Association of rare variants in genes of immune regulation with pediatric autoimmune CNS diseases

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Received: 26 June 2022 / Revised: 2 August 2022 / Accepted: 3 August 2022 / Published online: 12 August 2022
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Abstract

Background There is a gap in the literature regarding genetic underpinnings of pediatric autoimmune CNS diseases. This study explored rare gene variants implicated in immune dysregulation within these disorders.

Methods This was a single-center observational study of children with inflammatory CNS disorder who had genetic testing through next generation focused exome sequencing targeting 155 genes associated with innate or adaptive immunity. For in silico prediction of functional effects of single-nucleotide variants, Polymorphism Phenotyping v2, and Sorting Intolerant from Tolerant were used, and Combined Annotation Dependent Depletion (CADD) scores were calculated. Identified genes were analyzed using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results Of 54 patients, 42 (77.8%) carried variant(s), among which 12 (22.2%) had 3–8 variants. Eighty-eight unique single-nucleotide variants of 55 genes were identified. The most variants were detected in UNC13D, LRBA, LYST, NOD2, DOCK8, RNASEH2A, STAT5B, and AIRE. The majority of variants (62, 70.4%) had CADD > 10. KEGG pathway analysis revealed seven genes associated with primary immunodeficiency (Benjamini 1.40E−06), six genes with NOD-like receptor signaling (Benjamini 4.10E−04), five genes with Inflammatory Bowel Disease (Benjamini 9.80E−03), and five genes with NF-kappa B signaling pathway (Benjamini 1.90E−02).

Discussion We observed a high rate of identification of rare and low-frequency variants in immune regulatory genes in pediatric neuroinflammatory CNS disorders. We identified 88 unique single-nucleotide variants of 55 genes with pathway analysis revealing an enrichment of NOD2-receptor signaling, consistent with involvement of the pathway within other autoimmune conditions and warranting further investigation.

Keywords Autoimmune · Neuroinflammatory · Demyelinating · Genetics · Variants of unknown significance · Next-generation sequencing

Introduction

Autoimmune and neuroinflammatory central nervous system (CNS) disorders are being increasingly recognized in children as a complex group of disorders with a wide range of clinical manifestations [1]. The genetic basis of inflammatory disorders of the CNS remains largely unknown, and among these, multiple sclerosis (MS) has been the most widely investigated. Studies of MS genetic predisposition have historically focused on identifying common variants or single-nucleotide polymorphisms (SNPs) that are associated with increased risk of developing the disease. Genome-wide association studies (GWAS) have uncovered more than 230 such SNPs [2, 3]. Of the hundreds of susceptibility genetic loci implicated in MS, the Major Histocompatibility
Complex (MHC) locus constitutes the largest component of genetic risk [4]. Studies have shown that roughly 20% of MS heritability is explained by common variants from GWAS, while 5% are explained by coding, rare variants that are not identified through GWAS. Despite all efforts, 75% of MS is still unexplained, which underscores the remarkable genetic complexity of these conditions [3].

Many genes implicated in autoimmune and inflammatory disorders are pleotropic. Nearly a third of the genetic variants associated with MS also have been reported in other autoimmune diseases, and studies of multiple, different autoimmune diseases has shown that almost two-thirds of loci are shared between these diseases [4–6]. Identification of rare variants associated with different conditions could shed light on pathophysiologic mechanisms underlying these diseases.

Considering the sparsity of literature, especially in pediatric patient populations, the authors sought to explore rare variants of genes implicated in immune dysregulation in pediatric autoimmune and inflammatory CNS disorders.

Methods

Patient population

IRB approval was obtained through Children’s Hospital Los Angeles and University of Southern California. Patients were identified by auditing individuals evaluated in the Pediatric Neuroimmunology and Demyelinating Disorders Program at Children’s Hospital Los Angeles between July 2019 and December 2021 who had genetic testing. Inclusion criteria were (1) patients were <21 years of age at the time of first neuroinflammatory attack or clinical presentation and (2) had a confirmed neuroinflammatory disorder per the senior author, a fellowship trained pediatric neuroimmunologist (JS). Diagnostic criteria varied for each condition (e.g., McDonald’s 2017 or International Pediatric Multiple Sclerosis Study Group 2013 criteria for MS) although were considered standard of care for the condition assessed. Diagnosis was subsequently verified by a second pediatric-trained neuroimmunologist (NA). There were no exclusion criteria and all individuals with genetic testing as defined below were enrolled. As this study was retrospective in nature, consent and assent were waived.

Study design

Individuals meeting inclusion criteria had to have undergone genetic testing with either whole exome sequencing or a focused exome sequencing study (e.g., commercial autoinflammatory and autoimmunity syndromes panel) which was obtained for clinical purposes. Institutionally, all patients are advised to have genetic testing performed following confirmatory diagnosis of a neuroinflammatory condition, limiting severity bias. All studies were completed at the same laboratory.

Demographic data were obtained through chart review. Patient characteristics included age (at the time when results of genetic studies were obtained), sex, race, ethnicity (Hispanic/Latino vs. non-Hispanic/Latino), and clinical diagnosis.

Autoimmune and inflammatory CNS disorders include the following categories: demyelinating brain and spinal cord disorders, immune-mediated encephalopathies or encephalitis, systemic autoimmune conditions with CNS manifestations, CNS vasculitis, and neurodegenerative and genetic conditions with immune-mediated pathophysiology [1].

Next-generation sequencing and bioinformatic analysis

Next-generation sequencing was performed using a focused exome analysis targeting 155 genes associated with primary disorders of innate or adaptive immunity. In some patients, an additional 37 genes implicated in autoimmunity were tested when clinically indicated (atypical or severe presentations). Additional gene testing was never reflexive (added on when the initial panels were negative) and was only ordered at the time of the initial panel. These panels are designed to identify monogenic autoinflammatory syndromes, monogenic autoimmunity, periodic fever syndromes, familial cold autoinflammatory syndromes, familial Mediterranean fever, and monogenic inflammatory bowel disease. The list of genes included in the panels and relevant transcript(s) are included in the supplementary material (Appendix 1). Online Mendelian Inheritance in Man (OMIM®) database was used to identify the reported associated conditions and inheritance pattern.

