenovirus +	Severe meningoencephalitis and myelitis with symmetric involvement of the deep gray matter, thalami and basal ganglia. T2 hyperintensities in the cerebral cortex, cerebellum, brainstem and cervical spine, with some sparing of the parietal lobes and white matter	5
6	18 years	F	African American	–	Anti-NMDA receptor encephalitis	+	+	+	+	–	77	36	47	58	30	–	CSF EBV PCR+	Mild diffuse volume loss and nonspecific bifrontal white matter T2 hyperintensities	4
7	11 years	F	Caucasian	–	Febrile infection-related epilepsy syndrome	+	+	+	+	–	0: NA	0	35	72	32	–	–	Increased perfusion in bilateral anterior frontal, temporal lobes and hippocampi	4
8	13 years	M	Caucasian	History of epilepsy					Encephalitis vs. prolonged seizure	+	–	+	+	+	0: NA	1	17	68	NR
–	–	T2							hyperintensity and cortical edema involving left parietal and occipital lobe	1									
9	2 years	M	Caucasian	–	Parainfectious	+	+	+	+	+	134: 63N, 13L, 23M, 1B	17	27	62	3.8	–	SARS-CoV2 IgG Spike Protein+, PCR–	Reduced diffusion with decreased perfusion involving left frontal, temporal, parietal occipital lobes	1
10	10 months	F	Caucasian	–	Parainfectious	+	+	+	+	+	8: 8N, 58L, 29M, 5AL	160	31	80	NR	–	–	Symmetric punctate foci of restricted diffusion and T2 hyperintensities in bilateral caudate nucleus, putamen, globus pallidus and thalami, bilateral frontal and parietal lobe cortical gray matter, bilateral hippocampi and amygdala	1
11	3 years	M	Caucasian	–	Parainfectious	+	–	+	+	–	4: 4N, 52L, 40M, 1B, 2E, 1AL	2	35	88	25	IgG: 996mg/dl	NP RSV+	Diffuse patchy white matter T2 hyperintensity in brainstem, cerebellum, midbrain, bilateral thalami, subcortical, frontal and parietal regions	1

Normal ranges for CSF parameters are as follows: Glucose (40–75 mg/dl); Protein (<48 mg/dl); WBC (<4 cells/ul).

M male, *F* female, *PMHx* past medical history, *AMS* altered mental status, *EEG* electroencephalogram, *WBC* white blood cell count, *N* neutrophil, *L* lymphocyte, *M* monocyte, *B* band, *AL* atypical lymphocyte, *E* eosinophil, *RBC* red blood cell count, *Glu* glucose, *LP* lumbar puncture, *NP* nasopharyngeal, *OP* opening pressure, *PCR* polymerase chain reaction, *EBV* Epstein-Barr Virus, *SARS-CoV2 IgG* Severe Acute Respiratory Syndrome-Coronavirus-2 Immunoglobulin, *RSV* Respiratory Syncytial Virus, *MRI* magnetic resonance imaging, *FLAIR* fluid-attenuated inversion recovery, *PCPC* Pediatric Cerebral Performance Category (1: normal, 2: mild disability, 3: moderate disability, 4: severe disability, 5: coma or vegetative state, 6: death).

Table 2. Identified variants according to pathogenicity classification.

