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 polygenic 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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neuropathology [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/immunologic 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.Pro464Leu, CD46 p.Ala353Val, and CFHRS5 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 PMS9 p.Arg173Cys; HLH: UNC13D p.Arg292Cys and p.Ala730Val; and CFI p.Met795Thr (VUS); Primary immunodeficiency: IL2Rα p.Gly345Ser and p.Leu329Val (VUS); Cerebrotendinous xanthomatisis: CYP27A1 p.Pro384Leu; and Neuronal ceroid lipofuscinosis: CD46 p.Ala353Val and CLN6 p.Gly259Ser. Six subjects (55%) carried a risk polymorphism (Group 3), including two related to SLE: DNAE1 p.Arg25Ser and p.Gly127Arg. Other risk polymorphisms included Crohn’s disease: NOD2 p.Leu248Arg; FCN3 deficiency: FCN3 p.Leu117Ser; and 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 variant 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 anti-NMDA receptor antibodies 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.Gln888Lys. 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 PRFI, UNC13D, STXB2 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 nosopharyngeal 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 (years) | Sex | Race | PMHx | Diagnosis | Fever | Seizure | AMS | MRI findings | PCPC score |
|---------|-------------|-----|------|------|-----------|-------|---------|-----|--------------|------------|
| 1       | 6           | M   | Caucasian | – | Parainfectious | + | + | + | 1 | T2 hyperintensities in the pons, midbrain, bilateral thalamus, dorsomedial and pulvinar nuclei, Edema and scattered restricted diffusion in the bilateral frontal, temporal, parietal, and occipital lobes | 2 |
| 2       | 12          | F   | Caucasian | – | Acute necrotizing encephalitis | + | + | + | – | Extensive diffusion restriction in frontal, temporal, and parietal lobes, and bilateral caudate and putamen with white matter sparing | 1 |
| 3       | 13          | M   | Caucasian | – | Limbic encephalitis | – | + | + | – | T2 hyperintensities in the bilateral amygdala, hippocampi, insular cortices and gyrus recti | 2 |
| 4       | 2           | M   | African American | – | Parainfectious | + | – | + | – | Normal | 2 |
| 5       | 16          | M   | Caucasian | – | Parainfectious | + | – | + | – | Severe meningoencephalitis and myelitis with symmetric involvement of the deep gray matter, thalami and basal ganglia, T2 hypointensities in the cortical, cerebellum, brainstem and cervical spine, with some sparing of the parietal lobes and white matter | 5 |
| 6       | 18          | F   | African American | – | Anti-NMDA receptor encephalitis | + | + | + | – | Mild diffuse volume loss and nonspecific bilateral white matter T2 hypointensities | 4 |
| 7       | 11          | F   | Caucasian | – | Febrile infection-related epilepsy syndrome: epilepsy | + | + | + | – | Increased perfusion in bilateral anterior frontal, temporal lobes and hippocampi | 4 |
| 8       | 13          | M   | Caucasian | History of | Left T2 hyperintensity and cortical edema involving left parietal and occipital lobe | + | – | + | + | 0 | NR |
| 9       | 2           | M   | Caucasian | – | Parainfectious | + | + | + | – | Reduced diffusion with decreased perfusion involving left frontal, temporal, parietal, occipital lobes | 1 |
| 10      | 10 months   | F   | Caucasian | – | Parainfectious | + | + | + | – | Symmetric punctate foci of restricted diffusion and T2 hypointensities in bilateral caudate nucleus, putamen, globus pallidus and thalamus, bilateral frontal and parietal lobe cortical gray matter, bilateral hippocampi and amygdala | 1 |
| 11      | 3           | M   | Caucasian | – | Parainfectious | + | – | + | – | Diffuse patchy white matter T2 hypointensities in brainstem, cerebellum, midbrain, bilateral thalamus, 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, EEC 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 Immunoglobin, 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).
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.

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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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