Single nucleotide variants, exon-level deletions, coding exons duplications, and 10–20 base pair mutations of adjacent intronic sequences were reported [6]. The Single Nucleotide Polymorphism Database (dbSNP) reference SNP ID number (rs number) was reported when available. Variant frequencies were obtained using population frequency databases including the Genome Aggregation Database (gnomAD v.2.1.1) and Exome Aggregation Consortium (ExAC).

For in silico prediction of variant functional effects, we used Polymorphism Phenotyping v2 (PolyPhen-2), and
Sorting Intolerant from Tolerant (SIFT) with Genome Reference Consortium Human Build 37 (GRCh37/hg19) assembly input. Combined Annotation Dependent Depletion (CADD) scores were calculated using the GRCh37-v1.6 model.

Pathway analysis

To perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using clinical diagnosis gene lists, lists of gene names were first imported into the NIAID/NIH Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resources v.6.8 Analysis Wizard Tool. “OFFICIAL_GENE_SYMBOL” was selected in the Identifier field, Homo sapiens was inputted within the Species field, and “Gene List” was selected under List Type. Next, the imported gene list was analyzed using the DAVID Functional Annotation Tool set, specifically looking within the “Pathway” and “KEGG_Pathway” tools [7, 8].

Burden test analysis

This study assessed gene-based contribution of variants of unknown significance via weighted sum statistics (WSS) burden test[9] and the variance component C-alpha test [10], using previously established methods [11]. To assess the aggregate contribution of multiple rare genes in the disease processes studied, the authors performed burden testing analysis using high confidence variants and potentially pathogenic variants based on MAF or protein-prediction algorithms. Variants were identified by literature driven review in multiple sclerosis as other, more rare disorders, did not have sufficient genetic investigation to warrant phenotype/genotype differentiation [11–13]. Variants meeting criteria were considered qualifying variants and were applied in Test Rare vAriants with Public Data (TRAPD)[14] as a pathogenicity filter and subsequently analyzed against the gnomeAD database.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of patients included in this study. For KEGG pathway analysis, p values and Benjamini corrections were calculated. Benjamini values of < 0.05 were considered statistically significant. Analyses were performed using DAVID Bioinformatics Resources 6.8. For burden test analysis, the freely available TRAPD program was utilized. Data were reformatted to python format for conversion.

Results

We identified 54 patients with pediatric-onset autoimmune CNS disorders in whom autoimmune and autoinflammatory panels were obtained out of a total of 174 eligible patients (31%). The most frequent reasons for not having testing were: insurance denial (n = 103/120, 86%), family or patient declining testing (n = 10/120, 8%), and delays in obtaining testing at the time of study (n = 7/120, 6%). Of note, insurance denials were primarily commercial payors (n = 69/103, 67%) as opposed to state or federal payors (n = 34/103, 33%). Enrolled patients had higher rates of state or federal payors as a primary insurance (n = 40/54, 74%) which was significantly different (p < 0.001, 95% CI 0.08–0.36) compared to excluded patients. The mean age was 13.4 ± 5.31 years and 55% were female. Demographics and clinical diagnosis of patients are listed in Table 1.

Table 1 Demographics and clinical diagnosis

| Age Mean (year) | 13.4 ± 5.31 |
|----------------|-------------|
| Sex (n, %)     |             |
| Male           | 24 (44.4%)  |
| Female         | 30 (55.6%)  |
| Ethnicity (n, %)|         |
| Hispanic/Latino| 27 (50.0%)  |
| Not Hispanic/Latino | 11 (20.4%) |
| Not reported   | 16 (29.6%)  |
| Diagnosis (n, %)|       |
| MS             | 15 (27.8%)  |
| MOGAD          | 13 (24.0%)  |
| Autoimmune encephalitis | 5 (9.25%) |
| CNS vasculitis | 3 (5.56%)  |
| ADEM           | 2 (3.70%)   |
| Idiopathic transverse myelitis | 2 (3.70%) |
| Meningoencephalitis of unknown etiology | 2 (3.70%) |
| Post-infectious meningoencephalitis | 2 (3.70%) |
| CIS            | 1 (1.85%)   |
| Down syndrome regression disorder | 1 (1.85%) |
| Hemispheric inflammation | 1 (1.85%) |
| Inflammatory Stroke | 1 (1.85%) |
| MFS/Bickerstaff's brainstem encephalitis | 1 (1.85%) |
| Neuropsychiatric SLE | 1 (1.85%) |
| Neurosarcoïdosis | 1 (1.85%) |
| RIS            | 1 (1.85%)   |
| SLE cerebritis  | 1 (1.85%)   |
| Susac Syndrome | 1 (1.85%)   |

ADEM acute disseminated encephalomyelitis, CIS clinically isolated syndrome, CNS central nervous system, MFS Miller Fisher syndrome, MOGAD myelin oligodendrocyte glycoprotein antibody-associated disease, MS multiple sclerosis, RIS radiographically isolated syndrome, SLE systemic lupus erythematos
Forty-two patients (77.8%) carried variant(s) in immune dysregulation genes, among which 12 (22.2%) had 3–8 variants (Appendix 2). Eighty-eight unique single-nucleotide variants of 55 genes were identified (all heterozygous). Twelve patients (22.2%) had negative results. All variants were unique to each individual, except for two variants of NOD2 (p.Arg702Trp and p.Gly908Arg) that were each shared among two different individuals. The highest number of variants were detected in UNC13D (6 variants); LRBA, LYST, and NOD2 (4 variants); and DOCK8, RNASEH2A, STAT5B, and AIRE (3 variants).

Table 2 lists the gene variants categorized by clinical diagnosis. Two variants were deemed as increased risk alleles [NOD2 c.2104C > T (p.Arg702Trp) and NOD2 c.2722G > C (p.Gly908Arg)]. The rest of the variants (86, 97.7%) were classified as VUS. Seventy-seven (87.5%) variants were missense mutations in coding regions, four (4.5%) silent, three (3.4%) intronic, two (2.3%) in non-coding regions, and two (2.3%) resulted in a change in an RNA molecule that does not result in any protein product. Of note, no patients had any abnormalities on the 37 gene “add-on” testing that was performed in a minority (8/54, 15%) of patients.