Subject	Gene	HGVS c	HGVS p	Zygosity	MAF	Variant Class	IP	Related Phenotype	Supplemental Bibliography
P6: Anti-NMDA Encephalitis	*CFHR5*	c.434G>A	p.G145E	het	0.013580	DM?	AD	Atypical hemolytic uremic syndrome	[S1,S2]
	DNASE1	c.6G>T	p.R2S	het	0.015330	Risk polymorphism	-	Systemic lupus erythematosus	[S3–S5]
	DNASE1	c.379G>A	p.G127R	het	0.008660	Risk polymorphism	-	Systemic lupus erythematosus	[S3–S6]
P1: Parainfectious: No virus identified	*TOP3*	c.302A>G	p.K101R	synergistic het	0.009643	DM?	AD	Common variable immunodeficiency	[S7]
	LRBA	c.1399A>G	p.M467V	synergistic het	0.002190	DM	AR	Common variable immunodeficiency	[S8]
	NOD2	c.743T>G	p.L248R	het	0.000520	Risk polymorphism	-	Crohn's disease	[S9,S10]
	FCN3	c.349del	p.L117SfsTer65	het	0.016180	Risk polymorphism	-	FCN3 deficiency	[S11,S12]
P2: Acute Necrotizing Encephalitis	*CD46*	c.1058C>T	p.A353V	het	0.015410	DM?	AD	Atypical hemolytic uremic syndrome	[S13–S15]
	CFI	c.1912C>T	p.P64L	compound het	0.000224	DM	AD	Atypical hemolytic uremic syndrome	[S16,S17]
	UNC13D	c.2782C>T	p.R928C	compound het	0.016840	DM?	AR	Hemophagocytic lymphohistiocytosis	[S18–S20]
	UNC13D	c.2384T>C	p.M795T	compound het	0.000019	Unknown significance	-	-	-
	CD40	c.679C>G	p.P227A	het	0.020220	Risk polymorphism	-	Increased IL-6, TNF-alpha, Ig levels	[S21]
P4: Parainfectious: Nasopharyngeal	*ELANE*	c.770C>T	p.P257L	het	0.006283	DM?	AD	Congenital neutropenia	[S22–S24]
	TERT	c.1234C>T	p.H412T	het	0.003248	DM	AD	Aplastic anemia	[S25–S27]
P5: Parainfectious: Nasopharyngeal Adenovirus	*IL21R*	c.1033G>A	p.G345S	compound het	0.002330	DM?	AR	Primary immunodeficiency	[S28]
	IL21R	c.985C>G	p.L329V	compound het	0.000004	Unknown significance	-	-	-
	MASP2	c.359A>G	p.D120G	het	0.021830	Risk polymorphism	-	MASP2 deficiency	[S29,S30]
P7: Febrile Illness Related Encephalopathy	*CASP10*	c.1502C>T	p.P501L	het	0.001090	DM	AD	Autoimmune lymphoproliferative syndrome	[S31]
	CYP27A1	c.1151C>T	p.P384L	synergistic het	0.018290	DM?	AR	Cerebrotendinous xanthomatosis	[S32,S33]
	CLN6	c.775G>A	p.G259S	synergistic het	0.000024	DM	AR	Neuronal ceroid lipofuscinosis, late infantile	[S34]
P10: Parainfectious: No virus identified	*CASP10*	c.1337A>G	p.Y446C	het	0.030090	Risk polymorphism	-	Reduced apoptotic function	[S31,S35–S37]
P11: Parainfectious: Nasopharyngeal RSV	*CARD14*	c.1264G>A	p.Q422K	hom	0.022470	DM?	AD	Familial Mediterranean fever	[S38]
	SKIV2L	c.970C>T	p.R324W	synergistic het	0.008210	DM?	AR	Common variable immunodeficiency	[S7]
	PSMB9	c.517C>T	p.R173C	synergistic het	0.003055	DM?	AR	Common variable immunodeficiency	[S7]
	DARS2	c.1013G>A	p.G338Q	het	0.026350	Risk polymorphism	-	Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation	[S39]

Deleterious (DM) or potentially deleterious variants (DM?) for autosomal dominant (AD) disorders as classified by Qiagen's Human Genetic Mutation Database are shown in dark gray. Carrier states for autosomal recessive (AR) disorders when found in combination with other potential synergistic or compound heterozygous variants are shown in light gray. Finally, potentially contributing risk polymorphisms with previous functional evidence of phenotypic impact on immunity are shown in white. Primary publications regarding variant pathogenicity are provided in the Supplementary bibliography for reference. *AD* autosomal dominant, *AR* autosomal recessive, *DM* deleterious mutation, *DM* potentially deleterious mutation, *het* heterozygous, *hom* homozygous.

environmental factors and genetic background influencing phenotype expression. Additionally, it is difficult to estimate the frequency of implicated variants in healthy controls to determine if they are overrepresented in encephalitis. However, frequency limits and reports of pathogenicity guard against false positives. Another limitation is the lack of parental sampling which prevents determination of *cis* and *trans* positioning. Our study also did not perform confirmatory Sanger sequencing, and the filter also fails to identify regulatory, structural and copy number variants which may contribute to disease. Lastly, as sequencing was performed retrospectively, it was not possible to perform additional functional and immunologic testing on participants.

In this case series, we used WGS to identify immunogenetic risk in 8 of 11 children with unexplained CNS inflammation. As a small exploratory study, this report is hypothesis-generating and warrants larger studies that include functional testing to understand the prevalence and impact of immunogenetic variation in unexplained pediatric encephalitis.

DATA AVAILABILITY

The authors confirm that the data supporting the finding of this study are available within the article and its Supplementary Material. Raw data supporting the findings are available from the corresponding author upon reasonable request.