Most of the variants (85, 96.5%) had an allele frequency of less than 0.1% (MAF < 0.001) in the gnomAD database, including 68 variants (77.2%) < 0.01% (MAF < 0.0001). Fourteen variants (15.9%) were not reported in the gnomAD database.

Mean CADD score was 17.3 ± 9.45 (median 21.4, IQR 9.63–24.6). The majority of variants (62, 70.4%) had CADD score > 10. For seventeen rare variants of 13 genes (ACP5, ADAR, DEF6, LYST, NLRC4, NOD2, RAB27A, RFXANK, RNASEH2A, SLC7A7, TTC7A, UNC13D, and XIAP) available results of all platforms were in agreement predicting detrimental effect [deleterious/damaging based on PolyPhen and SIFT, moderate to highly conserved, and CADD > 15 (median 25.9, IQ 25.9–27.5)] (Table 2).

From the KEGG pathway analysis of the aggregated gene lists, seven genes associated with primary immunodeficiency (Benjamini 1.40 E − 06), six genes with NOD-like receptor signaling pathway (Benjamini 4.10 E − 04), five genes with inflammatory bowel disease (IBD) (Benjamini 9.80 E − 03), and five genes with NF-kappa B signaling pathway (Benjamini 1.90E − 02) (Table 3).

Burden testing analysis of rare variants in our cohort were compared to the gnomAD control database. No single gene in burden testing analysis was noted to be significant after multiple testing corrections (p = 0.38) with a similar non-statistically significant c-alpha score (p = 0.66).

## Discussion

To our knowledge, this is the first study of rare variants of immune regulation genes in a relatively large sample of pediatric patients with autoimmune CNS diseases. Using next-generation sequencing provides insight into rare variants that are not identified by GWAS.

We observed a high rate (77.4%) of identification of rare and low-frequency variants within immune dysregulation genes among pediatric patients with autoimmune CNS disorders. The majority of identified variants had a CADD score > 10, indicating the likelihood to be function-altering. The findings could shed light on pathophysiologic mechanisms of these conditions. Although the cohort-based gene test did not achieve statistical significance after correcting for multiple gene testing, the heterogeneity and small “n” in this inception cohort likely limited the ability to detect genes that may have contributed to the phenotypes recorded.

Table 4 lists immune dysregulation conditions associated with the 55 genes harboring the rare variants identified in our study. Several of these genes have been reported to be associated with neurological manifestations. Notably, TREX1, RNASEH2A, ADAR, and IFIH1 are among the genes associated with Aicardi–Goutieres syndrome [15]; STXBP2, UNC13D are associated with familial hemophagocytic lymphohistiocytosis (FHL) [16], which can cause neuroinflammation in up to 50% of patients [17]. Variants of NOD2 are most notably known for increased risk of Crohn’s disease [18], but are also reported in association with Rasmussen syndrome with CNS granulomatosis [19]. TNFAIP3 has been reported in association with a granulomatous neuro-inflammatory disorder of CNS [20], neuropsychiatric Systemic Lupus Erythematosus (SLE) [21], and Neuromyelitis Optica (NMO) [22]. Decreased TNFAIP3 gene expression was associated with Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) relapse [23]. LYST is associated with Chediak-Higashi syndrome, learning disorders, cerebellar deficits, polynuropathies, spasticity, cognitive decline, and parkinsonism [24]. RAG1, one of the genes involved in Severe Combined Immunodeficiency (SCID) [25], is also reported in association with refractory status epilepticus [26] and optic neuropathy [27]. Other associations include AIRE with autoimmune cerebellar degeneration [28]; RAB27A with developmental regression and seizures [29]; RTEL1 with microcephaly, developmental delay, spastic diplegia, and cerebellar dysfunction [30]; STAT1 with CNS aneurysms and inflammatory spinal cord lesions [31]; SMARCAL1 with microcephaly, developmental delays, and neuronal migration disorders [32]. TTC7A
Table 2  List of rare variants, allele frequency, and results of in silico predictions categorized by diagnosis

| Dx                               | Gene  | Variant                      | dbSNP    | ExAC AF | PolyPhen  | SIFT         | Conserv | CADD |
|----------------------------------|-------|------------------------------|----------|---------|-----------|--------------|---------|------|
| ADEM                             | ADAR  | c.577C > G                   | rs145588689 | 0.003   | NA        | NA           | Mod     | 23.5 |
|                                  | ADAR  | (p.Pro193Ala)                |          |         |           |              |         |      |
|                                  | AIRE  | c.722G > T                   | rs1260665653 | NA      | Probably damaging | Deleterious | Weak   | 5.897 |
|                                  | AIRE  | (p.Ser241Ile)                |          |         |           |              |         |      |
|                                  | DEF6  | c.1745 T > A                 | rs751075162 | 0.0001  | Possibly damaging | Deleterious | High   | 27.7 |
|                                  | DEF6  | (p.Leu582Gln)                |          |         |           |              |         |      |
|                                  | ITGB2 | c.1358G > A                  | rs138659490 | 0.0008  | NA        | NA           | High    | 9.234 |
|                                  | ITGB2 | (p.Ser453Asn)                |          |         |           |              |         |      |
|                                  | NOD2  | c.1151 T > A                 | rs777343284 | 0.0003  | Probably damaging | Tolerated   | High   | 25.9 |
|                                  | NOD2  | (p.Phe384Tyr)                |          |         |           |              |         |      |
| Autoimmune encephalitis         | AIRE  | c.1256G > A                  | rs756933733 | NA      | Possibly damaging | Tolerated   | Mod    | 19.08|
|                                  | AIRE  | (p.Cys419Tyr)                |          |         |           |              |         |      |
|                                  | IL21R | c.585C > G                   | rs773814550 | NA      | Possibly damaging | Tolerated   | Mod    | 24.6 |
|                                  | IL21R | (p.Ser195Arg)                |          |         |           |              |         |      |
|                                  | RNASEH2A | c.871C > T            | rs771858022 | 0.00006 | Probably damaging | Deleterious | High   | 24.6 |
|                                  | RNASEH2A | (p.Arg291Cys)            |          |         |           |              |         |      |
|                                  | STAT1 | c.1632 + 6G > A             | rs185216067 | 0.0008  | NA        | NA           | NA      | 5.658 |
|                                  | STAT1 | (p.Ala91Thr)                 |          |         |           |              |         |      |
|                                  | TNFRSF1A | c.271G > A               | NA      |         | Possibly damaging | Tolerated   | Mod    | 21.7 |
|                                  | TNFRSF1A | (p.Ala91Thr)           |          |         |           |              |         |      |
|                                  | XIAP  | c.844G > C                   | NA      |         | Probably damaging | Deleterious | High   | 37   |
|                                  | XIAP  | (p.Glu282Gln)                |          |         |           |              |         |      |
| CIS                              | CYBA  | c.553G > A                   | rs1158937022 | NA      | Tolerated | Tolerated    | Weak    | 15.57|
| CNS vasculitis                   | DOCK8 | c.4276A > G                  | rs755182322 | 0.0009  | Tolerated | Tolerated    | High    | 23.6 |
|                                 | DOCK8 | (p.Ser1426Gly)               |          |         |           |              |         |      |
|                                 | IL21  | c.470A > T                   | rs1326239267 | NA      | Tolerated | Tolerated    | Weak    | 12.68|
|                                 | IL21  | (p.His157Leu)                |          |         |           |              |         |      |
|                                 | SLC7A7 | c.187C > T                   | NA      |         | Possibly damaging | Deleterious | High   | 26   |
|                                 | SLC7A7 | (p.Leu63Phe)                 |          |         |           |              |         |      |
|                                 | UNC13D | c.652G > T                   | rs775666284 | 0.0001  | Possibly damaging | Deleterious | Mod    | 26.1 |
|                                 | UNC13D | (p.Gly218Trp)                |          |         |           |              |         |      |
| Down syndrome regression disorder| CTLA4 | c.23G > A                    | rs138279736 | 0.0005  | Tolerated | Tolerated    | Mod     | 17.97|
|                                 | CTLA4 | (p.Arg8Gln)                  |          |         |           |              |         |      |
|                                 | IRF7  | c.1405 T > C                 | rs746725871 | 0.0009  | Benign    | Tolerated    | Mod     | 4.558|
|                                 | IRF7  | (p.Trp469Arg)                |          |         |           |              |         |      |
|                                 | LYST  | c.1676G > A                  | rs138011756 | 0.0008  | Benign    | Tolerated    | Mod     | 16.15|
|                                 | LYST  | (p.Arg559His)                |          |         |           |              |         |      |
|                                 | SMARCAL1 | c.488C > A              | rs748188404 | 0.0003  | Tolerated | Tolerated    | Weak    | 6.197|
|                                 | SMARCAL1 | (p.Trp163Asn)           |          |         |           |              |         |      |
| Hemispheric inflammation         | RBCK1 | c.69 T > G                   | rs748386516 | 0.0007  | Possibly damaging | Tolerated   | High   | 13.08|
|                                 | RBCK1 | (p.Asp23Glu)                 |          |         |           |              |         |      |
|                                 | UNC13D | c.419 T > C                  | rs1181554837 | NA      | Probably damaging | Deleterious | Mod    | 25.9 |
|                                 | UNC13D | (p.Leu140Thr)                |          |         |           |              |         |      |
| Meningoencephalitis of unknown etiology | CARD14 | c.652C > T                   | NA      |         | NA        | NA           | Weak    | 24.7 |
|                                 | CARD14 | (p.Arg218Cys)                |          |         |           |              |         |      |
|                                 | CYBA  | c.274G > A                   | rs202179890 | 0.0002  | Benign    | Tolerated    | Weak    | 7.442|
|                                 | CYBA  | (p.Val92Ile)                 |          |         |           |              |         |      |
|                                 | DOCK8 | c.1817G > A                  | rs778451048 | 0.0003  | Benign    | Tolerated    | High    | 21.3 |
|                                 | DOCK8 | (p.Ser606Asn)                |          |         |           |              |         |      |
|                                 | PLCG2 | c.3092A > G                  | rs747605077 | 0.00001 | Benign    | Deleterious  | High    | 2.114|
|                                 | PLCG2 | (p.Asn1031Ser)               |          |         |           |              |         |      |
| Dx       | Gene   | Variant             | dbSNP    | ExAC AF | PolyPhen | SIFT     | Conserv | CADD  |
|---------|--------|---------------------|----------|---------|----------|----------|---------|-------|
| PSTPIP1 | c.831G > T  
(p.Glu277Asp) | rs99098606         | NA       | Tolerated | Tolerated | Mod     | 6.831   |
| RMRP    | n.189C > T  
(RNA change) | NA                 | NA       |          |          |          |         |
| STAT5B  | c.799C > T  
(p.Pro267Ser) | NA                 | NA       | Probably damaging | Tolerated | Mod     | 24.3    |
| TNFRSF13B | c.41G > A  
(p.Arg14His) | rs200309474         | 0.002    | Tolerated | Tolerated | Weak    | 0.258   |
| TNFSF12 | c.610G > A  
(p.Gly204Arg) | rs746979506         | 0.0009   | Probably damaging | Tolerated | Weak    | 14.18   |
| TREX1   | c.24G > A  
(Silent) | rs147463121         | 0.0001   | NA       | NA       | NA      | 3.279   |
| MOGAD   | ACP5    | c.249C > G  
(p.Asp83Glu) | rs563929774 | 0.0001 | Probably damaging | Deleterious | High    | 24.3   |
| ADA2    | c.1033G > A  
(p.Ala345Thr) | rs752798667         | 0.0002   | Benign   | Tolerated | Mod     | 26.6    |
| AIRE    | c.1438A > G  
(p.Thr480Ala) | NA                 | NA       | Benign   | Tolerated | Mod     | 21.6    |
| CTLA4   | c.309C > T  
(Silent) | NA                 | NA       | NA       | NA       | NA      | 35      |
| IFIH1   | c.1745C > T  
(p.Ala582Val) | rs889262310         | NA       | Benign   | Tolerated | Weak    | 12.41   |
| LRBA    | c.40A > G  
(p.Thr14Ala) | rs1200143430        | NA       | Probably damaging | Tolerated | Weak    | 21.4    |
| LRBA    | c.8479A > G  
(p.Me2827Val) | rs1276578449        | NA       | Probably damaging | Tolerated | Mod     | 19.67   |
| LRBA    | c.8476G > A  
(p.Ala2826Thr) | rs779604273         | 0.0009   | Probably damaging | Tolerated | Weak    | 23.7    |
| MEFV    | c.828A > C  
(p.Glu276Asp) | rs775020273         | 0.0005   | NA       | NA       | Weak    | 0.1     |
| NOD2    | c.2104C > T  
(p.Arg702Trp) | rs2066844           | 0.03     | Probably damaging | Deleterious | Mod     | 8.082   |
| RAG1    | c.656G > A  
(p.Arg219Gln) | rs764179803         | 0.0001   | Benign   | Tolerated | Mod     | 10.03   |
| RBCK1   | c.700G > C  
(p.Glu234Gln) | rs756811010         | 0.0001   | Benign   | NA       | High    | 40      |
| STAT5B  | c.2348C > T  
(p.Pro783Leu) | NA                 | Possibly damaging | Tolerated | Mod     | 23.6    |
| STIM1   | c.1367 T > C  
(p.Ile456Thr) | NA                 | NA       | Benign   | Deleterious | Mod     | 5.025   |
| STXB2   | c.1453-9G > A  
(Intronic) | rs372742473         | 0.0002   | NA       | NA       | NA      | 6.059   |
| TNFRSF13B | c.21C > G  
(p.Ser7Arg) | rs780461208         | 0.0002   | NA       | Tolerated | Weak    | 13.14   |
| UNC13D  | c.3022A > C  
(p.Thr1008Pro) | rs753816739         | 0.0002   | Probably damaging | Tolerated | Weak    | 24.5    |
| UNC13D  | c.2783G > A  
(p.Arg928His) | rs113461073         | 0.002    | Benign   | Tolerated | Weak    | 0.44    |
| ZAP70   | c.790+5C > T  
(Intronic) | rs56133341          | 0.0004   | NA       | NA       | NA      | 0.239   |
Table 2 (continued)