REFERENCES

1. Messacar K, Fischer M, Dominguez SR, Tyler KL, Abzug MJ. Encephalitis in US children. Infect Dis Clin North Am. 2018;32:145–62.
2. Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. Clin Infect Dis. 2003;36:731–42.
3. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. Clin Infect Dis. 2013;57:1114–28.
4. Blincoe A, Heeg M, Campbell PK, Hines M, Khojah A, Klein-Gitelman M, et al. Neuroinflammatory disease as an isolated manifestation of hemophagocytic lymphohistiocytosis. J Clin Immunol. 2020;40:901–16.
5. Ramirez GA, Lanzani C, Bozzolo EP, Citterio L, Zagato L, Casamassima N, et al. TRPC6 gene variants and neuropsychiatric lupus. J Neuroimmunol. 2015;288:21–4.
6. Guo Y, Audry M, Ciancanelli M, Alsina L, Azevedo J, Herman M, et al. Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency: TLR3 is otherwise redundant in protective immunity. J Exp Med. 2011;208:2083–98.
7. Fabric Genomics. Fabric Genomics Opal Genome Interpretation Platform®. 2022. https://fabricgenomics.com/.
8. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020;40:24–64.
9. McCreary D, Omoyinmi E, Hong Y, Mulhern C, Papadopoulou C, Casimir M, et al. Development and validation of a targeted next-generation sequencing gene panel for children with neuroinflammation. JAMA Netw Open. 2019;2:1914274.
10. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
11. Stenson PD, Ball EV, Mort M, Phillips AD, Shaw K, Cooper DN. The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. Curr Protoc Bioinformatics. 2012. https://doi.org/10.1002/0471250953.bi0113s39.
12. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University. Online Mendelian Inheritance in Man, OMIM®. https://omim.org/.
13. Goldberg BS, Ackerman ME. Antibody-mediated complement activation in pathology and protection. Immunol Cell Biol. 2020;98:305–17.
14. Sanders ME, Alexander EL, Koski CL, Frank MM, Joiner KA. Detection of activated terminal complement (C5b-9) in cerebrospinal fluid from patients with central nervous system involvement of primary Sjogren's syndrome or systemic lupus erythematosus. J Immunol. 1987;138:2095–9.
15. Jacob A, Hack B, Chiang E, Garcia JGN, Quigg RJ, Alexander JJ. C5a alters blood-brain barrier integrity in experimental lupus. FASEB J. 2010;24:1682–8.

16. Chua GT, Zhou D, Ho ACC, Chan SHS, Yu CY, Lau YL. A case report of complement C4B deficiency in a patient with steroid and IVIG-refractory anti-NMDA receptor encephalitis. BMC Neurol. 2020. https://doi.org/10.1186/s12883-020-01906-x.
17. Shu Y, Chen C, Chen Y, Xu Y, Chang Y, Li R, et al. Serum complement levels in anti-N-methyl-d-aspartate receptor encephalitis. Eur J Neurol. 2018;25:178–84.
18. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. Neurology. 2011;77:589–93.
19. Fidan K, Göknar N, Gülhan B, Melek E, Yıldırım ZY, Baskın E, et al. Extra-Renal manifestations of atypical hemolytic uremic syndrome in children. Pediatr Nephrol. 2018;33:1395–403.
20. Formeck C, Swiatecka-Urban A. Extra-renal manifestations of atypical hemolytic uremic syndrome. Pediatr Nephrol. 2019;34:1337–48.
21. Shields AM, Pagnamenta AT, Pollard AJ, Taylor JC, Allroggen H, Patel SY. Classical and non-classical presentations of complement factor I deficiency: two contrasting cases diagnosed via genetic and genomic methods. Front Immunol. 2019;10:1150.
22. Altmann T, Torvell M, Owens S, Mitra D, Sheerin NS, Morgan BP, et al. Complement factor I deficiency: a potentially treatable cause of fulminant cerebral inflammation. Neurol Neuroimmunol Neuroinflamm. 2020;7:e689.
23. Dai D, Wen F, Liu S, Zhou S. Brain damage resembling acute necrotizing encephalopathy as a specific manifestation of haemophagocytic lymphohistiocytosis-induced by hypersensitivity. Ital J Pediatr. 2016;42:79.
24. Cagdas D, Mayr D, Baris S, Worley L, Langley DB, Metin A, et al. Genomic spectrum and phenotypic heterogeneity of human IL-21 receptor deficiency. J Clin Immunol. 2021;41:1272–90.
25. Kotlarz D, Ziętara N, Uzel G, Weidemann T, Braun CJ, Diestelhorst J, et al. Loss-of-function mutations in the IL-21 receptor gene cause a primary immunodeficiency syndrome. J Exp Med. 2013;210:433–43.

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AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version. KFK and DM designed study, generated and analyzed the data, conceptualized, wrote and edited the manuscript. DWS, DSR, and KT analyzed the data and edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board at the University of Pittsburgh (#20010099). Written informed consent was obtained from one or more parents/guardians for each child. Written assent was garnered when the child was able. Written informed consent was obtained for participation in the study, as well as consent for publication of study results.

ADDITIONAL INFORMATION

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