| Dx  | Gene | Variant                        | dbSNP       | ExAC AF | PolyPhen     | SIFT         | Conserv   | CADD  |
|-----|------|--------------------------------|-------------|---------|--------------|--------------|-----------|-------|
| MS  | ACP5 | c.131C>T (p.Thr44Met)         | rs369804864 | 0.00003 | Probably damaging | NA          | High      | 7.842 |
|     | ADAM17 | c.53C>T (p.Pro18Leu)               | rs144458353 | 0.0006 | Benign       | Tolerated    | Mod       | 21.4  |
|     | BACH2 | c.2230A>G (p.Ile744Val)              | rs1321699864 | NA     | Benign       | Tolerated    | Weak      | 13.41 |
|     | CARD14 | c.2140G>A (p.Gly714Ser)             | rs151150961 | 0.0007 | NA           | NA           | Weak      | 6.068 |
|     | DOCK8 | c.268_270del (p.Asp90del)              | rs776468911 | 0.0003 | NA           | NA           | NA        | 26.2  |
|     | DUOX2 | c.1295G>A (p.Arg432His)               | rs530736554 | 0.0007 | NA           | NA           | High      | 24.3  |
|     | DUOX2 | c.1825C>T (p.Pro609Ser)               | rs201221237 | 0.0009 | NA           | NA           | High      | 25.6  |
|     | G6PC3 | c.1001T>C (p.Met334Thr)              | rs746741551 | 0.0002 | Benign       | Tolerated    | Weak      | 1.205 |
|     | G6PC3 | c.413G>A (p.Arg138His)               | rs763535974 | 0.0001 | Benign       | Tolerated    | Mod       | 15.17 |
|     | IL10  | c.434C>T (p.Ala145Val)               | rs774072665 | 0.00001 | Benign       | Tolerated    | Weak      | 14.84 |
|     | IL1RN | c.28G>C (p.Gly10Arg)                | rs770976676 | 0.0002 | Benign       | Deleterious  | Weak      | 33    |
|     | LRBA  | c.5149G>A (p.Val1717Met)             | rs143003767 | 0.0007 | Benign       | Tolerated    | Weak      | 16    |
|     | LYST  | c.2465C>T (p.Thr822Ile)              | rs199746236 | 0.0003 | Possibly damaging | Deleterious | Mod       | 26.4  |
|     | LYST  | c.6454A>C (p.Ser2152Arg)             | rs201317160 | 0.0003 | Tolerated    | Tolerated    | Mod       | 14.68 |
|     | NLRC4 | c.443G>T (p.Arg148Leu)               | rs377088692 | NA     | Possibly damaging | Deleterious | High      | 15.23 |
|     | NOD2  | c.1295C>T (p.Ala432Val)              | rs2076754   | 0.0002 | Probably damaging | Deleterious | High      | 16.34 |
|     | ORAI1 | c.14C>T (p.Pro5Leu)                  | rs549883296 | NA     | Tolerated    | Tolerated    | Weak      | 24.7  |
|     | RAB27A | c.543A>G (p.Ile181Met)               | rs139025012 | 0.0001 | Possibly damaging | Deleterious | High      | 22.4  |
|     | RFXANK | c.661G>A (p.Asp221Asn)               | NA         | Possibly damaging | Deleterious | Mod       | NA       |
|     | RMRP  | n.*70G>A (Non-coding)               | NA         | NA     | NA           | NA           | NA        | NA    |
|     | SH3BP2 | c.1135C>T (p.Pro379Ser)              | rs759054470 | 0.00003 | Benign       | Tolerated    | High      | 23.4  |
|     | STAT5B | c.2358A>G (Silent)                  | rs568497349 | 0.0002 | NA           | NA           | NA        | 23.6  |
|     | STIM1 | c.1773C>G (p.Asp591Glu)              | rs776241052 | 0.0002 | Benign       | Tolerated    | Weak      | 13.73 |
|     | TBX1  | c.1039C>A (p.Arg347Ser)              | NA         | Possibly damaging | Tolerated    | Mod       | NA       |
| Dx                          | Gene       | Variant                  | dbSNP         | ExAC AF | PolyPhen  | SIFT   | Conserv | CADD |
|-----------------------------|------------|--------------------------|---------------|---------|-----------|--------|---------|------|
| Neuropsychiatric SLE        | **TNFAIP3**| c.2117G > A (p.Arg706Gln)| rs3734553     | 0.0001  | Benign     | Deleterious | High   | 22.4 |
| Neurosarcoïdosis            | **UNC13D** | c.2795 T > C (p.Leu932Pro)| rs760552006   | 0.003   | Probably damaging | Deleterious | High   | 26.4 |
| Neuropsychiatric SLE        | **UNC13D** | c.681C > T (Silent)      | rs779543680   | 0.0003  | NA         | NA     | NA      | 11.53|
| Neuropsychiatric SLE        | SLC29A3    | c.146G > C (p.Arg49Pro)  | rs201610819   | 0.001   | Probably damaging | Tolerated | Weak   | 24.3 |
| Inflammatory stroke         | **RNASEH2A**| c.101A > G (p.As34Gly)  | rs762516714   | 0.001   | Probably damaging | Deleterious | High   | 27.5 |
|                             | **NOD2**   | c.2722G > C (p.Gly908Arg)| rs2066845     | 0.014   | Probably damaging | Deleterious | Mod    | 29.7 |
|                             | **RTEL1**  | c.2306G > A (p.Arg769His)| 0.0001       | Tolerated | Tolerated | Tolerated | Weak   | 11.48|
| RIS                         | **NOD2**   | c.2722G > C (p.Gly908Arg)| rs2066845     | 0.014   | NA         | NA     | Mod    | 29.7 |
|                             | **TTC7A**  | c.563G > A (p.Arg188His) | rs147471840   | 0.0002  | Probably damaging | Deleterious | Mod    | 25.2 |
| SLE cerebritis              | **CARD8**  | c.803A > G (p.Asn268Ser)| NA            | Tolerated | Tolerated | Tolerated | Mod    | NA   |
|                             | **LYST**   | c.7157A > G (p.His2386Arg)| rs758888571  | 0.0002  | Probably damaging | Tolerated | High   | 23.2 |
|                             | **NOD2**   | c.2104C > T (p.Arg702Trp)| rs2066844    | 0.03    | NA         | NA     | Mod    | 8.082|
| Susac syndrome              | **DCLRE1C**| c.212C > T (p.Thr71Met)  | rs147013097   | 0.0003  | Tolerated | Tolerated | Mod    | 24.6 |
| Transverse myelitis         | **IFIH1**  | c.2973C > A (p.Phe991Leu)| rs763358277   | NA      | Possibly damaging | Tolerated | Mod    | 21.6 |
|                             | **RNASEH2A**| c.821A > G (p.As274Ser) | rs373169862   | 0.0007  | Benign     | Tolerated | Weak   | 2.817|

Variants for which available results of all platforms were in agreement predicting detrimental effect are bolded

ADEM acute disseminated encephalomyelitis, CIS clinically isolated syndrome, CNS central nervous system, MOGAD myelin oligodendrocyte glycoprotein antibody-associated disease, MS multiple sclerosis, RIS radiographically isolated syndrome, SLE systemic lupus erythematos

with perisylvian polymicrogyria, cerebellar hypoplasia and arthrogryposis, severe microcephaly, refractory epilepsy, developmental delay, and hypomyelinating leukodystrophy [33]; and ZAP70 with silent brain infarcts [34].

Our analysis of KEGG-enriched pathways both reflects the nature of the screening tests used and provides mechanistic insights into the pathophysiology of these conditions. The most significantly enriched term was “Primary Immunodeficiency,” potentially a result of the autoimmune and autoimmune syndrome screening panels used in the study. By definition, autoimmune neurologic diseases represent disorders of immune regulation. Inflammatory pathways have already been linked to neural dysfunction; examples in epilepsy alone include the IL-1 receptor/Toll-like receptor (TLR) 4 axis, the arachidonic acid–prostanoid cascade, oxidative stress, and transforming growth factor-β (TGFβ) signaling associated with blood–brain barrier dysfunction, among others [82]. It is, therefore, not surprising that genes involved with immune development and regulation may also be involved with diseases of neuroinflammation.

What is surprising from these data is an identified enrichment in specific arms of the immune response, notably the Nucleotide Oligomerization Domain (NOD)-like Receptor...
The NOD-like Receptor protein family is one of several classes of germline-encoded pattern recognition receptors (PRR) used within the innate immune system [83]. Other example PRRs include TLRs and C-type lectin receptors, which interact with microbial ligands such as bacterial lipopolysaccharide and peptidoglycan or yeast β-glucans. In contrast to membrane-bound PRRs, NOD-like receptors are known to detect pathogen and danger-associated patterns within the cytoplasmic compartment and are involved with initial innate immune responses to cellular injury and stress [83].

NOD2 in particular is stimulated by bacterial peptidoglycan-related products to oligomerize, recruit receptor-interacting serine/threonine-protein kinase 2 (RIPK2), and ultimately activate downstream NF-κB and MAPK signaling to promote production of proinflammatory molecules [84] (Fig. 1). NOD2 has already been linked to conditions of immune dysregulation; NOD2 polymorphisms are the strongest genetic risk factors for the development of Crohn’s Disease, although the exact mechanisms by which are not yet clear [18]. In addition to Crohn’s disease, NOD2 mutations are associated with systemic and CNS inflammatory granulomatous diseases, such as Blau Syndrome, early-onset sarcoidosis [85], and CNS granulomatosis [19]. Moreover, expression of NOD is increased in astrocytes after exposure to bacterial pathogens of the CNS [86], promoting microglial inflammation in murine models of pneumococcal meningitis [87], as well as dopaminergic degeneration in a murine model of Parkinson’s Disease [88]. Our data suggest NOD2-receptor signaling may be an attractive candidate for further investigation and targeting in pediatric autoimmune-neuroinflammatory conditions.

This study is not without limitations. This study evaluated a population of children with heterogeneous rare and ultra-rare diseases making broad generalization of the results difficult. Accordingly, this study has a low total n. This undoubtedly contributed to the lack of statistical significance during burden testing analysis. In addition, there are several limitations to burden testing that affect its utility particularly in more modest sample sizes. Some of the well-known barriers include locus heterogeneity (several contributing genes each accounting for only a small percentage of cases), and high background rate of rare variants in the candidate genes [14]. Using a public sequencing database (e.g., gnomAD) as control imposes additional challenges. There is possibility of “contamination” of the control group, as these datasets might contain individuals with neurological and/or immune dysregulation conditions. Another important consideration is that the aggregate datasets include multiple different sequencing platforms and variant-calling processes that might be different from which was used for cases, affecting the validity of the comparison between the two groups. Moreover, due to lack of individual-level data in the aggregate datasets, an approximation is used, resulting in a more conservative test which overestimates the sum of variants in the population-based datasets, in turn underestimating the difference between cases and controls.

Our results highlight the need for more expansive testing of this population to further assess if the high frequency of

| KEGG Pathway                              | Genes                  | Count | %     | p value    | Benjamini |
|-------------------------------------------|------------------------|-------|-------|------------|-----------|
| Primary immunodeficiency                  | DCLE1C, ORM, TNFRSF13B, AIRE, RAG1, RFXANK, ZAP70 | 7     | 13    | 1.30E-08   | 1.40E-06  |
| NOD-like receptor signaling pathway       | MEFV, NLRC4, TNFAIP3, CARD8, NOD2, PSTPIP1  | 6     | 11.1  | 8.00E-06   | 4.10E-04  |
| Inflammatory bowel disease (IBD)          | IL10, IL21R, IL21, NOD2, STAT1  | 5     | 9.3   | 2.90E-04   | 9.80E-03  |
| NF-kappa B signaling pathway              | TNFAIP3, TNFRSF1A, XIAP, PLCG2, ZAP70 | 5     | 9.3   | 9.30E-04   | 1.90E-02  |
| Gene      | Associated immune dysregulation conditions                                                                 | Neurologic manifestations                                                                 |
|-----------|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| ACP5      | Spondyloenchondrodysplasia with immune dysregulation (MIM: 607,944), monogenic                            | Early onset recurrent strokes [44]                                                        |
| ADA2      | SLE, Sjögren’s syndrome, hemolytic anemia, thrombocytopenia, hypothyroidism, inflammatory myositis, Raynaud's disease, vitiligo [35] | AGS, Torsion dystonia [45], Bilateral striatal necrosis and spastic paraplegia [46]        |
| ADAM17    | Neonatal inflammatory skin and bowel disease (MIM: 614,328)                                              | Autoimmune cerebellar degeneration [47]                                                   |
| ADAR      | Dyschromatosis symmetrica hereditaria (MIM: 127,400)                                                     | Lymphocytic infiltration of brain, seizures and headache [48]                            |
| AIRE      | Autoimmune Polyendocrinopathy Syndrome type 1 (MIM: 240,300)                                            | AGS, rapid neuroregression, spastic-dystonic syndrome, spastic paraparesis [50]           |
| BACH2     | Immunodeficiency-60 and autoimmunity (inflammatory bowel disease and recurrent sinopulmonary infections) (MIM: 618,394) | Rasmussen syndrome with CNS granulomatosis [55], Multiple system atrophy [56]            |
| CARD14    | Pityriasis rubra pilaris (MIM: 173,200), Psoriasis 2 (MIM: 602,723)                                       |                                                                            |
| CARD8     | Crohn’s disease 30 (MIM 619,079)                                                                         |                                                                            |
| CTLA4     | Immune dysregulation with autoimmunity, immunodeficiency, and lymphoproliferation                          |                                                                            |
| CYBA      | CGD4 (MIM: 233,690)                                                                                      |                                                                            |
| DCLRE1C   | Omenn syndrome (SCID associated with erythrodermia, hepatosplenomegaly, lymphadenopathy, and alopecia) (MIM: 603,554, 602,450) |                                                                            |
| DEF6      | Immunodeficiency 87 and autoimmunity, increased susceptibility to EBV, hemolytic anemia (MIM: 619,573)   |                                                                            |
| DOCK8     | Hyper-IgE recurrent infection syndrome (MIM: 243,700)                                                     |                                                                            |
| DUOX2     | Partial iodide organification defect, thyroid dyshormonogenesis (MIM: 607,200)                            |                                                                            |
| G6PC3     | Dursun Syndrome, severe congenital neutropenia(MIM:612,541)                                             |                                                                            |
| IFI1H1    | AGS7 (MIM: 615,846), Singleton-Merton syndrome (MIM: 182,250)                                           |                                                                            |
| IL10      | Progression of RA (MIM: 180,300)                                                                         |                                                                            |
| IL1RN     | Osteomyelitis, sterile multifocal, with periostitis and pustulosis (MIM: 612,852)                         |                                                                            |
| IL21      | CVID 11(MIM:615,767)                                                                                     |                                                                            |
| IL2R      | Combined immunodeficiency due to interleukin 21 receptor deficiency (MIM: 615,207)                       |                                                                            |
| IFI7      | Severe influenza disease (MIM: 616,345)                                                                 |                                                                            |
| ITGB2     | Leukocyte adhesion deficiency 3 (MIM: 612,840)                                                           |                                                                            |
| LRBA      | CVID 8 with autoimmunity (MIM: 614,700), idiopathic thrombocytopenic purpura, autoimmune hemorrhagic anemia, and inflammatory bowel disease |                                                                            |
| LYST      | Chediak-Higashi syndrome (MIM:214,500)                                                                  |                                                                            |
| MEFV      | FMF (MIM: 134,610, 249,100), acute febrile neutrophilic dermatosis (MIM: 608,068)                          |                                                                            |
| NLRC4     | Familial cold autoinflammatory syndrome (MIM: 616,115), Autoinflammation with infantile enterocolitis (MIM: 616,050) |                                                                            |
| NOD2      | Increased risk Crohn’s disease, granulomatous diseases (Blau syndrome, early-onset sarcoidosis)[37], NOD2-associated autoinflammatory disease [38] |                                                                            |
| Gene       | Associated immune dysregulation conditions                                                                 | Neurologic manifestations                                                                 |
|------------|-------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| ORAI1      | Tubular aggregate myopathy 2 (MIM: 615,883), Immunodeficiency 9 (MIM: 612,782), Immunodeficiency 9 (MIM: 612,782) | Developmental regression, seizure [57–59]                                                  |
| PLCG2      | Familial cold autoinflammatory syndrome, autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation (MIM: 614,878) | Facial nerve palsy, seizure, and decreased consciousness [60], Optic neuropathy [61]       |
| PSTPIP1    | Pyogenic sterile arthritis, pyoderma gangrenosum, and acne syndrome (MIM:604,416)                          |                                                                                            |
| RAB27A     | Griscelli syndrome type 2 (affecting skin, hair, immune system) (MIM:607,624)                              |                                                                                            |
| RAG1       | Alpha/beta T-cell lymphopenia with gamma/delta T-cell expansion, severe cytomegalovirus infection, and autoimmunity (MIM: 609,889), Combined cellular and humoral immune defects with granulomas (MIM: 233,650), Omenn syndrome (SCID with hyperesoinophilia) (MIM:603,554), SCID, B cell-negative (MIM:601,457) | Facial nerve palsy, seizure, and decreased consciousness [60], Optic neuropathy [61]       |
| RBCK1      | Polymelanin body myopathy with or without immunodeficiency (MIM: 615,895)                                  |                                                                                            |
| RFXANK     | Hereditary MHC class II deficiency (MIM: 209,920)                                                           |                                                                                            |
| RMRP       | Cartilage-hair hypoplasia (MIM: 250,250), Anauxetic (spondylopaetaepiphysieal) dysplasia (MIM:607,095)     |                                                                                            |
| RNASEH2A   | AGS4 (MIM: 610,333)                                                                                         | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| RTE1L      | Dyskeratosis congenita (MIM: 615,190)                                                                       | Microcephaly, developmental delay, spastic diplegia, cerebellar dysfunction [63]          |
| SH3BP2     | Chorobism (MIM: 118,400)                                                                                    |                                                                                            |
| SLC29A3    | Histioctysis-lymphadenopathy plus syndrome (MIM: 602,782)                                                  | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| SLC7A7     | Lysinuric protein intolerance (MIM:222,700)                                                                 | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| SMARCAL1   | Schimke immunoosseous dysplasia (SIOD)(MIM: 242,900)                                                        | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| STAT1      | STAT1 Immune deficiencies (MIM: 614,892, 613,796, 614,162)                                                  | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| STAT5B     | Growth hormone insensitivity with immune dysregulation (MIM:245,590, 618,985)                               | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| STIM1      | Tubular aggregate myopathy (MIM: 160,565), Stormorken syndrome (thrombocytopenia, asplenia, skin rash, deep-set eyes with miosis, muscle weakness) (MIM: 185,070), STIM1 immunodeficiency (MIM:612,783) | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| STXB2P     | FHL5(MIM: 613,101)                                                                                         | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TRX1       | DiGeorge/Velocardiofacial syndrome (MIM: 217,095, 188,400, 187,500, 192,430)                                 | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TNFAIP3    | familial Behcet-like autoinflammatory syndrome (MIM: 616,744)                                             | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TNFRSF13B  | SCID (MIM: 240,500), autoimmune inflammatory disorder associated with COVID-19 [39]                        | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TNFRSF1A   | Tumor necrosis factor receptor-associated periodic syndrome (TRAPS)                                          | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TNFSF12    | CVID                                                                                                       | AGS, Developmental delay, intellectual disability, seizures and epileptic encephalopathy [62] |
| TREXI      | AGS1(MIM:225,750), Chilblain lupus (MIM: 610,448), susceptibility to SLE (MIM: 152,700), Retinal vasculopathy with cerebral leukodystrophy [40, 41] (MIM: 192,315) | AGS, white matter ring-enhancing lesions, stroke [78], tumor like lesions of the brain [79] |
rare and ultra-rare variants is contributory. However, the broad overlap of the dysfunctional pathways associated with identified gene abnormalities does shed light on the possibility of shared pathology among these disorders. The authors hope that these data serve as a proof of concept for the need for additional next-generation exome sequencing in individuals with pediatric neuroinflammatory disorders. Further study of more homogenous populations within this broad category (e.g., multiple sclerosis) would be particularly beneficial to best evaluate the nuances of the role of genes. There is a potential for severity bias in this study as well as not all patients at our center were able to obtain genetic testing and those who did have this testing may have had more significant or severe disease phenotypes. An important consideration in these data are that it was derived out of a focused exome panel which was limited to only genes already associated with inflammatory disease. Thus, pathway analysis is anticipated to be heavily influenced by the focused selection of genes that were analyzed, even beyond the variant level. Further study, with more broad, whole exome-based analysis, would be greatly beneficial for determining how enriched these pathways truly are. An additional limitation was that there was a statistically significant difference in the rate of commercial versus state/federal insurance for patients in the enrolled versus unenrolled groups, potentially skewing our results towards individuals who were more likely to come from lower socio-economic statuses although this was not assessed in this study. Additionally, given the geographic location of our center, there is a much higher rate of individuals of Hispanic or LatinX descent than at other centers nationally and this is of particular importance when assessing generalizability of these data. Finally, the potentially for epigenetic phenomenon or the interplay of environment, early childhood stress, and/or diet, was not assessed in this study and may be of use in future research.

Conclusions

The genetic basis of autoimmune and neuroinflammatory CNS disorders remains largely unknown, particularly in pediatric patient populations. We observed a high rate (77.4%) of identification of rare and low-frequency variants in immune regulatory genes in pediatric neuroinflammatory CNS disorders. We identified 88 unique single-nucleotide variants of 55 genes, including UNC13D, LRBA, LYST, NOD2, DOCK8, RNASEH2A, STAT5B, and AIRE. Finally, pathway analysis revealed an enrichment of NOD2-receptor signaling within this patient cohort, consistent with involvement of the pathway within other autoinflammatory conditions and warranting further investigation. This study provides the field a first glance at the genetic underpinning of pediatric autoimmune and inflammatory CNS disorders. The above gene variants, implicated in disorders of immune regulation, may play a role in pathogenesis of or predilection for autoimmune CNS disorders.
Fig. 1  A NOD2 and B Inflammatory bowel disease signaling pathways. Genes harboring rare variants are marked with a red star.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11325-2.

Fig. 1 (continued)

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.
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